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Inflammation-Induced Enhancement of the Visceromotor Reflex to Urinary Bladder Distention: Modulation by Endogenous Opioids and the Effects of Early-In-Life Experience with Bladder Inflammation

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Abstract

Abdominal electromyographic (EMG) responses to noxious intensities of urinary bladder distention (UBD) are significantly enhanced 24 hrs following zymosan-induced bladder inflammation in adult female rats. This inflammation-induced hypersensitivity is concomitantly inhibited by endogenous opioids because intraperitoneal (i.p.) naloxone administration before testing significantly increases EMG response magnitude to UBD. This inhibitory mechanism is not tonically active since naloxone does not alter EMG response magnitude to UBD in rats without inflammation. At the dose tested, naloxone does not affect bladder compliance in rats with or without inflammation. The effects of i.p. naloxone likely result from blockade of a spinal mechanism, because intrathecal (i.t.) naloxone also significantly enhances EMG responses to UBD in rats with inflammation. Rats exposed to bladder inflammation from P90-P92 prior to re-inflammation at P120 show similar hypersensitivity and concomitant opioid inhibition, with response magnitudes being no different from that produced by inflammation at P120 alone. In contrast, rats exposed to bladder inflammation from P14-P16 prior to re-inflammation at P120 show markedly enhanced hypersensitivity and no evidence of concomitant opioid inhibition. These data indicate that bladder inflammation in adult rats induces bladder hypersensitivity that is inhibited by an endogenous opioidergic mechanism. This mechanism can be disrupted by neonatal bladder inflammation.

Keywords

bladder; opioids; inflammation; neonatal; visceromotor reflex; pain

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Introduction

Mycotic and bacterial infections of the urinary tract often produce urinary bladder inflammation, but our understanding of the neural substrates that are altered by infectious processes and result in bladder pain is limited. Recently, we showed that brief intravesical administration of zymosan in adult rats led to urinary bladder inflammation that became progressively more severe in a time-dependent manner.²⁵ This treatment also led to a significant increase in arterial blood pressure and abdominal muscle electromyographic (EMG) responses to urinary bladder distention (UBD) when rats were tested 24 hrs following zymosan administration.^{25,26} These zymosan-induced increases in responses were observed primarily at suprathreshold intensities of UBD in the range believed to be noxious, and thus this hypersensitivity to UBD could be representative of a hyperalgesic state.^{25,26} In separate studies, we also found that the bladder hypersensitivity produced by zymosan treatment as an adult was markedly enhanced in rats that were also administered intravesical zymosan for three consecutive days as neonates (P14-P16).²⁶ This enhanced bladder response was paralleled by a more global bladder hypersensitivity, manifested as increased micturition frequency²⁶ and decreased thresholds for micturition reflexes during cystometry.⁸

Other studies of primary hyperalgesia and inflammation involving either the knee joint³¹ or the hindpaw^{28,39} have shown that descending inhibitory systems are progressively engaged during the first 24 hrs of acute inflammation and act to suppress somatic hyperalgesia (see also^{11,27,32-36}). These data suggested the possibility that the urinary bladder hypersensitivity produced by acute bladder inflammation in the adult rat may be concomitantly suppressed by inflammation-induced activation of an inhibitory system^{37,38} and that the enhancement effect we observed with neonatal exposure to zymosan might be due to impairment of this inhibitory system.²¹

To investigate these possibilities, and testing the hypothesis that an opioidergic inhibitory system was involved, we examined whether the bladder hypersensitivity produced by a single, acute exposure to intravesical zymosan in adult rats was augmented if the opioid receptor antagonist naloxone was present during UBD testing (Experiments 1, 4 and 5). We performed cystometrogams (CMG) to determine whether naloxone affected urinary bladder compliance, either in the presence or absence of bladder inflammation (Experiment 2), and we examined whether the opioid system was located spinally by examining the effects of intrathecal (i.t.) naloxone (Experiment 3). Finally, we examined whether the effects of naloxone on responses to acute bladder inflammation were influenced by a prior bout of bladder inflammation occurring either in adult rats one month prior to UBD testing (P90-P92; Experiment 4) or during the neonatal period (P14-P16; Experiment 5).

Materials and Methods – General

Animals and animal care

Adult female Sprague-Dawley rats (Harlan, Prattville, AL) were maintained in separate cages and were either age P90 or P120 at the time of their initial treatments. Timed-pregnant female Sprague-Dawley rats (Harlan, Prattville, AL) were also maintained in separate cages.

Following birth, only female pups were maintained and were age P14 at the time of their initial treatments. The rat pups remained housed with their mothers until weaning at ~P21. The light-dark cycle for all rats was 6:00-6:00. At the time of adult testing, no attempt was made to control for phase of estrous cycle. All studies were approved by the University of Alabama at Birmingham Animal Care and Use Committee.

Adult Zymosan Pretreatment Procedure

Twenty-four hrs prior to adult testing, all rats with the exception of groups AN and AV in Experiment 1 and group Z-N in Experiment 4 were pretreated with intravesical zymosan. Rats were anesthetized with inhaled isoflurane (5% induction, 2% maintenance) and a 22-gauge angiocatheter was placed into the urinary bladder via the urethra. Intravesical zymosan (1% solution in sterile water; 0.5 ml) was administered and left to dwell in the bladder for 30 min and drained prior to catheter removal. Each animal was administered ampicillin (100 mg/ml; 0.2 ml; subcutaneous, s.c.) and awakened. These treatments typically occurred beginning at ~7:30 AM.

Adult Surgical Preparation

On the day of adult testing with either UBD or CMG, the trachea was cannulated for artificial respiration under deep isoflurane anesthesia (4% in oxygen) and a 22-gauge angiocatheter was placed into the urinary bladder via the urethra and held in place by a tight suture around the distal urethral orifice. The animal was then moved to a recording area and maintained on 4% isoflurane until surgical procedures were completed. Body temperature was maintained throughout testing with a heating pad.

The timing of the UBD or CMG test session began when the animal was placed in the recording area. The left abdominal skin was incised and silver wires were placed into the left external oblique musculature of each animal for differential amplification of EMG activity. Following this procedure the anesthesia was reduced to 1% and maintained at this level for the duration of testing. All data were saved on a computer using Spike-2 software and associated hardware (Micro 1401; CED, Cambridge, UK). The EMG response was defined as: (rectified EMG activity during UBD - rectified baseline EMG prior to UBD) / rectified baseline EMG.

Urinary Bladder Distention

The UBD trials and the timing of UBD trials were identical across all experiments. Initially, three 20 sec duration, 60 mmHg distentions of the urinary bladder were administered (data not presented) to overcome a period of bladder sensitization that occurs prior to demonstration of vigorous and reliable VMRs.³ These distentions were followed by a fixed sequence of 20 sec duration graded distentions of the bladder at pressures of 10, 20, 30, 40, 50, 60, 70 and 80 mmHg, respectively (3 min intertrial interval). UBD consisted of distending the bladder via air pressure and monitoring intravesical pressure via an in-line, pneumatically-linked, low-volume pressure transducer.

Cystometrogram

The timing of the CMG session was identical for each rat that underwent CMG testing. The bladder was emptied using abdominal palpation prior to the onset of the saline infusion, which always occurred exactly 10 min after a naloxone or vehicle injection was given (see below). Room-temperature saline was infused into the bladder at a slow, steady rate (0.05 ml/min) for 40 min through polyethylene tubing threaded through a T-port extension and into the transurethral catheter. Intravesical pressure was measured through a sidearm of the T-port connected to a low-volume pressure transducer.

Specific Methods

Experiment 1

24 Hour Adult Pretreatment—This experiment was performed to determine if bladder hypersensitivity observed in the normal adult rat following acute exposure to intravesical zymosan was being concomitantly suppressed by opioid inhibition. Two groups of rats at age P120 were administered the adult zymosan pretreatment procedure described in the General Methods, and two additional groups received the same anesthesia protocol but in the absence of an intravesical treatment. Twenty-four hrs later, all rats underwent the adult surgical preparation and UBD testing procedure described in the General Methods. At the time of testing, half of the rats in each condition were injected with either naloxone hydrochloride (1 mg/kg, i.p.) or sterile water (1 mg/kg, i.p.; vehicle control). Ten min following the injection, the UBD testing procedure was initiated.

Experiment 2

CMG Testing of Passive Compliance—CMGs were performed to determine whether passive compliance of the urinary bladder was affected by naloxone administration in the presence or absence of inflammation. Two groups of rats at age P120 underwent the adult zymosan pretreatment procedure described in the General Methods. Twenty-four hrs later, the pretreated groups and an additional two groups of age-matched, untreated rats underwent CMG testing as described in the General Methods. Prior to the onset of CMG testing, half of the rats in each condition were injected with either naloxone hydrochloride (1 mg/kg, i.p.) or sterile water (1 mg/kg, i.p.; vehicle control). Ten min following the injection, the CMG testing procedure was initiated.

Experiment 3

i.t. Naloxone—This experiment was performed to determine whether the effect of i.p. naloxone observed in Experiment 1 could be due to a spinal locus of action. Two groups of adult rats at age P120 underwent the adult zymosan pretreatment procedure and 24 hrs later received the adult surgical procedure described in the General Methods. At the time of testing, a 7.8 cm i.t. catheter (PE10) was inserted via a slit in the atlanto-occipital membrane and threaded down the spinal column. Rats were administered either naloxone hydrochloride (1 µl/µg; 10 µg; i.t.) or vehicle (10 µl) followed by a 7 µl flush with vehicle. Two vehicles were used in separate groups of rats; physiological saline or sterile water. There were no significant differences in the functions generated with the two vehicles and so the data sets were combined. Ten min following the injection, the UBD testing procedure was initiated.

The i.t. catheters were targeted to the L5-S2 region of the spinal cord and catheter placement was sampled and verified in a subset of rats (N=4) with Evan's blue injections at the end of the testing procedure.

Experiment 4

Inflammation at P90-P92 and 24 Hour Adult Pretreatment—This experiment was performed to determine whether a history of exposure to bladder inflammation during adulthood one month prior to testing would affect the magnitude of the opioid inhibitory effect observed in Experiment 1. Specifically, the adult zymosan pretreatment procedure and UBD testing procedure were administered to adult rats (P120) with or without exposure to bladder inflammation for three consecutive days at age P90-P92. Groups of rats were administered the adult zymosan pretreatment procedure from P90-P92. One month later (P120), two of the pretreated groups and an additional two groups of age-matched, untreated rats were anesthetized and once again administered the adult zymosan pretreatment procedure followed 24 hrs later by the adult surgical preparation and UBD testing procedure. At the time of testing, half of the rats in each condition were injected with either naloxone hydrochloride (1 mg/kg, i.p.) or sterile water (1 mg/kg, i.p.; vehicle control). Ten min following the injection, the UBD testing procedure was initiated. Thus, the zymosan conditions of Experiment 1 were replicated exactly, and the effects of adult (P90-P92) intravesical zymosan administration followed by re-exposure to zymosan 24 hrs prior to UBD (P120) were also examined. A remaining group that was pretreated from P90-P92 was left untreated 24 hrs prior to UBD testing and was administered naloxone hydrochloride (1 mg/kg, i.p.) on the day of testing. This group was included to determine whether opioid inhibition could be permanently engaged following the intravesical zymosan treatments from P90-P92, or if this inhibition was evident only following re-exposure to zymosan 24 hrs prior to UBD testing.

Experiment 5

Inflammation at P14-P16 and 24 Hour Adult Pretreatment—This experiment was performed to determine whether a history of exposure to bladder inflammation during the neonatal period of development would affect the magnitude of the opioid inhibitory effect produced by a subsequent acute exposure to bladder inflammation. The adult zymosan pretreatment procedure and UBD testing procedure were administered to adult rats (P120) with or without prior exposure to bladder inflammation as neonates. For three consecutive days from age P14-P16, four groups of rat pups were anesthetized with inhaled halothane (5% induction followed by 2% maintenance). The external urethra of each rat pup was swabbed with betadine and a 24-gauge angiocatheter was placed into the urinary bladder via the urethra in two of the four groups. In these two groups, intravesical zymosan (1% solution in sterile water; 0.1 ml) was administered and left to dwell in the bladder for 30 min prior to being drained. The remaining two groups were only administered anesthesia. After 30 min, the angiocatheters were removed, and all rat pups were administered ampicillin (100 mg/ml; 0.05 ml; s.c.) and awakened.

At age P120, each group was administered the identical protocol described in Experiment 1. That is, all groups were administered the adult zymosan pretreatment procedure followed 24

hrs later by the adult surgical preparation and UBD testing procedure. At the time of testing, half of the rats in each group were administered either naloxone hydrochloride (1 mg/kg, i.p.) or sterile water (1 mg/kg, i.p.; vehicle control). Ten min following the injection, the UBD testing procedure was initiated.

Statistics—All data are presented as group mean $\pm$ standard error of the mean (SEM). In studies involving graded UBD testing, EMG responses were analyzed using repeated measures ANOVAs, and when significant main effects or relevant interactions (pressure $\times$ group) were obtained, post-hoc comparisons of means were performed using Holm's procedure¹⁵ to maintain family-wise α at 0.05. Group mean bladder weights were compared using ANOVA. Statistical significance was defined as p values $\leq$ 0.05.

Group Designations—Experimental groups are described in the text and Figure Legends according to their neonatal (P14-P16), adult (P90-P92), and/or P120 treatment. The drug treatment administered on the day of testing is also designated. For example, “ZN” describes adult rats that received treatment with zymosan (Z) 24 hrs prior to testing in the presence of naloxone (N). Alternatively, in Experiments 4 and 5 involving either neonatal or adult treatments before treatment 24 hrs prior to testing, “ZZN” describes rats that were administered zymosan (Z) as either neonates (P14-P16) or adults (P90-P92), treated with zymosan (Z) 24 hrs prior to testing, and treated with naloxone (N) on the day of testing. A dash (–) indicates no treatment.

Results

Experiment 1

24 Hour Adult Pre-Treatment—Experiment 1 was performed to determine if the bladder hypersensitivity observed in the adult rat following acute exposure to intravesical zymosan at P120 is concomitantly being suppressed by opioid inhibition. Figure 1 presents group mean EMG responses during tests with graded UBD. An ANOVA revealed a significant between-groups effect, $F(3,37)=7.24$, ($p<0.001$); significant pressure effect, $F(7,21)=87.65$, ($p<0.001$); and significant groups $\times$ pressure interaction, $F(7,259)=4.04$, ($p<0.001$). In groups receiving vehicle on the day of UBD testing, post-hoc comparisons revealed that the group receiving pre-treatment with zymosan (group ZV) had significantly greater EMG responses than the control group receiving pre-treatment with anesthesia alone (group AV) to distending pressures of 40-60 mmHg. These data are consistent with previous findings.^{25,26} In groups receiving naloxone on the day of testing, post-hoc comparisons revealed that the group receiving pre-treatment with zymosan (group ZN) had significantly greater EMG responses than the control group receiving pre-treatment with anesthesia alone (group AN) to all distending pressures between 30-80 mmHg. Finally, the effectiveness of the zymosan pre-treatment in enhancing EMG responses to UBD was increased further by naloxone administration; group ZN had significantly greater EMG responses than group ZV to distending pressures of 50-80 mmHg. The EMG responses of the two groups pre-treated with anesthesia only (groups AN and AV) did not differ at any distending pressure, indicating no tonic activation of an opioidergic system.

Experiment 2

CMG Testing of Passive Compliance—Experiment 2 was performed to determine whether naloxone affected passive compliance of the urinary bladder either in the presence or absence of inflammation. Figure 2 presents group mean intravesical pressures obtained during CMG testing. Intravesical pressure increased similarly in all groups and there was only a significant effect of pressure, $F(9,27)=63.03$, ($p<0.001$). There was no significant between-groups effect, $F(3,12)=0.05$, ($p=0.98$) or group $\times$ pressure interaction, $F(9,108)=0.30$, ($p=0.96$). These data indicate that the differences in EMG responses observed with i.p. administration of naloxone in Experiment 1 cannot be attributed either to naloxone-induced changes in bladder compliance or to an interaction between naloxone and zymosan-induced inflammation on bladder compliance.

Experiment 3

I.t. Naloxone—Experiment 1 indicated that the bladder hypersensitivity observed in the adult rat following acute exposure to intravesical zymosan is being suppressed by opioid inhibition. To determine whether the effect of i.p. naloxone observed in Experiment 1 could be due to a spinal locus of action, Experiment 3 used i.t., instead of i.p., administration of naloxone and vehicle. Figure 3 presents group mean EMG responses during graded UBD. An ANOVA revealed a significant between-groups effect, $F(1,21)=11.85$ ($p<0.01$); a significant pressure effect, $F(1,7)=38.32$ ($p<0.001$); and a significant group $\times$ pressure interaction $F(7,147)=10.05$ ($p<0.001$). Post-hoc comparisons of means revealed that the EMG responses of group ZN were significantly greater than those of group ZV to distending pressures of 50-80 mmHg. These data are consistent with the view that the effect of i.p. naloxone in enhancing the EMG responses of zymosan pre-treated rats to UBD in Experiment 1 could be due to a spinal locus of action.

Experiment 4

Inflammation at P90-P92 and 24 Hour Adult Pre-Treatment—Experiment 4 was performed to determine whether a history of zymosan-induced bladder inflammation during adulthood (P90-P92) would affect the magnitude of the opioid inhibitory effect resulting from an acute exposure to zymosan at P120. Figure 4 presents EMG responses obtained during graded UBD. An ANOVA revealed a significant between-groups effect, $F(4,27)=4.608$ ($p<0.01$); a significant pressure effect, $F(7,21)=66.63$ ($p<0.001$); and a significant group $\times$ pressure interaction, $F(7,189)=3.05$ ($p<0.001$). Post-hoc contrasts revealed that the EMG responses of group ZN were significantly greater than those of group ZV at distending pressures of 50-70 mmHg. Similarly, the EMG responses of group ZZN were significantly greater than those of group ZZV at distending pressures of 50-70 mmHg. There were no significant differences between groups ZV and ZZV, nor between groups ZN and ZZN. Group Z-N received zymosan at P90-P92 and naloxone on the day of testing but without acute re-inflammation at P120, and did not differ from either groups ZV or ZZV. These data are consistent with the outcomes shown in Experiment 1 and indicate that a prior bout of zymosan exposure in adulthood did not influence the magnitude of opioid inhibition produced by a subsequent acute exposure to zymosan.

Experiment 5

Inflammation at P14-P16 and 24 Hour Adult Pre-Treatment—Experiment 5 was performed to determine whether a history of zymosan-induced bladder inflammation during the neonatal period would impair the opioid inhibitory effect resulting from an acute exposure to zymosan during adulthood. Figure 5 presents EMG responses during graded UBD. An ANOVA revealed a significant between-groups effect, $F(3,32) = 2.95$ ($p=0.05$); a significant pressure effect, $F(7,21)=53.95$ ($p<0.001$); and a significant groups $\times$ pressure interaction, $F(7,224)=2.06$ ($p<0.005$). Naloxone administration revealed the opioid inhibitory effect in rats given anesthesia as neonates and the acute zymosan treatment (group AZN versus AZV), but failed to have any effect in rats that received neonatal exposure to zymosan (group ZZN versus ZZV). Specifically, in groups receiving anesthesia only at P14-P16, post-hoc comparisons showed that the EMG responses of group AZN were significantly greater than group AZV at UBD distending pressures of 30-80 mmHg. However, there were no significant differences in the EMG responses of groups ZZV and ZZN at any distending pressure, nor were there any differences between these groups and group AZN. Finally, the neonatal zymosan treatment was effective in enhancing the EMG responses (group ZZV versus group AZV) at UBD distending pressures of 50, 70, and 80 mmHg ($p=0.054$ at 60 mmHg).

Bladder Weights—At the termination of Experiments 1, 4, and 5, each animal's bladder was removed and the tissue was blotted dry. Table 1 shows there were no significant between-groups differences in bladder weights. These data rule out the possibility that bladder hypertrophy occurred in rats receiving intravesical zymosan on P14-P16, P90-P92, or P120.

Discussion

Urinary bladder pain is extremely common in the female population, with a lifetime incidence rate of >50%. While bladder inflammation resulting from mycotic and bacterial infections accounts for most of these cases, there is also a substantial number of individuals that experience bladder pain from diseases like painful bladder syndrome (PBS)/interstitial cystitis (IC), when no microbial infection is present. In previous and present studies, we demonstrated that briefly exposing adult female rats to intravesical zymosan, a mycotic inflammogen, resulted in modest but significant bladder hypersensitivity during graded UBD 24 hrs later²⁵ and we interpreted this hypersensitivity as possibly representing a hyperalgesic state. Bladder hypersensitivity was markedly enhanced when rats also experienced three consecutive days of exposure to intravesical zymosan during the neonatal period (P14-P16), but not if that treatment was administered during the adolescent period (P28-P30).²⁶ We theorized that neonatal inflammation “primes” or “predisposes” a rat to develop a hypersensitive bladder, especially when it is re-exposed to an additional bladder insult as an adult, and we suggested that experience with neonatal bladder inflammation may be one potential etiology for disorders like PBS/IC.²⁶

Independent studies of primary hyperalgesia involving the knee joint³¹ or hindpaw^{28,39} have demonstrated progressive engagement of descending inhibitory systems during the first 24

hrs of acute inflammation that act to suppress somatic hyperalgesia. This prompted us to consider whether bladder hypersensitivity produced by acute bladder inflammation in the adult rat may be concomitantly suppressed by inflammation-induced activation of an inhibitory system, and whether neonatal exposure to zymosan might, in some fashion, impair this system from functioning effectively in adulthood.

Experiment 1 demonstrated that a brief exposure to intravesical zymosan in the adult rat resulted in a bladder hypersensitivity that was being suppressed by an opioid inhibitory system because administration of i.p. naloxone prior to UBD testing significantly increased the magnitude of EMG responses to tests of graded UBD. This effect was replicated in Experiments 4 and 5. The opioid inhibition was triggered by bladder inflammation and does not appear to be tonically active, since naloxone had no effect on EMG responses to UBD as compared to vehicle in the non-inflamed condition.

While naloxone has been reported to affect bladder capacity in chloralose- or ketamine-anesthetized cats,¹ our results cannot be explained by naloxone-induced changes in bladder compliance. Specifically, Experiment 2 showed that intravesical pressures during CMG testing were not differentially affected by naloxone or vehicle administration in zymosan-treated rats or rats without inflammation. We intentionally infused 2.0 ml of volume to achieve distending pressures equivalent to the maximal pressures tested in studies of graded UBD. The fact that i.t. administration of naloxone in Experiment 3 produced similar findings to i.p. administration of naloxone also suggests that naloxone-induced changes in bladder compliance are unlikely to account for our results. Shimizu et al.³⁰ also failed to find any influence of naloxone (0.5 mg/kg; i.v.) alone on various cystometric parameters and similar negative results have been reported in a conscious rat preparation.²³

Cruz and Downie⁶ have shown that abdominal muscle contractions are present during spontaneous voiding and during voiding evoked by saline infusion within the physiological range of distending pressures in both conscious and urethane-anesthetized rats. It should, therefore, be acknowledged that the VMR utilized in the present study is not necessarily specific for nociceptive events, but this response does increase in a graded fashion to intensities of UBD that are above the non-painful, normal physiological range of intensities. This suggests that it is representative of primary afferent processing related to noxious intensities of UBD. The demonstration of opioid-related effects further supports the use of this model as representative of nociceptive sensory processing, as opioids have limited conscious effects apart from pain modulation.^{3,22}

Experiment 3 suggested that the effects of systemic naloxone were likely due to blockade of a spinal opioid system since i.t. administration of naloxone significantly enhanced the EMG responses to UBD in a manner remarkably similar to i.p. naloxone. The length of i.t. catheters was intended to deliver naloxone to the L5-S2 region of the cord and we believe this to be the primary locus of action. However, we cannot rule out possible rostral spread of naloxone to other spinal regions important for bladder control (e.g., T12-L1). In addition, there also could be a peripheral opioid component because enkephalins are present in parasympathetic ganglia on the bladder.^{7,14,17}

The effects observed in the present studies suggest that two opposing processes contribute to bladder hypersensitivity resulting from acute bladder inflammation. One process is enhanced input, perhaps mediated by inflammation-induced sensitization of primary nociceptive afferents^{12,13,19} and/or central sensitization of second-order spinal dorsal horn neurons.^{4,40} A second process involves inflammation-induced activation of a reactive opioid system that opposes enhanced input from the bladder. In our studies of adult rats treated with a single brief bout of bladder inflammation, opioid inhibition was present, but it was not sufficient to completely suppress the enhanced input resulting from bladder inflammation. Modest bladder hypersensitivity was the resultant state. This spinal opioid inhibitory influence may reflect a system of interneurons localized to the spinal cord, since enkephalinergic neurons are prevalent throughout the regions of hypogastric and pelvic afferent spinal input.¹⁸

We then considered whether prior activation of this opioid inhibitory system might alter how it was manifested if reactivated later-in-life. We examined whether three successive days of urinary bladder inflammation at P90-P92, followed by re-exposure to brief bladder inflammation at P120, influenced the magnitude of opioid inhibition. We found no influence of the P90-P92 treatment over and above that produced by exposure to bladder inflammation 24 hrs prior to UBD testing at P120. The magnitude of bladder hypersensitivity and opioid inhibition were no different from that produced by the adult 24 hr bladder inflammation pre-treatment alone.

Finally, we examined whether three successive days of urinary bladder inflammation at P14-P16 impaired the capacity to activate this opioid inhibitory system following re-exposure to brief bladder inflammation in adulthood, thus contributing to the enhanced bladder hypersensitivity observed in our previous studies.²⁶ Naloxone had no effect on EMG responses to UBD over and above vehicle in neonatal zymosan-treated rats in Experiment 5 (Groups ZZN and ZZV did not differ). These data support the view that neonatal bladder inflammation, unlike adult bladder inflammation, impairs the functioning or effectiveness of an opioid inhibitory system.

We considered the possibility that the failure to see an enhancement of EMG responses following naloxone administration in neonatal zymosan-treated rats was due to a ceiling effect in our EMG response measure, but several factors make this interpretation unlikely. First, we have obtained substantially larger EMG responses in previous studies than those obtained in the present studies,²⁶ suggesting that a response ceiling was not attained in the present studies. Second, there were no differences in the EMG responses of groups ZZN and ZZV in the lower range of graded distending pressures (e.g., 30–60 mmHg), where the EMG responses were approximately 50% of the maximal responses observed. If naloxone was having an effect in group ZZN, then it should have been observable at these submaximal response levels. We also considered the possibility that neonatal bladder inflammation produced histological changes that contributed to these outcomes. However, we have performed histological analyses on a limited number of bladders obtained from adult rats treated with either intravesical zymosan or anesthesia alone from P14-P16 in a manner identical to Experiment 5, but in the absence of any additional adult treatments. We have observed no major histological differences between animals treated in this manner at this point in our analyses.

Finally, we considered whether failure of the adult P90-P92 zymosan treatment to alter the functioning of the opioid inhibitory system was due to the possibility that three consecutive days of inflammation at P90-P92 may not be comparable to three consecutive days of inflammation at P14-P16, e.g., the adult treatment resulted in less severe inflammation. Data from our previous studies suggest that this is unlikely. We have demonstrated that adolescent rats treated in a similar fashion for either three or fourteen consecutive days both fail to show bladder hypersensitivity in adulthood following brief re-exposure to zymosan.²⁶ The primary focus of administering fourteen days of inflammation in those studies was to determine whether a longer duration of exposure to zymosan was required later during development to produce similar outcomes to three days of inflammation during the neonatal period.

The issue of how neonatal exposure to bladder inflammation impairs opioid inhibition is a matter of speculation. First, it may interfere with the development of either opioid inhibitory interneurons or a descending system that utilizes spinal opioids in modulating spinal nociceptive transmission from bladder afferents. For example, neonatal bladder inflammation might reduce μ - or δ -opioid receptor expression in the spinal cord. Similar views have been advanced to account for the loss of spinal morphine suppression of somatic nociception and tactile allodynia following tight ligation of L5/L6 nerves.²⁴ There also is abundant evidence that descending inhibitory systems derived from either the rostroventral medulla (RVM)^{11,32} or the locus coeruleus (LC)³³⁻³⁶ are recruited following inflammation of somatic tissue. While these descending systems are present at birth, they are only partially functional by P10-P12 and become functionally mature by P21.^{2,9,10} Perhaps the neonatal treatment impaired the development of one of these descending inhibitory systems. Alternatively, it may be the case that the neonatal treatment enhances the density and spinal segmental distribution of bladder afferents, as has been demonstrated for somatic afferents in studies of neonatal hindpaw inflammation.²⁹ If nociceptive input was markedly enhanced, and a developing inhibitory system was not altered in order to compensate for this enhanced input, then the effectiveness of the inhibitory system would be functionally reduced.

An alternative that is functionally equivalent to these views is that neonatal bladder inflammation might augment the operation of a descending system whose role is to inhibit a spinal opioid inhibitory circuit, i.e., disinhibition or facilitation. This notion is consistent with data of MacArthur et al.,²⁰ which showed that removal of descending spinal influences by mid-thoracic spinal transection resulted in increases in both dynorphin and enkephalin mRNA in rats receiving unilateral hindpaw injection of CFA. They concluded that the net effect of descending afferents on spinal nociceptive circuits was to suppress the response of opioid-containing neurons to noxious stimulation from the periphery. Hence, augmentation of this descending influence via neonatal bladder inflammation would functionally operate to produce the same outcome as simple impairment of a descending inhibitory system.

Coutinho et al.⁵ have provided an interesting parallel to the present findings in their studies of maternal separation in producing visceral hypersensitivity. They measured the VMR to graded colorectal distention and found that i.p. naloxone administration produced a parallel leftward shift in the stimulus-response function of adult rats that were not handled or maternally separated as neonates, but had no effect in adult rats that had previously

undergone maternal separation as neonates. The maternally separated rats also showed a diminished stress-induced cutaneous analgesia compared to non-handled controls suggesting a compromised ability of the former rats to activate endogenous opioidergic pain inhibitory systems. While our studies controlled for maternal separation, these studies more generally demonstrate that opioid inhibitory mechanisms can be impaired in rats by exposing them to a neonatal challenge, and are consistent with a greater activation of these systems in rats that do not undergo neonatal challenges.

In conclusion, our data indicate that urinary bladder inflammation induces a bladder hypersensitivity that is concomitantly opposed by spinal opioid inhibition. More importantly, this inhibition can be disrupted by neonatal exposure to bladder inflammation, suggesting that some painful bladder disorders, such as those triggered by either mycotic inflammation or PBS/IC, may in part be a consequence of early-in-life experiences with bladder inflammation, as we have argued elsewhere.²⁶ Since a variety of other disorders, such as irritable bowel syndrome and fibromyalgia, also have been attributed to a diminution in the effectiveness of inhibitory systems, it would be interesting to see whether epidemiological data supports the occurrence of some similar type of neonatal event that contributed to the etiology of those disorders.

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Perspective

The present study observed that bladder hypersensitivity resulting from acute bladder inflammation is being suppressed by an opioid inhibitory mechanism. Experiencing bladder inflammation during the neonatal period can impair the expression of this opioid inhibitory mechanism in adulthood. This suggests that bladder insults experienced during development may permanently alter visceral sensory systems and may represent one etiology of painful bladder disorders.

Figure 1.

Group mean EMG responses during graded UBD. Groups received either anesthesia alone (A) or zymosan (Z) 24 hrs prior to UBD testing in the presence of either vehicle (V) or naloxone (N). *Group ZN significantly different from group ZV, $p < 0.05$; +Group ZN significantly different from group AN, $p < 0.05$; #Group ZV significantly different from group AV, $p < 0.05$.

Figure 2.

Group mean intravesical pressure in mmHg during CMG testing. Groups received either no treatment or zymosan (Z) 24 hrs prior to slow infusions with saline (0.05 ml/min) in the presence of either vehicle (V) or naloxone (N). ANOVAs revealed no significant between-groups differences.

Figure 3.

Group mean EMG responses during graded UBD. Groups received zymosan (Z) 24 hrs prior to UBD testing in the presence of either i.t. vehicle (V) or i.t. naloxone (N). *Group ZN significantly different from group ZV, $p < 0.05$. The insets "a" and "b" show representative EMG traces for rats in the naloxone and vehicle group, respectively. These were obtained at 70 mmHg of distending pressure in rats with approximate transformed EMG values of 3 (naloxone) and 1 (vehicle), in order to match the approximate group mean values at 70 mmHg. Dotted lines signify pressure onset and termination of the 20 sec distending stimulus.

Figure 4.

Group mean EMG responses during graded UBD. Groups ZZV, ZZN, and Z-N received zymosan (Z) from P90-P92, followed by either zymosan (Z) or no treatment (–) 24 hrs prior to UBD testing at P120 in the presence of either vehicle (V) or naloxone (N). Groups ZV and ZN received zymosan (Z) 24 hrs prior to UBD testing at P120 in the presence of either vehicle (V) or naloxone (N). *Group ZN significantly different from group ZV, $p < 0.05$; # Group ZZN significantly different from group ZZV, $p < 0.05$. Groups ZV, ZZV, and Z-N did not differ. Groups ZN and ZZN did not differ.

Figure 5.

Group mean EMG responses during graded UBD. Groups received either anesthesia alone (A) or zymosan (Z) from P14-P16, followed by zymosan (Z) 24 hrs prior to UBD testing at P120 in the presence of either vehicle (Z) or naloxone (N). *Group AZN significantly different from group AZV, $p < 0.05$; #Group ZZV significantly different from group AZV, $p < 0.05$. Groups ZZV, ZZN, and AZN did not differ at any distending pressure.

Table 1

Group mean bladder weights in grams ($\pm$ SEM) obtained from rats in Experiments 1, 4, and 5. ANOVAs revealed no significant between-groups differences.

Experiment 1	Experiment 4	Experiment 5
ZN 0.16 ± 0.02	ZN 0.14 ± 0.01	ZZN 0.18 ± 0.01
ZV 0.14 ± 0.02	ZV 0.16 ± 0.01	ZZV 0.17 ± 0.02
AN 0.15 ± 0.02	ZZN 0.14 ± 0.01	AZN 0.14 ± 0.01
AV 0.13 ± 0.01	ZZV 0.14 ± 0.01	AZV 0.16 ± 0.01
	Z-N 0.14 ± 0.01	