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Rostral Ventral Medulla Modulation of the Visceromotor Reflex Evoked by Urinary Bladder Distension in Female Rats

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Abstract

The present studies examined the involvement of the rostral ventral medulla (RVM) in modulating the visceromotor response (VMR) evoked by urinary bladder distension (UBD) in adult female rats. The VMR was indexed by electromyographic (EMG) responses of the abdominal external oblique muscle to UBD. Experiment 1 showed that the predominant effect of electrical stimulation of the RVM in normal rats was to produce intensity-dependent inhibition of the VMR (54% of sites sampled). Facilitatory, biphasic, or no effects were obtained at the remaining sites. Experiment 2 showed that RVM-induced inhibition of the VMR was significantly attenuated by intraperitoneal (i.p.) administration of naloxone, but not saline vehicle. In Experiment 3, we examined the effect of lesions of the RVM in rats with inflamed bladders since previous research has shown that an endogenous opioid inhibitory system is engaged by bladder inflammation. Electrolytic lesions of the RVM, but not sham-lesions of the RVM, significantly increased the VMR to graded UBD in rats with augmented VMRs induced by prior inflammation of the bladder. The present data suggest that the RVM can inhibit the VMR to UBD acting in part via an opioid inhibitory system and that bladder inflammation can recruit the RVM to produce a net inhibitory effect on the VMR to UBD.

Keywords

bladder; rostral ventromedial medulla; visceromotor reflex; pain; inhibition

Perspective

Stimulation of the RVM resulted in inhibitory, facilitatory, and biphasic modulation of the visceromotor reflex to urinary bladder distension. Inhibitory effects of stimulation were attenuated by naloxone and lesions of the RVM enhanced the VMR in rats with inflamed bladders. These data indicate an important role of the RVM in modulating bladder pain.

Introduction

Intravesical administration of zymosan in adult rats leads to a time-dependent increase in urinary bladder inflammation¹⁴ and a significant increase in abdominal muscle

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electromyographic (EMG) and arterial blood pressure (ABP) responses to urinary bladder distension (UBD).^{4,14} The latter responses are components of the visceromotor response (VMR) to UBD and have been used to assess bladder hypersensitivity.^{2,4,14,15} In normal rats, the bladder hypersensitivity produced by intravesical zymosan is concomitantly suppressed by an opioid inhibitory system because either intraperitoneal (i.p.) or intrathecal (i.t.) administration of naloxone at the time of UBD testing significantly increases the magnitude of EMG responses to UBD in rats with inflamed bladders.⁴ These outcomes obtained with inflammation of the bladder are similar to those obtained in rats with either knee joint¹⁸ or hindpaw^{16,23} inflammation where the hyperalgesia is concomitantly suppressed by an inhibitory system.^{c.f., 21,22} However, the source of the inhibitory influence in studies of bladder inflammation is unknown.

The RVM is well-established as a final common pathway for spinal modulation of nociception. Bladder distension can alter the activity of identified raphespinal neurons in cat and rostral raphe neurons in rat^{3,19} and formalin-induced bladder inflammation results in c-Fos expression in RVM neurons.¹⁷ Transneuronal tracing studies also suggest that the bladder could be subject to descending modulation from neurons located in the RVM.¹² Independent studies of somatic tissue have shown that electrical or chemical stimulation of RVM neurons can inhibit spinal nociceptive transmission, and in some studies, the inhibition can be eliminated by either i.t. administration of the non-selective opioid receptor antagonist naloxone^{9,10,29} or the selective δ_2 opioid receptor antagonist naltriben.⁷ These findings suggest that peripheral inflammation of the bladder could potentially recruit neurons located in the RVM and mediate the opioid inhibitory effect observed previously.⁴

To date, no functional studies have been performed demonstrating that RVM neurons either participate in the modulation of bladder pain in normal rats or are recruited by bladder inflammation to alter bladder pain. In order to address the hypothesis that the RVM plays a role in bladder-associated pain, the following studies examined whether (1) electrical stimulation in the RVM modulated EMG responses evoked by UBD in normal rats, (2) the RVM-induced inhibition of EMG responses evoked by UBD in normal rats was opioidergic, and (3) lesions of the RVM altered the magnitude of EMG responses evoked by graded UBD in rats with or without inflammation of the bladder.

Materials and Methods

Animals and animal care

Adult female Sprague-Dawley rats were obtained from Harlan (Prattville, AL) and maintained in separate cages (6:00-6:00 light-dark cycle). Female rats were used for comparisons with our previous studies of bladder inflammation and interstitial cystitis. All studies were approved by the University of Alabama at Birmingham Animal Care and Use Committee and conformed to the NIH guidelines for the care and use of laboratory animals.

Experiment 1 - Effects of RVM Stimulation

The trachea of each adult female rat (N=21) was cannulated for artificial respiration under deep isoflurane anesthesia (4%) and a 22-gauge angiocatheter was inserted into the urinary bladder via the urethra and secured with a tight suture. The animal was placed on a ventilator and remained on 4% isoflurane. Platinum wire EMG electrodes were placed into the left external oblique musculature for differential amplification and recording of EMG activity. Rats were placed in a stereotaxic apparatus and a stimulating electrode was inserted through a midline burr hole (−10.50 mm from bregma) and lowered to a starting location in the RVM −8.5 mm below the surface of the brain. Anesthesia was reduced to 1% and maintained at this level. A minimum of six, but up to ten, 60 mmHg distensions of the bladder were administered with an

intertrial interval (ITI) of 3 min. These distensions were used to overcome an initial period of bladder sensitization resulting from UBD.² Testing proceeded after three consecutive EMG control responses to distension were obtained that varied by less than 10% of their mean. The effect of RVM stimulation on the EMG response evoked by a 60 mmHg UBD test stimulus was then examined. At any given site, RVM stimulation began 20 sec prior to the onset of UBD and continued through the entire 20 sec, 60 mmHg UBD stimulus. The procedure was then repeated as RVM stimulation intensity was increased in a fixed sequence (10, 20, 30, 40, and 50 μ A for 14 sites and 10, 20, 30, 40, 50, and 80 μ A for 17 sites; 200 μ s pulses and 100 Hz). An additional control trial (60 mmHg distension in the absence of RVM stimulation) was evaluated between the tests of 30-40 μ A to ensure that the baseline levels had not changed. In a few cases, it was not possible to test the entire sequence of intensities because stimulation itself increased EMG activity. In some rats, after the sequence of intensities was examined the electrode was lowered 0.5 mm, a new baseline was established, and the stimulation procedure was repeated. However, in the majority of cases only a single site of stimulation was tested. At the end of the experiment, the lowest site of stimulation was lesioned and the sites of stimulation mapped using Paxinos and Watson.¹³ In a few animals, we also examined whether initiating RVM stimulation approximately 10 sec after the start of UBD would produce rapid inhibition of the EMG response although no formal latencies to onset were recorded.

Experiment 2 - Effect of Naloxone on RVM-induced Inhibition

In a separate set of two groups of animals (N=7/group), an electrode was directed to the RVM and the same procedure described in Experiment 1 was used to establish a baseline VMR to the 60 mmHg UBD stimulus. We then identified a single RVM site where we could obtain stimulation-produced inhibition of the EMG response evoked by a 60 mmHg UBD test stimulus (this often required moving the electrode until a suitable site was found). The intensity-testing procedure described in Experiment 1 (10, 20, 30, 40, and 50 μ A; 200 μ s pulses; 100 Hz) was then used to establish a control inhibitory stimulus-response function with the 60 mmHg UBD stimulus. Naloxone (1 mg/kg i.p.) or saline vehicle in (1 ml/kg) was administered to separate groups of rats and followed 10 minutes later by two, 60 mmHg control trials and re-testing of the RVM stimulation intensities. Sites of stimulation were lesioned at the end of the experiment and mapped as described above.

Experiment 3 - Effects of RVM Lesions

Electrolytic or sham lesions of the RVM were made two weeks prior to testing. Rats were placed in a stereotaxic apparatus under deep isoflurane anesthesia (4%) and a lesion electrode was targeted to the RVM (−10.5 mm from bregma, −8.5 mm ventral, and midline). The NRM region of the RVM was specifically targeted and lesions were made by passing anodal current through the electrode (0.8 mA, 7-9 s). The identical procedure was used for sham lesions except that no current was passed through the electrode. The skin was closed with wound clips and rats were allowed to recover.

Twenty-four hours prior to EMG testing, rats were pretreated with 30 min of exposure to either intravesical zymosan (1% solution in saline; 0.5 ml) under isoflurane anesthesia (5% induction followed by 2%) or isoflurane anesthesia alone (5% induction followed by 2%). The zymosan was administered via a 22-gauge angiocatheter inserted into the urinary bladder via the urethra. After 30 min the zymosan solution was drained, the angiocatheter removed, and each animal injected with ampicillin (20 mg).

On the following day, all rats received the general surgical treatment described in Experiment 1 for placement of EMG electrodes and were placed unrestrained on a heating pad. Anesthesia was reduced to 1% for the duration of testing and the first UBD trial occurred 10 min later. Three 20 sec duration, 60 mmHg distensions of the urinary bladder were administered to

overcome the initial period of bladder sensitization and were followed by a sequence of 20 sec duration graded distensions of the bladder at pressures of 10, 20, 30, 40, 50, 60, 70 and 80 mmHg, respectively (3 min ITI). There were four groups of rats (N=7-8/group) based on type of lesion (RVM lesion or sham lesion) and 24 h pre-treatment (zymosan or anesthesia). At the termination of the recording session the brain was removed and placed in formalin for later histological verification of lesion site.

Data Analysis and Statistics

The EMG response was defined as: (rectified EMG activity during UBD - rectified baseline EMG prior to UBD) / rectified baseline EMG as described previously.^{14,15} Group data are presented as means $\pm$ S.E.M. In studies of RVM stimulation, the mean of each rat's last three control trials had to vary by less than 10% of their mean before RVM stimulation was tested. The following criteria were then used to categorize an animal's response to RVM stimulation. Inhibition: RVM stimulation produced consistent decreases relative to baseline; facilitation: RVM stimulation produced consistent increases relative to baseline; inhibition/facilitation: RVM stimulation produced at least two initial decrease of greater than 10% of baseline followed by increases relative to baseline on all remaining tests; and facilitation/inhibition: RVM produced at least two initial increases of greater than 10% of baseline followed by decreases relative to baseline on all remaining tests; no effect: RVM stimulation produced no consistent increases or decreases. One- or two-way ANOVAs were performed. Appropriate post-hoc contrasts were performed for each trial using Holm's procedure⁸ to maintain family-wise α at 0.05. Statistical significance was defined as p values ≤ 0.05 .

Results

Experiment 1 - Effects of RVM Stimulation

The predominant effect of electrical stimulation of the RVM was to inhibit the EMG response to the UBD test stimulus and current-dependent inhibition of the EMG response was obtained at 17/31 sites (54%) tested. A representative individual example of current-dependent inhibition of the EMG response to the 60 mmHg UBD test stimulus is shown in Figure 1A. The inhibitory effect of RVM stimulation was virtually immediate in onset and an example is shown in Figure 1B. Group mean data for inhibition are shown in Figure 2A. The sites of inhibition were distributed throughout the RVM (Figure 1C) and included both the nucleus raphe magnus (NRM; 11 sites) and the nucleus reticularis pars alpha (Gi α ; 6 sites).

In 5/31 sites (16%), significant facilitation of the EMG response was obtained and group data are shown in Figure 2C. We typically observed approximately a 50% increase in the EMG response to UBD at the greater intensities. However, in some cases, RVM stimulation alone evoked EMG activity and data from these trials were not used. Facilitatory sites (4/5) were located in the Gi α (Figure 1C).

Inhibition followed by facilitation (Figure 2B) was obtained in 2/31 sites (6%), facilitation followed by inhibition (Figure 2D) was obtained in 3/31 sites (10%); and no effect was found in 4/31 sites (13%).

Experiment 2 - Effect of Naloxone on RVM-induced Inhibition

Figure 3 shows RVM stimulation-produced inhibition of the EMG response evoked by a 60 mmHg UBD test stimulus before and after administration of either 1 mg/kg of naloxone or saline vehicle. In the pre-drug tests, current-dependent inhibition of the EMG response was similar to that reported in Experiment 1 and there were no significant differences between the pre-naloxone and pre-saline intensity functions. Only naloxone administration significantly reduced the magnitude of RVM stimulation-produced inhibition. A two-way ANOVA yielded

a significant group effect ($F=8.81$; $p=0.012$; naloxone versus vehicle), drug effect ($F=15.21$; $P=0.002$; pre-drug versus post-drug), and their interaction ($F=3.81$; $P=0.075$). These were followed by repeated measures ANOVAs within each group. The naloxone-treated group showed a significant pre-post drug effect ($F=21.61$; $P=0.004$). The magnitude of the EMG control responses obtained before and after naloxone administration was not significantly different. Sites of stimulation are shown in the inset of Figure 2.

Experiment 3 - Effects of RVM Lesions

Figure 4 presents group mean EMG responses during tests with graded UBD. Lesions of the RVM significantly increased the magnitude of EMG responses to UBD when compared to sham-lesioned rats, but only in rats that were pre-treated with zymosan 24 h prior testing. A two-way ANOVA indicated significant effects of type of lesion ($F=5.84$; $P=0.023$), type of pre-treatment ($F=20.92$, $P<0.001$), UBD pressure ($F=35.91$, $P<0.001$), and all interactions were significant. In groups pre-treated with intravesical zymosan, follow-up one-way ANOVAs and post-hoc comparisons revealed that the means of RVM-lesioned rats were significantly greater than the means of RVM sham-lesioned rats at UBD pressures of 50-80 mmHg (p 's range from 0.004 - 0.03). In groups pre-treated with anesthesia alone, a follow-up one-way ANOVA revealed no significant between-group or interaction effects between RVM-lesioned and sham-lesioned groups at any distending pressure. Also shown in Figure 4 are data from a group of rats pre-treated with zymosan with lesions that entirely missed the RVM ($N=5$; most lesions were small lesions in the pyramids). A separate ANOVA indicated that these rats did not significantly differ from the zymosan pre-treated rats that received sham lesions and provide additional indirect evidence that the lesions of the RVM and not surrounding tissue are critical for increasing the EMG response following zymosan pre-treatment. Histological analyses showed that RVM lesions always encompassed the NRM and either the lesion or gliosis from the lesion encompassed the G_{α} at the largest part of the lesion. In many cases, the lesion also extended into the medial ventral aspects of the nucleus reticularis gigantocellularis (Gi). The lesions typically extended from -10.04 to -11.30 mm from bregma and most were centered at -10.80 mm from bregma. An example of a lesion is shown in the inset of Figure 4.

Discussion

In previous studies, we demonstrated that briefly exposing rats to intravesical zymosan results in bladder hypersensitivity^{4,14,15} and concomitantly activates an opioid inhibitory system that serves to suppress this bladder hypersensitivity.⁴ It is believed that the net nociceptive response to bladder inflammation reflects a complex summation of excitatory and inhibitory processes at peripheral, spinal, and central levels^{4,5,6,11,25} and we now know that this includes the RVM. The present study is the first to demonstrate that the RVM modulates bladder nociception in the normal rat and can be recruited under conditions of bladder inflammation. We targeted the RVM because electrical or chemical stimulation of the RVM inhibits spinal nociceptive transmission from somatic tissue, and in some studies, these RVM-inhibitory effects have been demonstrated to be mediated by spinal opioids.^{7,9,10,29}

We found that inhibitory influences were predominant at the RVM stimulation sites we sampled. The inhibitory effects were potent with 50 μ A of current producing upwards of 80% inhibition of the control response to a 60 mmHg distending stimulus. RVM stimulation produced a virtually immediate inhibitory effect and this effect dissipated within 3 min. Experiment 2 showed that i.p. administration of naloxone, but not saline vehicle, significantly attenuated the inhibitory effects of RVM stimulation indicating partial mediation by endogenous opioids. Complete blockade of inhibition by naloxone was not observed. One could speculate that this partial blockade resulted from RVM stimulation activating additional,

non-opioid inhibitory systems that would be unaffected by naloxone, e.g., a 5-HT inhibitory system, or that the 1 mg/kg dose of naloxone was not of sufficient to achieve complete blockade. We have previously shown in CMG studies that this dose and route of administration of naloxone does not change bladder compliance.⁴

Like other studies of RVM influences on spinal nociceptive processing^{26,27,28}, we also observed both facilitatory and biphasic effects of electrical stimulation. The relative percentage of these effects was low, as compared to inhibitory effects, but comparable to those reported by Zhuo and Gebhart²⁸ for RVM influences on EMG responses evoked by colorectal distension (CRD). However, their sites of stimulation were primarily in the Gi, rather than the Gi_α, and we did not examine this more dorsal region. We assume that the biphasic effects obtained with electrical stimulation reflect current spread with increasing intensities of stimulation, where lesser intensities engage one descending system, e.g. inhibitory or facilitatory, which is then countered by an opposing system as current spread increases with intensity. In summary, these RVM stimulation and naloxone data show that the response to bladder distension in the normal rat can be modulated by descending systems from the RVM and provide independent support for the notion that bladder inflammation could engage a descending inhibitory system utilizing opioids.

Experiment 3 provided direct evidence that the RVM region is part of the system engaged by bladder inflammation since lesions of the RVM significantly increased the EMG response to graded UBD in rats with inflamed bladders. These data are consistent with the hypothesis that a RVM descending inhibitory system is activated by bladder inflammation and mediates the spinal opioid inhibitory effects we observed in our previous study.⁴ RVM lesions had no significant effect in rats without bladder inflammation, but who had received prior exposure to the anesthesia protocol. These data indicate that the RVM system recruited by bladder inflammation is not tonically active, but rather is a reactive system. The lack of tonic activity in this system is supported by data obtained in our naloxone study of normal rats. Pre- and post-naloxone control responses to the 60 mmHg UBD test stimulus were not different. Only stimulation-produced inhibition of the VMRs to UBD were affected by naloxone. The lesion and naloxone data that bear on the lack of a tonic opioid activity are also internally consistent with the naloxone data of our previous experiment⁴ in which we found no evidence of tonic opioid activity in anesthesia-treated rats administered either naloxone or saline in tests of graded UBD. These RVM lesions were centered in the NRM, but also encompassed all of the Gi_α and in many cases extended into the medial ventral Gi.

At the present time, the time-course and duration of bladder inflammation-induced activation of the RVM inhibitory system remain to be established. We assume that it would parallel the development of inflammation in the bladder. In previous time-course studies, we have shown that bladder inflammation produced by intravesical zymosan administration, as indexed by plasma extravasation of Evan's Blue, progressively increases and to a peak at 24 hours and returns to normal by 72 hours¹⁴. However, it is possible that the RVM inhibitory system might remain engaged once bladder inflammation subsides. Further, we don't know if this system is unique to the bladder in terms of endogenous factors that will engage the system.

Our RVM lesion study revealed a net inhibitory influence of this CNS site in animals with inflamed bladders. As noted previously, however, we were able to obtain facilitatory effects of RVM stimulation in non-inflamed animals. Therefore, it is possible that a descending RVM facilitatory system could also be engaged under conditions of bladder inflammation and is being masked by a stronger inhibitory effect. The interaction between facilitatory and inhibitory influences from the Gi and NRM are complex^{1,20}, and for example, simply extending a lesion from the Gi into the NRM can eliminate the ability to detect an inflammatory-induced facilitatory influence from the Gi.²⁴

The present studies also bear on work with early-in-life exposure to bladder inflammation affecting adult bladder sensitivity. In previous studies, we showed that female rats exposed to intravesical zymosan on P14-P16 had enhanced EMG responses to UBD following re-exposure to intravesical zymosan in adulthood.^{4, 15} However, these rats failed to respond to naloxone administration in adulthood after being administered intravesical zymosan, unlike normal adult rats exposed to intravesical zymosan but without early-in-life exposure to zymosan.⁴ We suggested that early-in-life exposure to bladder inflammation might impair the functioning of a reactive opioid inhibitory system seen in normal adults. The present data are consistent with these views in that the early-in-life treatment could affect a descending RVM inhibitory system utilizing spinal opioids.

Overall, the present data indicate that bladder hypersensitivity resulting from bladder inflammation can be thought of as the net result of activating many processes, including peripheral sensitization of primary afferents^{5,6,11}, central sensitization of second-order dorsal horn neurons²⁵, and as suggested by our studies, inflammation-induced activation of a reactive opioid inhibitory system utilizing neurons in the RVM.

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Figure 1.

Panel A: Representative tracings of RVM-induced inhibition of EMG responses to graded UBD in an individual rat. Control trial (60 mmHg) is shown at the top with successive increments in RVM stimulation intensity. Inhibition is evident by 30 uA and maximal by 50 uA with recovery back to control levels within the 3 minute ITI. Panel B: RVM stimulation induces a rapid inhibitory effect as shown in a representative example in which RVM stimulation (arrow) was initiated approximately 10 sec into a 20 sec period of distension with 60 mmHg UBD stimulus (dotted lines represent trial onset and termination of 60 mmHg stimulus). Panel C: A summary of the RVM stimulation sites that inhibited (○), facilitated (●), biphasic excited/inhibited (△), biphasic inhibited/excited (▲), or produced no effect (▼) on the VMR evoked by a 60 mmHg UBD test stimulus for the data shown in Figure 2. Only two anatomical planes (−10.04 and −11.30 mm from bregma) are used to represent the sites of stimulation for ease of data presentation, but stimulation sites occurred anywhere between −9.80 to −11.60 mm from bregma.

Figure 2.

RVM stimulation-induced changes in the EMG response evoked by a 60 mmHg UBD test stimulus expressed as a group mean % change from control ($\pm$ SEM). Panel A - inhibition (N=17 sites), Panel B - inhibition/facilitation (N=2 sites), Panel C - facilitation (N=5 sites), and Panel D - facilitation/inhibition (N=3 sites). Data are derived from 15 animals.

Figure 3.

RVM stimulation (10-50 μ A) was first shown to inhibit the EMG response to a 60 mmHg UBD test stimulus in a pre-drug condition for two groups of rats. (N=7/group; $\circ$ pre-naloxone and Δ pre-saline vehicle). A significant attenuation of this RVM stimulation-produced inhibition was obtained following i.p. administration of 1 mg/kg of naloxone ($\bullet$), but not with i.p. administration of saline vehicle ($\blacktriangle$). Data are expressed as group mean % change from control ($\pm$ SEM) and post-hoc tests indicate significant differences between pre- and post-measures for the naloxone group (* $p < .05$). Inset shows composite representation of the sites of stimulation ($\circ$ naloxone group and Δ saline vehicle group).

Figure 4.

Group mean EMG responses ($\pm$ SEM) during tests with graded UBD. Group zymosan-lesion was significantly greater than group zymosan-sham lesion at UBD pressures of 50 - 80 mmHg (* $p < 0.05$). Group anesthesia-lesion did not differ from group anesthesia-sham lesion. $N = 7-10$ rats/group). A group of rats ($N = 5$) that received the zymosan pretreatment, but with lesions that missed the RVM (group zymosan-lesion (miss)) was not significantly different from group zymosan-sham lesion. The inset shows a representative example of a RVM lesion centered in the NRM with gliosis extending into the Glia.