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Research article

Spinal mechanisms of pudendal nerve stimulation-induced inhibition of bladder hypersensitivity in rats

Timothy J. Ness[a,*], Cary DeWitte[a], Jamie McNaught[a], Buffie Clodfelder-Miller[a], Xin Su[b]

[a] Department of Anesthesiology and Perioperative Medicine, University of Alabama at Birmingham, United States of America
[b] Neuromodulation Research, Medtronic, Inc, United States of America

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ABSTRACT

Bilateral electrical pudendal nerve stimulation (bPNS) reduces bladder hypersensitivity in rat models of bladder pain and anecdotally reduces pain in humans with pelvic pain of urologic origin. The spinal neurochemical mechanisms of this antinociception are unknown. In the present study, bladder hypersensitivity was produced by neonatal bladder inflammation in rat pups coupled with a second inflammatory insult as an adult. Visceromotor responses (VMRs; abdominal muscle contractions) to urinary bladder distension (UBD) were used as a nociceptive endpoint under urethane-isoflurane anesthesia. bPNS consisted of bilateral biphasic electrical stimulation of the mixed motor/sensory component of the pudendal nerves. Following determination of the inhibitory effect of bPNS on VMRs, pharmacological antagonists were administered via an intrathecal catheter onto the lumbosacral spinal cord and bPNS effects on VMRs redetermined. bPNS resulted in statistically significant inhibition of VMRs to UBD in hypersensitive rats that was statistically reduced by the intrathecal administration of methysergide, WAY100636, CGP35348 and strychnine but was unaffected by naloxone, bicuculline, phentolamine, ondansetron and normal saline. This study suggests that inhibitory effects of bPNS may include serotonergic, GABA-B-ergic and glycinergic mechanisms suggesting the potential for interaction of the neuromodulatory effect with concommitant drug therapies.

1. Introduction

Bilateral electrical pudendal nerve stimulation (bPNS) has been demonstrated to inhibit nociceptive responses to urinary bladder distension (UBD) in rats made hypersensitive to bladder stimuli [1]. Coupled with multiple anecdotal and case series reports in which the use of nerve stimulation resulted in improved pain control in people with the diagnosis of interstitial cystitis/bladder pain syndrome (IC/BPS) [2–4] these findings suggest the need for a controlled clinical trial examining this therapeutic modality. Previous studies related to the spinal mechanisms of pudendal nerve stimulation have suggested a link to multiple inhibitory neurotransmitters including opioids, serotonin, glycine and GABA [5–10]. However, these other studies were predominantly in feline models and/or studied systemic drug effects (rather than spinal) using cystometric measures as primary endpoints rather than models more commonly associated with nociception. Further study appears warranted as concomitant drug use which could alter these inhibitory systems, could also potentially alter clinical responses to the pain-relieving effects of this manipulation.

In our previous studies we identified optimal stimulation parameters and sites of electrical stimulation for the pudendal nerves (which arise from the same spinal segments as the lumbosacral nerves which contain afferents from the bladder) [1]. We studied these effects in a model of bladder hypersensitivity in which rats experience neonatal bladder inflammation and then receive a second bladder inflammatory challenge as adults [11]. It is thought that this model may be particularly relevant to the classic form of IC/BPS [12], in that it is associated with multiple features of IC/BPS including the presence of increased micturition rates, altered cystometry indicating a functionally small capacity, hypersensitive bladder, altered bladder neurochemistry, the presence of vascular fragility of submucosal tissues following prolonged hydrodistention, the presence of increased pelvic floor muscular

Abbreviations: 5HT, 5-hydroxytryptamine (serotonin); AUC, Area-Under-the-Curve; bPNS, bilateral pudendal nerve stimulation; GABA, gamma amino butyric acid; IC/BPS, interstitial cystitis/bladder pain syndrome; i.v., intravenous; s.c., subcutaneous; T, stimulation intensity threshold; UBD, urinary bladder distension; VMR, visceromotor response

* Corresponding author at: Department of Anesthesiology, University of Alabama at Birmingham, BMR2-208, 901 19th St. S Birmingham, AL, 35222, United States of America.
E-mail address: tness@uabmc.edu (T.J. Ness).

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tone and increased responsiveness to intravesical cold and potassium-containing fluids [11,13–16]. Using a before-after design, the following study examined the role of known spinal inhibitory neurotransmitters in the effects of bPNS by intrathecally administering selective antagonists to receptors activated by these substances.

2. Materials and methods

2.1. Animal subjects/anesthesia

All studies were approved by the UAB Institutional Animal Care and Utilization Committee. Subjects were adult, female Sprague-Dawley rats, raised from birth; the maternal animal source was Harlan Laboratories (Sprattville, AL). For all surgical procedures or pretreatments, rats were deeply anesthetized using deep isoflurane (1–3%) inhaled anesthesia. In order to measure EMG responses, rats were maintained in a lightly anesthetized state (urethane 1.2 gm/kg s.c. with 0-0.5% isoflurane in oxygen delivered by tight-fitting mask within a ventilation hood.) In these experiments isoflurane anesthesia was lowered (typically to $\leq$0.2%) until flexion reflexes were present in the hind limbs, but spontaneous escape behaviors were absent.

2.2. Pretreatments producing bladder hypersenstivity

Following a previously published protocol [11], rat pups, on three consecutive days (Postnatal Days P14-P16), were anesthetized with inhaled isoflurane, treated with s.c. ampicillin, swabbed with an iodine/povidone solution and had their urethras cannulated using a 24 gauge angiocatheter; intravesical zymosan (1% in saline, 0.1 ml) was then instilled for 30 min. They were kept warm on a heating pad and returned to their mothers following this treatment. As adults (12–15 weeks of age), these same rats received additional pretreatments one day prior to their terminal experiments. They were similarly anesthetized with inhaled isoflurane, ampicillin and iodine/povidone solution. They had their urethras cannulated using a 22 gauge angiocatheter and intravesical zymosan (1% in saline, 0.5 ml) was instilled for 30 min. They were allowed to recover from anesthesia and studied 24 h later for bPNS effects.

2.3. Pudendal nerve electrical stimulation

As previously reported [1], while deeply anesthetized with inhaled isoflurane, the mixed motor/sensory pudendal nerves were exposed immediately after they exited the lumbosacral plexus as they started to pass anteriorly into the pelvis. Pudendal nerves had double hook electrodes placed around each nerve which were held in place with polysiloxane gel. Grounding electrodes were placed dorsally for all rat groups. Electrical stimuli consisted of trains of biphasic pulses (100 μsec; 10 Hz) delivered at 1 T or 3 T (1 x or 3 x motor threshold, T; the minimal current needed to evoke any observable skeletal muscle contraction). Stimulation intensities to each side were adjusted independently and were typically in the range of 0.5–1.0 mA.

2.4. Measure of VMRs to UBD

The abdominal muscle electromyographic (EMG) activity to UBD has been widely used to evaluate bladder pain-related responses. While lightly anesthetized with a combination of injected urethane and inhaled isoflurane, VMRs remain remarkably stable over time [1,17]. For this measurement, electrodes (silver wire) were inserted into the left lower external oblique musculature immediately superior to the inguinal ligament. Contraction of the abdominal musculature, recorded as EMG activity, was measured via the electrodes using standard differential amplification and rectification and saved on a computer as quantified as previously described (Grass Inc, P511 AC amplifiers; 50x_amplification, 60 Hz clipping, low filter setting 10 Hz–high filter setting 3 kHz) [1]. Following surgery for nerve stimulation and intrathecal catheter placement (described below), a 22-gauge polytetrafluoroethylene angiocatheter was placed into the bladder via the urethra and held in place by a tight suture around the distal urethral orifice. Following surgery, UBDs for 20 s were produced using compressed air, and intravesical pressure was monitored using an in-line pressure transducer. Approximately 15 min after initial anesthesia induction, EMG activity to repeated presentations of 60 mm Hg UBD at 3-minute intervals was recorded until responses to UBD were stable (+/−20%).

2.5. Intrathecal drug protocol

Using the technique of Yaksh and Rudy [18] the atlanto-occipital membrane was incised allowing a 7.5 cm PE10 catheter to be inserted and advanced to the level of the lumbosacral spinal cord. Two rats demonstrating neurological deficits after this insertion were discarded from additional study. Since it would be difficult to observe IT drug effects on bPNS-induced inhibition unless such an inhibitory effect of bPNS were present and due to the complex nature of the experimental preparation and fragility of the pudendal nerves which can lead to technical failures of the preparation, a subset of rats (64 of 72 rats in the total sample) which demonstrated robust inhibitory effects of bPNS were selected for further pharmacological study. All of these rats were characterized for the effects of bPNS on VMRs evoked by graded UBD (10–60 mm Hg, 20 s with 1 min inter-trial periods). The following drugs were then administered intrathecally in a 10 μl volume followed by a 10 μl normal saline flush: naloxone hydrochloride (10 μg; a nonselective opioid receptor antagonist), bicuculline methiodide (0.5 μg; a GABA-A receptor antagonist), CGP35348 (30 μg; Tocris Pharmaceuticals, a GABA-B receptor antagonist), methysergide maleate (30 μg; a nonselective serotonergic antagonist), WAY100636 maleate (10 μg; a 5 H T1A receptor antagonist), ondansetron HCl (10 μg; a 5 H T3 receptor antagonist), phentolamine HCl (30 μg; a nonselective alpha adrenoceptor antagonist), strychnine HCl (1 μg; a glycinergic receptor antagonist) and normal saline (vehicle). The source of drugs utilized was Sigma/Aldrich (St. Louis, MO) unless otherwise noted. The doses employed were selected based upon published literature in rats [e.g.,17,18,19,20,21,22,23].

2.6. Study protocol

The upper panel of Fig. 1 is a diagrammatic description of the study. Rats which had experienced the bladder inflammatory pretreatments described above were prepared surgically and allowed to establish stable responses to UBD. Briefly, using the same protocol as previously published [1], the effects of bPNS on the VMRs to three repeated 60 mm Hg, 20 s UBDs (3 min intertrial interval) and graded (10–60 mm Hg, 20 s) UBD were assessed after 10 min of stimulation at 1 T or 3 T intensities of bPNS and after a 10-minute period of No Stimulation (0 T). Each stimulation period was followed by a 10-minute recovery period with no bPNS. The ordering of 0 T, 1 T and 3 T measurement sessions was randomized from rat to rat. Electrical stimulation was briefly stopped prior to each UBD to avoid artifact in the EMG signal and restarted immediately following each UBD until each set of VMRs was obtained. Following completion of Pre-Drug measures, the intrathecal drug was administered and 15 min later the protocol described above was repeated and Post-Drug Measures obtained. Panels A & B in Fig. 1 graphically present an individual example of a rat treated with intrathecal methysergide maleate.

2.7. Statistics and sample size calculations

All data represents mean ± SEM unless otherwise stated. An ANOVA with Tukey's HSD post hoc tests were used to characterize pre-drug responses to bPNS. Effects of drug administration were compared