Sympathetic β-adrenergic Mechanism in Pudendal Inhibition of Nociceptive and Non-nociceptive Reflex Bladder Activity

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ABSTRACT

This study investigated the role of the hypogastric nerve and β-adrenergic mechanisms in the inhibition of nociceptive and non-nociceptive reflex bladder activity induced by pudendal nerve stimulation (PNS). In α-chloralose anesthetized cats the non-nociceptive reflex bladder activity was induced by slowly infusing saline into the bladder, while the nociceptive reflex bladder activity was induced by replacing saline with 0.25% acetic acid (AA) to irritate the bladder. PNS was applied at multiple threshold (T) intensities for inducing anal sphincter twitching. During saline infusion, PNS at 2T and 4T significantly (p<0.01) increased bladder capacity to 184.7±12.6% and 214.5±10.4% of the control capacity. Propranolol (3 mg/kg, i.v.) had no effect on PNS inhibition, but MTEP (1-3 mg/kg, i.v.) significantly (p<0.05) reduced the inhibition. During AA irritation, the control bladder capacity was significantly (p<0.05) reduced to about 22% of saline control capacity. PNS at 2T and 4T significantly (p<0.01) increased bladder capacity to 406.8±47% and 415.8±46% of the AA control capacity. Propranolol significantly (p<0.05) reduced the bladder capacity to 276.3±53.2% (at 2T PNS) and 266.5±72.4% (at 4T PNS) of AA control capacity, while MTEP removed the residual PNS inhibition. Bilateral transection of the hypogastric nerves produced an effect similar to that produced by propranolol. This study indicates that the hypogastric nerve and a β-adrenergic mechanism in the detrusor play an important role in PNS inhibition of nociceptive but not non-nociceptive reflex bladder activity. In addition to this peripheral mechanism, a CNS mechanism involving metabotropic glutamate 5 receptors also has a role in PNS inhibition.

KEYWORDS: pudendal, neuromodulation, propranolol, hypogastric, cat
INTRODUCTION

Overactive bladder (OAB) is a constellation of symptoms that is characterized by urinary urgency or frequency, with or without urinary incontinence (1). OAB affects 15-27% of men and 33-43% of women in the United States (6). Anticholinergic medications are typically the first line treatment for OAB, but they are often limited in their efficacy and can have an adverse side effect profile that limits medication compliance (3, 5). For this reason, there has been an interest in alternative therapies for the treatment of OAB, including sacral, tibial, or pudendal neuromodulation (24, 25, 29). While these neuromodulation therapies have shown efficacy in improving symptoms and quality of life in OAB patients, the underlying mechanisms of action are not fully understood.

Although an influence on the central neural control of the bladder by targeting synaptic transmission in the spinal cord or brain is likely to play a major role in the effects of neuromodulation (18, 32), peripheral mechanisms involving the activation of adrenergic inhibitory mechanisms in the bladder are also possible. Electrical stimulation or reflex activation of sympathetic efferent axons in the hypogastric nerve inhibits bladder contractions via activation of β-adrenergic receptors in the detrusor muscle and modulates synaptic transmission in bladder parasympathetic ganglia via activation of α-adrenergic receptors (7, 8, 10). Furthermore, stimulation of somatic afferent axons in the pudendal nerves of rats (29) and cats (19) or in nerves passing from the hind limb to the lumbosacral spinal cord in cats (10) activates efferent pathways in the hypogastric nerve. Therefore, it is possible that neuromodulation affects the bladder muscle directly via sympathetic nerve activation of a β-adrenergic inhibitory mechanism as well as indirectly by altering the parasympathetic excitatory outflow from the sacral spinal cord.
A previous study (11) reported that PNS in cats inhibits small amplitude non-micturition contractions induced by distension of the bladder with a volume of saline below the threshold volume for eliciting a micturition reflex. Because the inhibitory effects on non-micturition contractions were abolished by bilateral transection of the hypogastric nerves, it was concluded that PNS modulation of bladder activity involves in part peripheral inhibition of the bladder mediated by reflex activity in sympathetic nerves. However, recent studies in cats showed that inhibition of distention-induced reflex bladder activity by PNS (21) or by sacral root stimulation (34) is not affected by bilateral transection of the hypogastric nerves. A study in rats (14) also showed that bladder inhibition induced by mechanical stimulation of the perineum, which activates pudendal nerve afferents, is not mediated by sympathetic reflex activity in the hypogastric nerve. These studies raise doubts about the earlier proposal that a sympathetic β-adrenergic mechanism in the detrusor plays an important role in PNS inhibition.

The purpose of this study in cats was to clarify the role of hypogastric nerve and β-adrenergic mechanisms in PNS inhibition of reflex bladder activity induced by nociceptive and non-nociceptive stimulation. Saline distention of the bladder was used to activate the non-nociceptive Aδ afferents and induce normal reflex bladder activity, while dilute (0.25%) acetic acid (AA) was used to irritate the bladder, activate the nociceptive C-fiber afferents (13) and induce overactive bladder reflexes (18, 32). Propranolol (a β-adrenergic receptor antagonist, i.v.) and bilateral transection of the hypogastric nerves were used to evaluate the role of sympathetic reflex activity in PNS inhibition. In addition, MTEP (a metabotropic glutamate 5 receptor antagonist, i.v.), which we showed in previous experiments (18, 26) to suppress a spinal inhibitory mechanism of PNS inhibition, was administered after propranolol or hypogastric nerve transection to examine a possible CNS mechanism of the residual PNS inhibition.
METHODS

All protocols involving the experimental use of animals in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical Procedures

The experiments were conducted in a total of 22 cats (12 male, 10 female, 3.0-4.6 kg, Liberty Research, Waverly, NY) under isoflurane (3-5% in O2) anesthesia during surgery followed by α-chloralose anesthesia (65 mg/kg i.v. and supplemented as needed) during data collection. Systemic blood pressures were monitored throughout the experiment via a catheter inserted into the right carotid artery. Heart rate and oxygen saturation levels were monitored by a pulse oximeter (9847V, NONIN Medical, Plymouth, MN) attached to the tongue. A tracheotomy was performed and an endotracheal tube was inserted to ensure airway patency. A catheter for intravenous infusion of fluids and drugs was inserted into the left cephalic vein.

Through a midline abdominal incision the ureters were isolated, ligated, cut, and drained externally. The hypogastric nerves were identified bilaterally in 7 cats and loosely encircled with a suture so that the nerves could be transected by pulling out the suture during the experiment. The urethra was then exposed, and a double lumen catheter was inserted through an urethrotomy into the bladder and secured by a ligature around the urethra to prevent leakage. One lumen of the catheter was connected to a pressure transducer to monitor intravesical pressures, while the other lumen was connected to a pump to slowly infuse (1-2 mL/min) the bladder with either saline or 0.25% AA.

The pudendal nerve was then isolated in the region of right sciatic notch. A tripolar cuff electrode (NC223pt, MicroProbe Inc., Gaithersburg, MD) was implanted around this nerve for
stimulation. The cuff electrode was connected to a stimulator (S88; Grass Medical Instruments, Quincy, MA) via a constant voltage stimulus isolator (SIU5; Grass Medical Instruments, Quincy, MA).

Experimental Protocol

Based on our previous studies (18, 32), uniphasic rectangular pulses of 0.2 ms pulse width and 5 Hz frequency were used for PNS. The stimulation threshold (T), which is defined as the minimal intensity for inducing an anal twitch, was determined at the beginning of the experiment and ranged from 0.3 V to 2.8 V. PNS intensities of 2T or 4T were used in this study to inhibit bladder activity and increase bladder capacity.

Initially, cystometrograms (CMGs) were performed by slowly infusing the bladder with saline to determine the control bladder capacity, which was defined as the bladder volume threshold required to induce a reflex micturition contraction of a large amplitude (>30 cmH₂O) and a long duration (>20 s). The bladder was emptied manually after each CMG by withdrawing the saline through the catheter. Multiple CMGs were performed to ensure reproducibility of the saline control bladder capacity. Then, the animals were divided into three experimental groups.

In the first group of cats (N = 8, 5 male and 3 female), four saline CMGs were performed: (1) control CMG without PNS, (2) CMG during 2T PNS, (3) CMG during 4T PNS, (4) control CMG without PNS. After the 4 CMGs, the animals received propranolol (3 mg/kg, i.v.) followed by MTEP (1 mg/kg and then 3 mg/kg, i.v.). After each dose of drug was administered, the four saline CMGs (control, 2T PNS, 4T PNS, and control) were repeated again. The same protocol was also performed in the second group of cats (N = 7, 4 male and 3 female). However, instead of saline the bladder was infused with 0.25% AA in order to irritate the bladder and induce
overactive reflex bladder activity. In the third group of cats (N = 7, 3 male and 4 female), the bladder was also infused with 0.25% AA but the experimental protocol was modified by replacing propranolol treatment with bilateral transection of hypogastric nerves.

**Data Analysis**

Bladder capacity measured during each CMG in the same animal was normalized to the control bladder capacity prior to propranolol treatment (first and second groups) or hypogastric transection (third group). Measurements were averaged across the animals for the same experimental condition. The results from animals within the same experimental groups are reported as mean ± SE. Statistical significance (p<0.05) was detected by a repeated-measures analysis of variance (ANOVA) followed by Dunnett’s (one-way) or Bonferroni’s (two-way) multiple comparison. The investigators doing the data analysis were not blinded regarding the experimental protocol used in each group of cats.

**RESULTS**

**PNS inhibition of non-nociceptive reflex bladder activity during saline distention**

PNS inhibited non-nociceptive reflex bladder activity during saline CMGs (Fig.1A) and significantly (p<0.01) increased bladder capacity to 184.7±12.6% and 214.5±10.4% of the saline control capacity (11±1.9 mL) at 2T and 4T intensities, respectively (Fig 1B, N=8 cats). The bladder capacity returned to control level after PNS (see Fig.1), indicating that the repeated PNS did not induce post-stimulation inhibition.

After administration of propranolol (3 mg/kg, i.v.) there was no significant change in bladder capacity during CMGs with/without PNS (Fig.2). However, after administration of
MTEP (1 mg/kg, i.v.) the control bladder capacity was significantly (p<0.05) increased while the bladder capacities measured during PNS were significantly (p<0.05) reduced, resulting in a reduction in the change in bladder capacity measured during the 2T PNS CMG (Fig.2B). An additional dose of MTEP (3 mg/kg, i.v.) completely removed the inhibition induced by 4T PNS (Fig.2B, N=8 cats).

**PNS inhibition of nociceptive reflex bladder activity during AA irritation**

In contrast to the lack of effect of propranolol on PNS inhibition during saline CMGs, the drug has a significant impact on PNS inhibition during AA CMGs. Infusion of 0.25% AA irritated the bladder and significantly (p<0.05) reduced bladder capacity to 21.7±6.9% of saline control capacity. PNS inhibited the nociceptive reflex bladder activity induced by AA irritation and significantly (p<0.01) increased bladder capacity to 406.8±47% (at 2T) and 415.8±46% (at 4T) of AA control capacity (2.3±0.6 mL, Fig.3). Propranolol (3 mg/kg, i.v.) did not change the AA control bladder capacity but significantly (p<0.05) reduced the increases in bladder capacity induced by both 2T and 4T PNS (Fig.3). However, after the propranolol treatment PNS still significantly (p<0.01) increased bladder capacity to 276.3%±53.2% (at 2T) and 266.5±72.4% (at 4T) of AA control capacity (Fig.3B). MTEP (1 mg/kg, i.v.) significantly (p<0.05) reduced the residual inhibition induced by 2T PNS but not 4T PNS (see Fig.3B, N=7 cats), while 3 mg/kg MTEP did not elicit an additional change in the inhibition (Fig.3).

Similar to propranolol treatment, bilateral transection of the hypogastric nerves also significantly reduced PNS inhibition during AA CMG (Fig.4). In this group of cats (N=7), infusion of 0.25% AA irritated the bladder and significantly (p<0.05) reduced bladder capacity to 22±3.7% of saline control capacity. PNS inhibited the AA irritation-induced bladder overactivity
and significantly (p<0.01) increased bladder capacity to 396.7%±87% (at 2T) and 382.4±71.2% (at 4T) of AA control capacity (2.4±0.3 mL, Fig.4). Bilateral transection of the hypogastric nerves did not change the AA control bladder capacity but significantly (p<0.05) reduced the increases in bladder capacity induced by both 2T and 4T PNS and caused 2T PNS unable to significantly increase the bladder capacity (Fig.4). Additional MTEP (1 mg/kg or 3 mg/kg, i.v.) produced a further reduction in PNS inhibition and removed the significant increase in bladder capacity by 4T PNS (Fig.4).

DISCUSSION

This study shows that the increase in bladder capacity induced by PNS (termed PNS inhibition) in normal bladders during saline CMGs (Fig.1) is not sensitive to propranolol (Fig.2), while PNS inhibition of overactive bladder activity induced by AA irritation is significantly reduced by propranolol (Fig.3) or by bilateral transection of the hypogastric nerves (Fig.4). These results indicate that reflex activation of a sympathetic efferent pathway in the hypogastric nerves by PNS followed by release of norepinephrine in the bladder and stimulation of post-junctional β-adrenergic receptors plays a major role in PNS inhibition of nociceptive reflex bladder activity but does not play a role in PNS inhibition of non-nociceptive reflex bladder activity. In addition, administration of MTEP can further reduce PNS inhibition of non-nociceptive (Fig.2) or nociceptive (Figs.3-4) reflex bladder activity after propranolol treatment or hypogastric nerve transection, indicating that the metabotropic glutamate 5 receptors in the CNS also play a role in PNS inhibition.

Our study extends the previous observations (11) that experimental conditions can influence the mechanisms underlying inhibitory effects of PNS on bladder activity. It is clear as
previously described (11) and confirmed in a subsequent investigation (21) that PNS inhibition of large amplitude micturition reflex contractions during distension of the bladder with saline is not dependent on sympathetic reflex activity (Fig.2). However, sympathetic reflexes do play a role either when the bladder is made overactive by intravesical infusion of dilute AA (Figs.3-4) or when the bladder is partially filled with saline to induce small amplitude non-micturition contractions (11). It is tempting to speculate that the latter two inhibitory effects reflect activation of a sympathetic reflex and the same peripheral β-adrenergic receptor mechanism. It is probable that bladder capacity after AA infusion is reduced in part by sensitization and increased firing of bladder afferents. It would also be reasonable to expect that the afferent firing is increased by the non-micturition contractions that occur during bladder filling and become more prominent in the AA irritated bladder (Fig.3). As noted in the previous study (11), the non-micturition contractions are suppressed by PNS. This should reduce afferent firing and in turn increase bladder capacity; and thus provide one explanation for the selective effect of PNS on bladder capacity in the irritated bladder versus the normal bladder.

Another possible explanation for the difference in the PNS effect on irritated and normal bladders is that PNS activation of reflex activity in the hypogastric nerve is enhanced by bladder irritation and that under normal conditions the reflex sympathetic activity to the bladder is minimal and does not produce sufficient activation of β-adrenergic receptors to influence bladder capacity. Therefore, propranolol does not alter PNS inhibition under normal conditions. One concern with this explanation is that PNS evokes reflexes on the hypogastric nerve in the absence of bladder irritation (19, 28) and yet does not increase bladder capacity. However, it should be recognized that the hypogastric nerve contains the sympathetic innervation of blood vessels and various organs (e.g., the reproductive organs, distal bowel, urethra and anal canal) in
addition to the bladder (15) and that stimulation of many somatic and visceral afferents evokes reflex firing on the hypogastric nerve (8, 10) without eliciting changes in bladder activity presumably because the firing targets other organs (10). Thus, increased bladder afferent firing induced by bladder irritation with AA may facilitate the PNS evoked sympathetic reflex activity targeting the bladder. This mechanism is probable because activation of mechano-sensitive bladder afferents enhances hypogastric efferent input to the bladder and electrical stimulation of these afferents produces inhibitory responses in the bladder mediated by β-adrenergic receptors (10). Thus, convergence and synergistic interactions of bladder C-fiber afferents and pudendal afferent inputs onto the spinal circuitry controlling the lumbar sympathetic inhibitory pathway to the bladder may account for the emergence of propranolol-sensitive PNS inhibition after bladder irritation. This central mechanism as well as the peripheral mechanism discussed above may contribute to the selectivity of pudendal neuromodulation of overactive bladder.

The enhancement of the spinal sympathetic inhibitory pathway to the bladder by AA irritation is supported by our recent study (27) showing that propranolol can enhance spinal reflex bladder activity elicited by AA irritation in acute spinal cord transected cats and reduce the PNS inhibition of spinal reflex bladder activity. In these experiments sympathetic nerve activity must tonically inhibit bladder contractile activity and/or bladder afferent firing because block of β-adrenergic receptors with propranolol enhances reflex bladder contractions presumably by removing the inhibition. However, in spinal cord intact animals in the present experiments bladder reflex activity induced by saline distension or AA irritation was not affected by propranolol (Figs.2-4) indicating that the tonic sympathetic inhibition is minimal under these conditions possibly due to suppression of the spinal sympathetic reflex pathway by supraspinal mechanisms. This idea is supported by an earlier study (7) which showed that the reflex firing on
the hypogastric nerve elicited by electrical stimulation of bladder afferents is enhanced by transection of the thoracic spinal cord.

The proposed enhancement of the PNS to hypogastric nerve reflex by bladder irritation raises the possibility that this reflex pathway is also suppressed under normal conditions. In decerebrate rats bilateral transection of the hypogastric nerves reduces bladder capacity (33); whereas this procedure in rats with an intact neuraxis does not affect bladder capacity (22), suggesting that decerebration enhances tonic sympathetic inhibitory input to the bladder. In decerebrate cats, PNS also suppressed the amplitude of bladder contractions induced by electrical stimulation of the pontine micturition center; and propranolol administration eliminated the PNS inhibition (20). These experiments were conducted during saline distension of the bladder, a condition in which propranolol has failed to influence PNS inhibition in the present and earlier experiments (21). These data raise the possibility that decerebration in the cat eliminates a tonic supraspinal inhibitory control of the hypogastric nerve reflex evoked by PNS and unmasks the propranolol-sensitive PNS inhibition of the bladder.

It is worth noting that nerve transection disrupts both efferent and afferent sympathetic pathways in the hypogastric nerve, while propranolol treatment only blocks the sympathetic efferent pathway to the bladder. Therefore, hypogastric nerve transection may produce effects in addition to those elicited by propranolol treatment. Although previous studies have shown that bladder distention/irritation can activate hypogastric afferent pathways (15, 23), transection of hypogastric nerves in this study had no effect on bladder capacity (Fig.4) indicating that hypogastric afferents from the bladder may not play a role in regulating the micturition reflex. Since our previous studies have shown that activation of sympathetic efferent axons in the hypogastric nerve inhibits bladder contractions via activation of β-adrenergic receptors in the
detrusor muscle (7,9,10) and in the present study hypogastric nerve transection and propranolol treatment produced a similar reduction in PNS inhibition (Figs.3-4), it is reasonable to conclude that hypogastric nerve transection reduced PNS inhibition by interrupting the sympathetic efferent pathway to the bladder and preventing the activation of β-adrenergic receptors in the detrusor muscle. However, the physiological role of hypogastric afferent from bladder still needs to be determined because our result indicates that it does not regulate bladder capacity (Fig.4).

In contrast to the peripheral β-adrenergic inhibitory action of PNS which is mediated by a sympathetic reflex and is only detectable during nociceptive reflex bladder activity, a CNS mechanism of PNS inhibition involving metabotropic glutamate 5 receptors can suppress non-nociceptive as well as nociceptive reflex bladder activity in cats (18) (Figs.2-4). However, spinal GABA_A receptors are involved in the inhibition of nociceptive reflex bladder activity, but are not involved in PNS inhibition of non-nociceptive bladder activity (32). Together, these results indicate that PNS acts via multiple peripheral and central mechanisms to suppress the activity of the normal and irritated overactive bladder.

It is known that metabotropic glutamate 5 receptors can act at peripheral nerve terminals to modulate nociception (4, 31). Although MTEP was administered intravenously in this study, it is less likely to act at the peripheral nerve terminals in the bladder. First, MTEP did not change bladder capacity during AA irritation (Figs.3-4) indicating that metabotropic glutamate 5 receptors do not play a role in the nociceptive bladder reflex. Second, PNS inhibition is less likely due to activation of metabotropic glutamate 5 receptors in the bladder nerve terminals because pudendal-to-bladder reflexes are mediated by efferent pathways in the pelvic and hypogastric nerves, which utilize cholinergic and adrenergic mechanisms but not glutamatergic...
mechanism. Therefore, the MTEP effects on PNS inhibition as shown in this study (Figs.2-4) could only be attributed to changes in metabotropic glutamate 5 receptors in CNS.

In summary, this study resolved the different views of previous investigators regarding the role of sympathetic efferent pathways in PNS inhibition of bladder activity. While the mechanisms of action of PNS inhibition may vary between species and in different pathological conditions, the present results suggest obvious clinical studies (e.g., testing PNS after the administration of a β3-adrenergic receptor blocking agent) that could extend the animal studies to humans. Although the detrusor in cats is extensively innervated by sympathetic nerves (30), it is well known that the sympathetic innervation is dense in bladder neck area but is sparse in the detrusor in humans (12, 16), rats (2), and guinea pigs (17). Whether the β-adrenergic mechanism in PNS inhibition as identified in this animal study applies to other species still needs to be determined. The peripheral and central mechanisms of PNS inhibition revealed in this study could also occur in other neuromodulation therapies such as tibial or sacral neuromodulation (25, 29). Understanding the mechanisms of bladder neuromodulation could promote the development of new neuromodulation therapies for bladder disorders (3, 5).

GRANTS

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DISCLOSURES

The authors declare no actual or potential conflicts of interest.
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FIGURE CAPATION

Fig.1. During saline infusion, pudendal nerve stimulation (PNS) significantly increased bladder capacity. A. Representative CMG traces. Black bar under the bladder pressure trace indicates the duration of PNS. T – threshold intensity for inducing anal twitching. B. Summarized results (N = 8 cats). * indicates significantly (p < 0.01, posttest) different from the control by 1-way ANOVA (p < 0.0001, F = 68.24). PNS: 5 Hz frequency, 0.2 ms pulse width, T = 0.3-2.8 V.

Fig.2. During saline infusion, the effects of propranolol and MTEP were tested on bladder inhibition induced by pudendal nerve stimulation (PNS). A.) Representative CMG traces that were performed in sequence from left to right in each row and then from top to bottom for different treatments. Black bar under the bladder pressure trace indicates the duration of PNS. T – threshold PNS intensity for inducing anal twitching. B.) Summarized results (N = 8 cats). * indicates significantly (p < 0.001, posttest) different from the data obtained without PNS by 2-way ANOVA (between groups: p < 0.0001, F = 58.61; Interaction: p = 0.0007, F = 4.672). @
indicates significantly (p < 0.05, posttest) different from the control in the same treatment group by 1-way ANOVA (4T PNS group: p = 0.04, F = 7.98; 2T PNS group: p = 0.01, F = 8.28; Without PNS group: p = 0.08, F = 2.68). PNS: 5 Hz frequency, 0.2 ms pulse width, T = 0.3-2.8 V.

Fig.3. During acetic acid infusion, the effects of propranolol and MTEP were tested on bladder inhibition induced by pudendal nerve stimulation (PNS). A.) Representative CMG traces that were performed in sequence from left to right in each row and then from top to bottom for different treatments. Black bar under the bladder pressure trace indicates the duration of PNS. T – threshold PNS intensity for inducing anal twitching. B.) Summarized results (N = 7 cats). * indicates significantly (p < 0.01, posttest) different from the data obtained without PNS by 2-way ANOVA (between groups: p < 0.0001, F = 30.42; Interaction: p = 0.017, F = 2.901). @ indicates significantly (p < 0.05, posttest) different from the control in the same treatment group by 1-way ANOVA (4T PNS group: p < 0.0001, F = 14.06; 2T PNS group: p < 0.0001, F = 19.99). # indicates significantly different from the propranolol data (p < 0.05, 1-way ANOVA). PNS: 5 Hz frequency, 0.2 ms pulse width, T = 0.5-1.4 V.

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