Sympathetic β-adrenergic mechanism in pudendal inhibition of nociceptive and non-nociceptive reflex bladder activity

Brian T. Kadow,[1] Timothy D. Lyon,[1] Zhaocun Zhang,[1,2] Vladimir Lamm,[1] Bing Shen,[1] Jicheng Wang,[1] James R. Roppolo,[3] William C. de Groat,[3] and Changfeng Tai[1,3]

[1]Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania; [2]Department of Urology, Qilu Hospital, Shandong University, Jinan, China; and [3]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

Kadow BT, Lyon TD, Zhang Z, Lamm V, Shen B, Wang J, Roppolo JR, de Groat WC, Tai C. Sympathetic β-adrenergic mechanism in pudendal inhibition of nociceptive and non-nociceptive reflex bladder activity. Am J Physiol Renal Physiol 311: F78–F84, 2016. First published May 11, 2016; doi:10.1152/ajprenal.00180.2016.—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, non-nociceptive reflex bladder activity was induced by slowly infusing saline into the bladder, whereas 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 $184.7 \pm 12.6\%$ and $214.5 \pm 10.4\%$ of the control capacity. Propranolol (3 mg/kg iv) had no effect on PNS inhibition, but 3-[(2-methyl-4-thiazolyl)ethynyl]pyridine (MTEP; 1–3 mg/kg iv) significantly ($P < 0.05$) reduced the inhibition. During AA irritation, the control bladder capacity was significantly ($P < 0.05$) reduced to ~22% of the saline control capacity. PNS at 2T and 4T significantly ($P < 0.01$) increased bladder capacity to $406.8 \pm 47\%$ and $415.8 \pm 46\%$ of the AA control capacity. Propranolol significantly ($P < 0.05$) reduced the bladder capacity to $276.3 \pm 53.2\%$ (at 2T PNS) and $266.5 \pm 72.4\%$ (at 4T PNS) of the AA control capacity, whereas MTEP (a metabotropic glutamate 5 receptor antagonist) removed the residual PNS inhibition. Bilateral transection of the hypogastric nerves produced an effect similar to that produced by propranolol. This study indicates that hypogastric nerves 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 central nervous system mechanism involving metabotropic glutamate 5 receptors also has a role in PNS inhibition.

pudendal; neuromodulation; propranolol; hypogastric; cat

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 firstline 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 symp-toms 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 hindlimb 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 pudendal nerve stimulation (PNS) (21) or by sacral root stimulation (34) is not affected by bilateral transection of the hypogastric nerves. A study (14) in rats 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 the present 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 non-nociceptive Aδ afferents and induce normal reflex bladder activity, whereas dilute (0.25%) acetic acid (AA) was used to irritate the bladder, activate nociceptive C-fiber afferents (13), and induce OAB reflexes (18, 32).

Address for reprint requests and other correspondence: C. Tai, Dept. of Urology, Univ. of Pittsburgh, 700 Kaufmann Bldg., Pittsburgh, PA 15213 (e-mail: cftai@pitt.edu).

Propranolol (a β-adrenergic receptor antagonist, intravenous) and bilateral transection of the hypogastric nerves were used to evaluate the role of sympathetic reflex activity in PNS inhibition. In addition, 3-[(2-methyl-4-thiazolyl)ethynyl]pyridine (MTEP; a metabotropic glutamate 5 receptor antagonist, intravenous), which we have shown 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 central nervous system (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 of the University of Pittsburgh.

Surgical procedures. Experiments were conducted in a total of 22 cats (12 male cats and 10 female cats, 3.0–4.6 kg, Liberty Research, Waverly, NY) under isoflurane (3–5% in O_2) anesthesia during surgery followed by α-chloralose anesthesia (65 mg/kg iv and supplemented as needed) during data collection. Systemic blood pressure was monitored throughout the experiment via a catheter inserted into the right carotid artery. Heart rate and O_2 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 the 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 seven 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, whereas 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, 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).

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 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 cmH$_2$O) 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. Animals were then divided into three experimental groups.

In the first group of cats (n = 8, 5 male cats and 3 female cats), four saline CMGs were performed: *1*) a control CMG without PNS, *2*) a CMG during 2T PNS, *3*) a CMG during 4T PNS, and *4*) a control CMG without PNS. After the four CMGs, animals received propranolol (3 mg/kg iv) followed by MTEP (1 mg/kg and then 3 mg/kg iv).

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 cats and 3 female cats). However, instead of saline, the bladder was infused with 0.25% AA to irritate the bladder and induce overactive reflex bladder activity. In the third group of cats (n = 7, 3 male cats and 4 female cats), the bladder was also infused with 0.25% AA but the experimental protocol was modified by replacing propranolol treatment with bilateral transection of the hypogastric nerves.

Data analysis. The bladder capacity measured during each CMG in the same animal was normalized to the control bladder capacity before 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 means ± SE. Statistical significance ($P < 0.05$) was detected by repeated-measures 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. 1*A*) 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 (n = 8 cats; Fig. 1*B*). The bladder capacity returned to the control level after PNS (see Fig. 1), indicating that the repeated PNS did not induce poststimulation inhibition.

After the administration of propranolol (3 mg/kg iv), there was no significant change in the bladder capacity during CMGs with/without PNS (Fig. 2). However, after the administration of MTEP (1 mg/kg iv), 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 the bladder capacity measured during the 2T PNS CMG (Fig. 2*B*). An additional dose of MTEP (3 mg/kg iv) completely removed the inhibition induced by 4T PNS (n = 8 cats; Fig. 2*B*).

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 had a significant impact on PNS inhibition during AA-infused CMGs. Infusion of 0.25% AA irritated the bladder and significantly ($P < 0.05$) reduced the bladder capacity to 21.7 ± 6.9% of the saline control capacity. PNS inhibited the nociceptive reflex bladder activity induced by AA irritation and significantly ($P < 0.01$) increased the bladder capacity to 406.8 ± 47% (at 2T) and 415.8 ± 46% (at 4T) of the AA control capacity (2.3 ± 0.6 ml; Fig. 3). Propranolol (3 mg/kg iv) did not change the AA control bladder capacity but significantly ($P < 0.05$) reduced the increases in the bladder capacity induced by both 2T and 4T PNS (Fig. 3). However, after the propranolol treatment, PNS still significantly ($P < 0.01$) increased the bladder capacity to 276.3% ± 53.2% (at 2T) and 266.5 ± 72.4% (at 4T) of the AA control capacity (Fig. 3*B*). MTEP (1 mg/kg iv) significantly ($P < 0.05$) reduced the residual inhibition induced by 2T PNS but not 4T PNS (n = 7 cats; see Fig. 3*B*), whereas 3 mg/kg MTEP did not elicit an additional change in the inhibition (Fig. 3).

Fig. 1. During saline infusion, pudendal nerve stimulation (PNS) significantly increased bladder capacity. *A*: representative cystometrogram (CMG) traces. The solid horizontal bar under the bladder pressure trace indicates the duration of PNS. T is the threshold intensity for inducing anal twitching. *B*: summarized results (*n* = 8 cats). *Significantly (*P* < 0.01 by posttest) different from the control by one-way ANOVA (*P* < 0.0001, *F* = 68.24). PNS: 5-Hz frequency, 0.2-ms pulse width, T = 0.3–2.8 V.
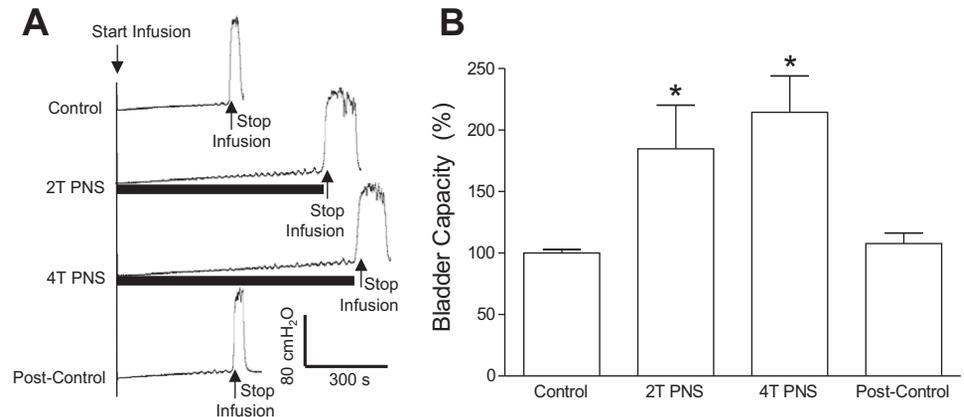
Similar to propranolol treatment, bilateral transection of the hypogastric nerves also significantly reduced PNS inhibition during AA-infused CMG (Fig. 4). In this group of cats (*n* = 7), infusion of 0.25% AA irritated the bladder and significantly (*P* < 0.05) reduced the bladder capacity to 22 ± 3.7% of the saline control capacity. PNS inhibited the AA irritation-induced bladder overactivity and significantly (*P* < 0.01) increased the bladder capacity to 396.7% ± 87% (at 2T) and 382.4 ± 71.2% (at 4T) of the 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 the bladder capacity induced by both 2T and 4T PNS and prevented 2T PNS from significantly increasing the bladder capacity (Fig. 4). Additional MTEP (1 or 3 mg/kg iv) produced a further reduction in PNS inhibition and removed the significant increase in the bladder capacity by 4T PNS (Fig. 4).

DISCUSSION

This study shows that the increase in the bladder capacity induced by PNS (termed PNS inhibition) in normal bladders during saline-infused CMGs (Fig. 1) is not sensitive to propranolol (Fig. 2), whereas 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 the release of norepinephrine in the bladder and stimulation of postjunctional 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 and 4) reflex bladder activity after propranolol treatment or hypogastric nerve transection, indicating that 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 and 4) or when the bladder is partially filled with saline to induce small amplitude nonmicturition 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 nonmicturition contractions that occur during bladder filling and become more prominent in the AA-irritated bladder (Fig. 3). As noted in the previous study (11), nonmicturition 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, 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 PNS-evoked sympathetic reflex activity targeting the bladder. This mechanism is probable because activation of mechanosensitive 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
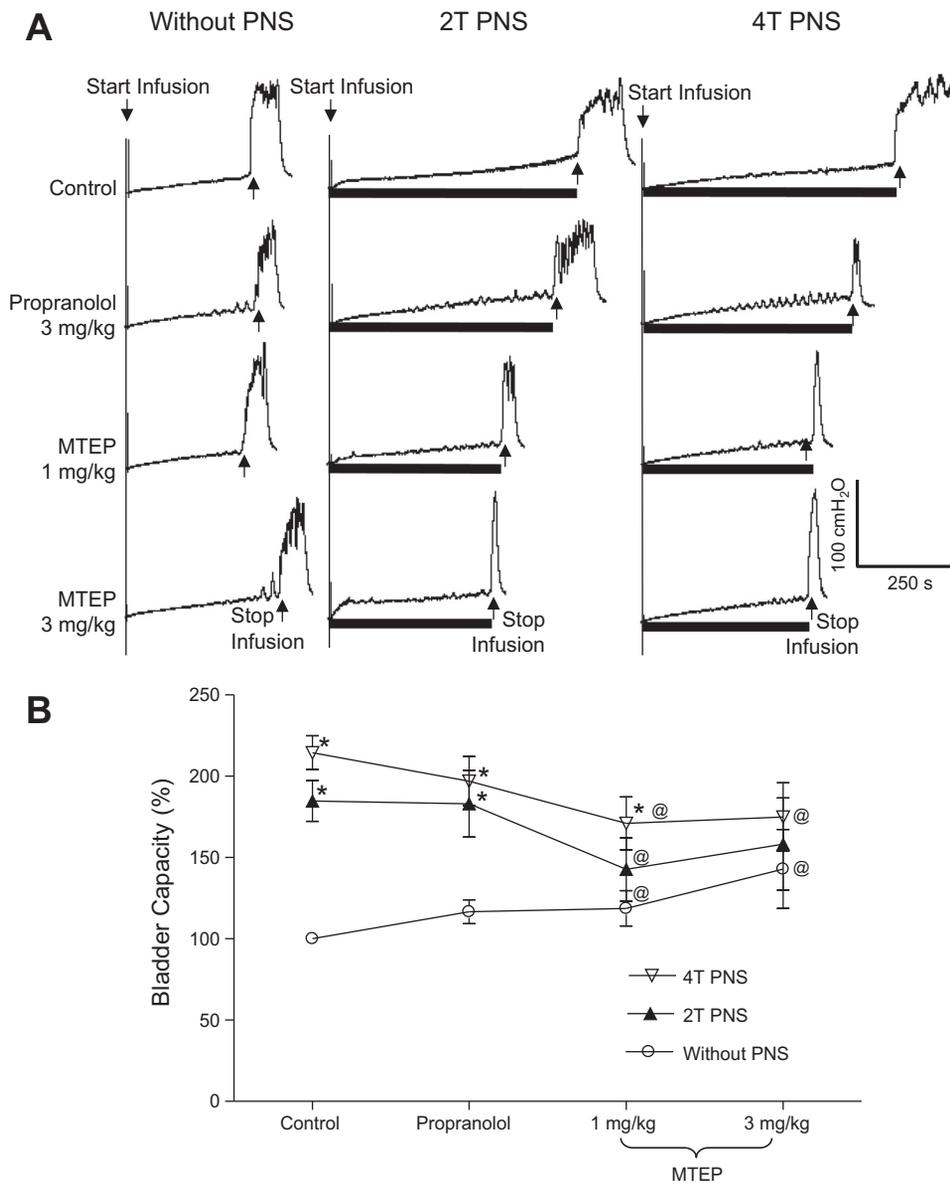
Fig. 2. During saline infusion, the effects of propranolol and 3-[(2-methyl-4-thiazolyl)ethynyl]pyridine (MTEP) were tested on bladder inhibition induced by PNS. *A*: representative CMG traces that were recorded in sequence from *left* to *right* in each row and then from *top* to *bottom* for different treatments. The solid horizontal bar under the bladder pressure trace indicates the duration of PNS. *B*: summarized results ($n = 8$ cats). *Significantly ($P < 0.001$ by posttest) different from the data obtained without PNS by two-way ANOVA (between groups: $P < 0.0001$, $F = 58.61$; interaction: $P = 0.0007$, $F = 4.672$); @significantly ($P < 0.05$ by posttest) different from the control in the same treatment group by one-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.

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 OAB.

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) that 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
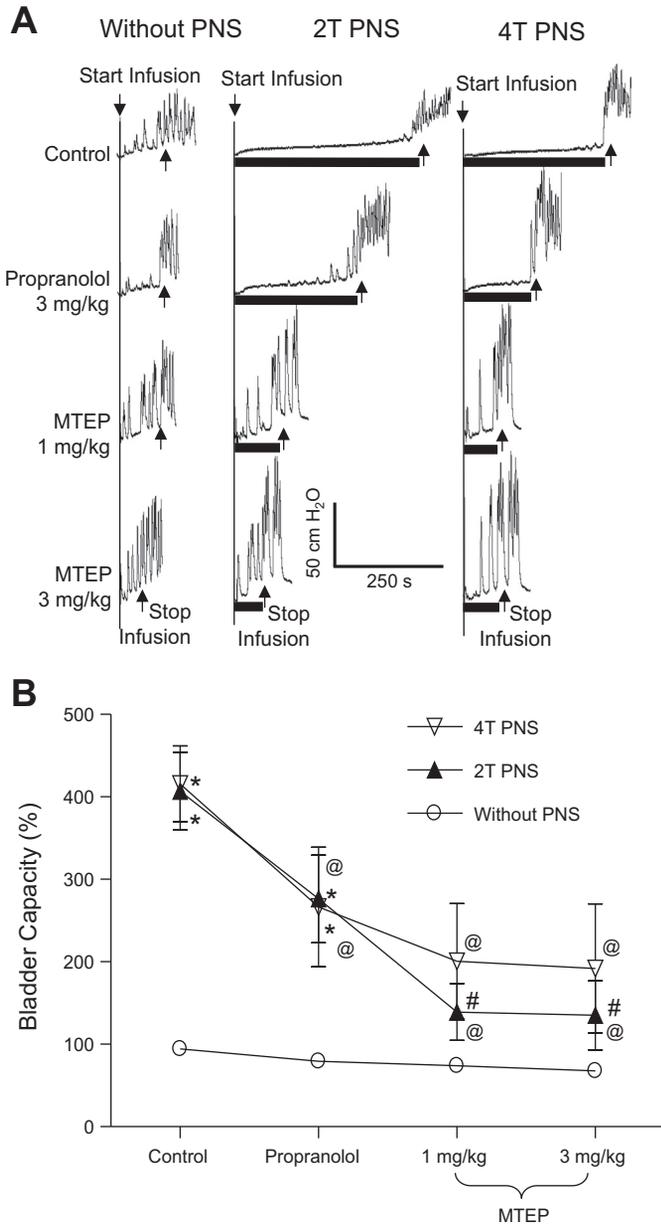
Fig. 3. During AA infusion, the effects of propranolol and MTEP were tested on bladder inhibition induced by PNS. *A*: representative CMG traces that were recorded in sequence from *left* to *right* in each row and then from *top* to *bottom* for different treatments. The solid horizontal bar under the bladder pressure trace indicates the duration of PNS. *B*: summarized results ($n = 7$ cats). *Significantly ($P < 0.01$ by posttest) different from the data obtained without PNS by two-way ANOVA (between groups: $P < 0.0001$, $F = 30.42$; interaction: $P = 0.017$, $F = 2.901$); @significantly ($P < 0.05$ by posttest) different from the control in the same treatment group by one-way ANOVA (4T PNS group: $P < 0.0001$, $F = 14.06$; 2T PNS group: $P < 0.0001$, $F = 19.99$); #significantly different from the propranolol data ($P < 0.05$ by one-way ANOVA). PNS: 5-Hz frequency, 0.2-ms pulse width, T = 0.5–1.4 V.

thetic 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 re-
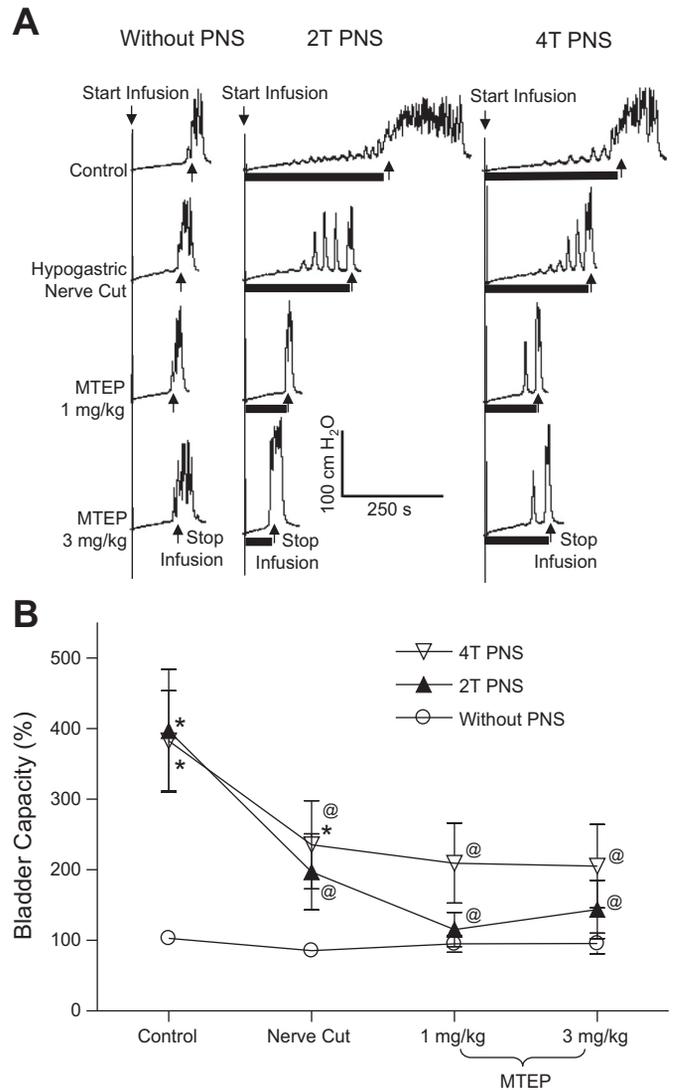

Fig. 4. During AA infusion, the effects of hypogastric nerve transection and MTEP were tested on bladder inhibition induced by PNS. *A*: representative CMG traces that were recorded in sequence from *left* to *right* in each row and then from *top* to *bottom* for different treatments. The solid horizontal bar under the bladder pressure trace indicates the duration of PNS. *B*: summarized results ($n = 7$ cats). *Significantly ($P < 0.01$ by posttest) different from the data obtained without PNS by two-way ANOVA (between groups: $P < 0.0001$, $F = 22.86$; interaction: $P = 0.01$, $F = 3.13$); @significantly ($P < 0.05$ by posttest) different from the control in the same treatment group by one-way ANOVA (4T PNS group: $P < 0.0001$, $F = 17.31$; 2T PNS group: $P = 0.0004$, $F = 10.17$). PNS: 5-Hz frequency, 0.2-ms pulse width, T = 0.6–1.6 V.

(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, whereas propranolol treatment only blocks the sympa-

duction in PNS inhibition (Figs. 3 and 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 afferents from bladder still needs to be determined because our result indicates that they do 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 normal and irritated OABs.

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 peripheral nerve terminals in the bladder. First, MTEP did not change the bladder capacity during AA irritation (Figs. 3 and 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 bladder nerve terminals because pudendal to bladder reflexes are mediated by efferent pathways in the pelvic and hypogastric nerves, which use cholinergic and adrenergic mechanisms but not glutamatergic mechanisms. Therefore, 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 the 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 under different pathological conditions, the present results suggest obvious clinical studies (e.g., testing PNS after the administration of a β$_3$-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 sympathetic innervation is dense in the bladder neck area but 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).

AUTHOR CONTRIBUTIONS

B.T.K., T.D.L., Z.Z., V.L., B.S., J.W., J.R.R., W.C.d.G., and C.T. performed experiments; B.T.K., T.D.L., Z.Z., V.L., B.S., J.W., J.R.R., W.C.d.G., and C.T. analyzed data; B.T.K., T.D.L., Z.Z., V.L., B.S., J.W., J.R.R., W.C.d.G., and C.T. interpreted results of experiments; B.T. K., T.D.L., Z.Z., V.L., B.S., J.W., J.R.R., W.C.d.G., and C.T. prepared figures; B.T.K., T.D.L., Z.Z., V.L., B.S., J.W., J.R.R., W.C.d.G., and C.T. drafted manuscript; B.T.K., T.D.L., Z.Z., V.L., B.S., J.W., J.R.R., W.C.d.G., and C.T. edited and revised manuscript; B.T.K., T.D.L., Z.Z., V.L., B.S., J.W., J.R.R., W.C.d.G., and C.T. approved final version of manuscript; J.R.R., W.C.d.G., and C.T. conception and design of research.

REFERENCES

1. **Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, van Kerrebroeck PE, Victor A, Wein AJ.** The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *Neurourol Urodyn* 21: 167–178, 2002.
2. **Alm P, Elmér M.** Adrenergic and cholinergic innervation of the rat urinary bladder. *Acta Physiol Scand* 94: 36–45, 1975.
3. **Andersson KE, Pehrson R.** CNS involvement in overactive bladder: pathophysiology and opportunities for pharmacological intervention. *Drugs* 63: 2595–2611, 2003.
4. **Bhave G, Karim F, Carlton SM, Gereau RW.** Peripheral group I metabotropic glutamate receptors modulate nociception in mice. *Nat Neurosci* 4: 417–423, 2001.
5. **Chapple CR, Khullar V, Gabriel Z, Muston D, Bitoun CE, Weinstein D.** The effects of antimuscarinic treatments in overactive bladder: an update of a systematic review and meta-analysis. *Eur Urol* 54: 543–562, 2008.
6. **Coyne KS, Sexton CC, Irwin DE, Kopp ZS, Kelleher CJ, Milsom I.** The impact of overactive bladder, incontinence and other lower urinary tract symptoms on quality of life, work productivity, sexuality and emotional well-being in men and women: results from the EPIC study. *BJU Int* 101: 1388–1395, 2008.
7. **de Groat WC, Lalley PM.** Reflex firing in the lumbar sympathetic outflow to activation of vesical afferent fibres. *J Physiol* 226: 289–309, 1972.
8. **de Groat WC, Lalley PM.** Reflex sympathetic firing in response to electrical stimulation of the carotid sinus nerve in the cat. *Brain Res* 80: 17–40, 1974.
9. **de Groat WC, Saum WR.** Sympathetic inhibition of the urinary bladder and of pelvic ganglionic transmission in the cat. *J Physiol* 220: 297–314, 1972.
10. **de Groat WC, Theobald RJ.** Reflex activation of sympathetic pathways to vesical smooth muscle and parasympathetic ganglia by electrical stimulation of vesical afferents. *J Physiol* 259: 223–237, 1976.
11. **Fall M, Erlandson BE, Carlsson CA, Lindström S.** The effect of intravaginal electrical stimulation on the feline urethra and urinary bladder: neuronal mechanisms. *Scand J Urol Nephrol Suppl* 44: 19–30, 1977.
12. **Gosling JA, Dixon JS, Jen PY.** The distribution of noradrenergic nerves in the human lower urinary tract. A review. *Eur Urol* 36, *Suppl* 1: 23–30, 1999.
13. **Habler HJ, Jänig W, Koltzenburg M.** Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat. *J Physiol* 425: 545–562, 1990.
14. **Hotta H, Watanabe N.** Gentle mechanical skin stimulation inhibits micturition contractions via the spinal opioidergic system and by decreasing both ascending and descending transmissions of the micturition reflex in the spinal cord. *PloS One* 10: e0135185, 2015.
15. **Jänig W, Schmidt M, Schnitzler A, Wesselmann U.** Differentiation of sympathetic neurones projecting in the hypogastric nerves in terms of their discharge patterns in cats. *J Physiol* 437: 157–179, 1991.
16. **Klück P.** The autonomic innervation of the human urinary bladder, bladder neck and urethra: a histochemical study. *Anat Rec* 198: 439–447, 1980.
17. **Kunze A, Neuhaus J, Stolzenburg JU.** Quantitative immunohistochemical study of the innervation of the guinea-pig lower urinary tract. *BJU Int* 98: 424–429, 2006.

18. **Larson JA, Ogagan PD, Chen G, Shen B, Wang J, Roppolo JR, de Groat WC, Tai C.** Involvement of metabotropic glutamate receptor 5 in pudendal inhibition of nociceptive bladder activity in cats. *J Physiol* 589: 5833–5843, 2011.

19. **Lindström S, Fall M, Carlsson CA, Erlandson BE.** The neurophysiological basis of bladder inhibition in response to intravaginal electrical stimulation. *J Urol* 129: 405–410, 1983.

20. **Lyon TD, Ferroni MC, Kadow BT, Slater RC, Zhang Z, Chang V, Lamm V, Shen B, Wang J, Roppolo JR, de Groat WC, Tai C.** Pudendal but not tibial nerve stimulation inhibits bladder contractions induced by stimulation of pontine micturition center in cats. *Am J Physiol Regul Integr Comp Physiol* 310: R366–R374, 2016.

21. **McGee MJ, Danziger ZC, Bamford JA, Grill WM.** A spinal GABAergic mechanism is necessary for bladder inhibition by pudendal afferent stimulation. *Am J Physiol Renal Physiol* 307: F921–F930, 2014.

22. **Morikawa K, Kakiuchi M, Fukuoka M, Kato H, Ito Y, Gomi Y.** Effects of various drugs on bladder function in conscious restrained-denervated rats placed in a restraining cage and produced by transection of the hypogastric nerve. *Japan J Pharmacol* 52: 405–411, 1990.

23. **Moss NG, Harrington WW, Tucker MS.** Pressure, volume, and chemosensitivity in afferent innervation of urinary bladder in rats. *Am J Physiol Regul Integr Comp Physiol* 272: R695–R703, 1997.

24. **Peters KM, Feber KM, Bennett RC.** Sacral versus pudendal nerve stimulation for voiding dysfunction: a prospective, single-blinded, randomized, crossover trial. *Neurourol Urodyn* 24: 643–647, 2005.

25. **Peters KM, Macdiarmid SA, Wooldridge LS, Leong FC, Shobeiri SA, Rovner ES, Siegel SW, Tate SB, Jarnagin BK, Rosenblatt PL, Feagins BA.** Randomized trial of percutaneous tibial nerve stimulation versus extended-release tolterodine: results from the overactive bladder innovative therapy trial. *J Urol* 182: 1055–1061, 2009.

26. **Reese JN, Rogers MJ, Xiao Z, Shen B, Wang J, Schwen Z, Roppolo JR, de Groat WC, Tai C.** Role of spinal metabotropic glutamate receptor 5 in pudendal inhibition of the nociceptive bladder reflex in cats. *Am J Physiol Renal Physiol* 308: F832–F838, 2015.

27. **Rogers MJ, Xiao Z, Shen B, Wang J, Schwen Z, Roppolo JR, de Groat WC, Tai C.** Propranolol, but not naloxone, enhances spinal reflex bladder activity and reduces pudendal inhibition in cats. *Am J Physiol Regul Integr Comp Physiol* 308: R42–R49, 2015.

28. **Steers WD, Mallory B, de Groat WC.** Electrophysiological study of neural activity in penile nerve of the rat. *Am J Physiol Regul Integr Comp Physiol* 254: R989–R1000, 1988.

29. **van Kerrebroeck PE, van Voskuilen AC, Heesakkers JP, Lycklama á Nijholt AA, Siegel S, Jonas U, Fowler CJ, Fall M, Gajewski JB, Hassouna MM, Cappellano F, Elhilali MM, Milam DF, Das AK, Dijkema HE, van den Hombergh U.** Results of sacral neuromodulation therapy for urinary voiding dysfunction: outcomes of a prospective, worldwide clinical study. *J Urol* 178: 2029–2034, 2007.

30. **Wakabayashi Y, Makiura Y, Tomoyoshi T, Kitahama K, Geffard M, Maeda T.** Adrenergic innervation of the urinary bladder body in the cat with special reference to structure of the detrusor muscle: an immunohistochemical study of noradrenaline and its synthesizing enzymes. *Arch Histol Cytol* 57: 277–289, 1994.

31. **Walker K, Reeve A, Bowes M, Winter J, Wotherspoon G, Davis A, Schmid P, Gasparini F, Kuhn R, Urban L.** mGlu5 receptors and nociceptive function. II. mGlu5 receptors functionally expressed on peripheral sensory neurones mediate inflammatory hyperalgesia. *Neuropharmacol* 40: 10–19, 2001.

32. **Xiao Z, Reese J, Schwen Z, Shen B, Wang J, Roppolo JR, de Groat WC, Tai C.** Role of spinal GABA$_A$ receptors in pudendal inhibition of nociceptive and nonnociceptive bladder reflexes in cats. *Am J Physiol Renal Physiol* 306: F781–F789, 2014.

33. **Yoshiyama M, de Groat WC.** Effects of intrathecal administration of pituitary adenylate cyclase activating polypeptide on lower urinary tract functions in rats with intact or transected spinal cords. *Exp Neurol* 211: 449–55, 2008.

34. **Zhang F, Zhao S, Shen B, Wang J, Nelson DE, Roppolo JR, de Groat WC, Tai C.** Neural pathways involved in sacral neuromodulation of reflex bladder activity in cats. *Am J Physiol Renal Physiol* 304: F710–F717, 2013.