Propranolol, but not naloxone, enhances spinal reflex bladder activity and reduces pudendal inhibition in cats

Marc J. Rogers,1 Zhiying Xiao,1,2 Bing Shen,1 Jicheng Wang,1 Zeyad Schwen,1 James R. Ropper,3 William C. de Groat,3 and Changfeng Tai1,3

1Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania; 2Department of Urology, The Second Hospital, Shandong University, Jinan, Peoples Republic of China; and 3Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

Submitted 29 August 2014; accepted in final form 9 November 2014

OVERACTIVE BLADDER (OAB) IS a significant clinical problem that is estimated to affect more than 30 million adults in the United States (2). OAB is characterized by urinary urgency, frequency, and often incontinence, which can significantly impact the quality of life. Currently, antimuscarinic drugs are first-line therapy for OAB, but these drugs are associated with significant side effects and moderate efficacy causing a mediocre long-term compliance (1, 4). The U.S. Food and Drug Administration recently approved mirabegron (a β3-adrenergic receptor agonist) for the treatment of OAB with reduced side effects (3). In addition to the drug treatments, both pudendal and tibial neuromodulation have also been shown to be effective in OAB treatment (19, 20). However, the mechanisms underlying neuromodulation therapies for OAB are currently not fully understood.

Previous studies in cats have shown that both pudendal nerve stimulation (PNS) and tibial nerve stimulation (TNS) can inhibit bladder overactivity induced by bladder irritation with intravesical acetic acid (14, 15, 23). Opioid receptors play a major role in TNS inhibition of bladder overactivity but have no role in PNS inhibition (15, 23). However, PNS inhibition can involve multiple neurotransmitter mechanisms, including glutamate (14), GABA (25), and serotonin (22). These neurotransmitter mechanisms could occur at the spinal and/or supraspinal sites. Identification of the site of action of different neurotransmitter mechanisms involved in PNS or TNS inhibition of bladder overactivity in animals may provide insights into the mechanisms underlying the clinical use of pudendal or tibial neuromodulation for treatment of OAB.

The purpose of this study is to determine the role of β-adrenergic and opioid receptors in spinal reflex bladder activity in α-chloralose-anesthetized cats. A spinal cord transection (SCT) was performed at the thoracic T9/T10 spinal level to eliminate the nociceptive afferent Aδ-fiber-mediated supraspinal micturition reflex (7, 11). Acute SCT cats were studied instead of chronic SCT cats to investigate the effect of neuromodulation on the spinal bladder reflex that was not changed/reorganized by chronic SCT. Dilute (0.25%) acetic acid was then used to irritate the bladder, activate the nociceptive bladder afferent C-fibers (12), and induce the spinal reflex bladder contractions. PNS or TNS was applied to inhibit these spinal reflex bladder contractions. Propranolol (a β1/β2-adrenergic receptor antagonist with weaker affinity for β3-adrenergic receptors) or naloxone (an opioid receptor antagonist) was administered intravenously to examine the role of these receptors in nociceptive bladder afferent C-fiber-mediated spinal reflex and PNS/TNS inhibition of this spinal reflex.

METHODS

The protocol and animal use in this study were approved by Animal Care and Use Committee at the University of Pittsburgh.

Surgical procedure. Ten cats (5 males and 5 females, 3.0–4.1 kg, Liberty Research, Waverly, NY) were anesthetized with isoflurane (2–3% in oxygen) during surgery and then with α-chloralose (65 mg/kg iv and supplemented as needed) during data collection. A tube was inserted via a tracheotomy to keep the airway patent. A catheter was placed into the right carotid artery to monitor blood pressure. Another catheter was placed into the right cephalic vein to allow for the intravenous injection of drugs or fluids. A pulse oximeter (9847V, NONIN Medical, Plymouth, MN) was placed on the tongue to monitor heart rate and oxygen saturation.

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).
Through an abdominal incision, the ureters were isolated, tied, and cut for external drainage of urine. A double lumen catheter was inserted into the bladder through a small cut at the proximal urethra and secured by a ligature around the urethra. One lumen of the catheter was used to infuse (1–2 ml/min) either saline or dilute (0.25%) acetic acid (AA), while the other lumen was used to record intravesical pressure. The same infusion rate was used in the same animal throughout the entire experiment. A small incision on the medial side of the left ankle exposed the tibial nerve for implanting a bipolar cuff electrode (NC222pt, MicroProbe, Gaithersburg, MD). Another incision was made lateral to the tail in the right sciatic notch to expose the right pudendal nerve for implanting an isovolumetric bladder capacity (%) was normalized to initial saline control capacity prior to SCT in the same animal. The amplitude of maximal bladder contractions was measured before and after a drug treatment to indicate the effect on spinal reflex bladder activity. Measurements were averaged across the animals for the same conditions and reported with standard error of the mean. Statistical significance was determined by a paired t-test or ANOVA followed by Dunnett (one-way) or Bonferroni (two-way) post hoc tests.

RESULTS

Effects of propranolol and naloxone on spinal reflex bladder contractions. During saline CMGs, micturition contractions of large amplitude (>30 cmH2O) and long duration (>20 s) could be induced after filling the bladder to a volume ranging from 9.1 to 18 ml, i.e., the bladder capacity (average 13.7 ± 0.7 ml, n = 10 cats, Fig. 1A). After a complete SCT at T9/T10 level, this large micturition reflex disappeared during saline CMGs, even after the baseline bladder pressure reached more than 40 cmH2O at a much larger bladder volume (157.8 ± 11.8% of the saline control capacity; Fig. 1A). Infusing AA into the bladder, which is known to activate nociceptive afferent C-fibers, induced small-amplitude (<30 cmH2O) and short-dura-

Figure 1. Spinal reflex bladder activity induced by bladder irritation after acute spinal cord transection (SCT) at T9/T10 level. A: cystrometogram (CMG) during saline infusion demonstrates no reflex bladder contractions after acute SCT. B: bladder capacity before and after a drug treatment to indicate the effect on spinal reflex bladder activity. Measurements were averaged across the animals for the same conditions and reported with standard error of the mean. Statistical significance was determined by a paired t-test or ANOVA followed by Dunnett (one-way) or Bonferroni (two-way) post hoc tests.
tion (<20 s) bladder contractions at a significantly \( P < 0.0001 \) smaller bladder volume ranging from 5.3 to 11.3 ml (average 57.1 ± 5.1\% of the saline control capacity, Fig. 1, A and B). These small bladder contractions were not detected during saline infusion after SCT (see the middle trace in Fig. 1A).

The small-amplitude bladder contractions were not only induced during AA infusion but also during isovolumetric

**Fig. 2.** Effects of propranolol or naloxone on spinal reflex bladder activity induced by acetic acid irritation. A–C: propranolol’s effects on contraction amplitude and bladder capacity (\( n = 5 \) cats). D–F: naloxone’s effects on contraction amplitude and bladder capacity (\( n = 5 \) cats). The downward arrow on CMG traces in A and D indicates where the bladder capacity is measured. *Significant \( (P = 0.0019) \) difference (paired Student’s \( t \)-test). Note: Parts A–C and D–F were from different cats.
conditions after stopping the AA infusion at bladder volumes about 20–30% above the AA bladder capacity (Fig. 2, A and D). Under these conditions, propranolol (3 mg/kg iv) significantly \( (P = 0.0019) \) increased the maximal amplitude of the bladder contractions from \( 14.9 \pm 3.3 \) to \( 21.6 \pm 3.7 \text{ cmH}_2\text{O} \) (Fig. 2B). However, it did not change the bladder capacity during CMGs (Fig. 2, A and C). Naloxone (1 mg/kg iv) did not alter either the maximal contraction amplitude or bladder capacity (Fig. 2, D–F). At the end of the experiment during AA-induced isovolumetric contractions, administration of a ganglionic blocking agent (hexamethonium, 10 mg/kg iv) significantly \( (P < 0.05) \) reduced the maximal contraction amplitude (>50%) in both propranolol and naloxone-pretreated cats (Fig. 3), showing that these bladder contractions were mediated by spinal reflex mechanisms since hexamethonium-sensitive peripheral ganglion reflexes have not been detected in the feline bladder.

**Effects of propranolol and naloxone on PNS inhibition of spinal reflex bladder contractions.** Before any drug treatment, PNS alone applied during AA CMGs significantly \( (P < 0.05) \) increased bladder capacity from 58.0 ± 4.7% to 85.8 ± 10.3% and 96.5 ± 10.7% at 2T and 4T intensity, respectively (Fig. 4 A and B). After administering propranolol (3 mg/kg i.v.), PNS inhibition was significantly \( (P < 0.05) \) reduced (Fig. 4, A and B). However, PNS still significantly \( (P < 0.05) \) increased the bladder capacity from 51.8 ± 7.6% of saline control to 64.5 ± 9.5% (2T) and 64.7 ± 7.3% (4T) after propranolol treatment (Fig. 4, A and B). In another group of cats, naloxone (1 mg/kg iv) did not alter the PNS inhibition (Fig. 4C).

TNS did not inhibit spinal reflex bladder contractions. TNS at both 2T and 4T intensity did not significantly inhibit the spinal reflex bladder activity during AA CMGs (Fig. 5, A and B). Propranolol or naloxone treatment did not change the TNS effect on spinal reflex bladder activity (Fig. 5, B and C).

**DISCUSSION**

This study shows that in cats, spinal reflex bladder contractions can be induced after acute SCT by bladder irritation using 0.25% acetic acid (Figs. 1 and 3). PNS can inhibit these spinal reflex contractions (Fig. 4), but TNS has no effect (Fig. 5). Propranolol can significantly increase the amplitude of spinal reflex contractions (Fig. 2, A and B) and greatly reduce PNS inhibition (Fig. 4, A and B), but naloxone has no effect on either the spinal reflex contractions (Fig. 2, D–F) or PNS inhibition of these contractions (Fig. 4C).

It is known that bladder reflexes are initiated by two types of afferent nerves: Aδ-fibers and C-fibers (7, 11). During saline infusion, bladder distention activates the nonnociceptive afferent Aδ-fibers that trigger a spinobulbospinal micturition reflex (8), while the nociceptive afferent C-fibers are silent (12). Therefore, acute SCT at T9/T10 level eliminates the supraspinal reflex bladder contraction during saline distention (Fig. 1A). However, changing bladder infusion from saline to AA induces bladder contractions of small amplitude \( (<30 \text{ cmH}_2\text{O}) \) and short duration \( (<20 \text{ s}) \) (Figs. 1A and 2, A and D). These small bladder contractions are mediated by spinal reflexes because they are sensitive to hexamethonium (a ganglionic blocking agent, Fig. 3) that blocks the efferent pathway from the spinal cord to the bladder. Although propranolol or naloxone was administered in this study before hexamethonium, our previous study showed the same sensitivity to hexamethonium in untreated cats (26). In addition, these small bladder contractions can be largely removed by lidocaine injection into the sacral spinal cord or transection of the sacral spinal roots and spinal cord (26). It is also known that the afferent C-fibers, which are inactive during saline distention, become activated during bladder irritation (12). Previous studies in cats have further demonstrated that the spinal C-fiber reflex can be activated after acute (5, 16) and chronic (7) SCT by electrical stimulation of bladder afferents and recording of efferent activity on the pelvic nerve. Therefore, it would be logical to conclude that in this study, AA irritation triggers a spinal
bladder reflex by activating the nociceptive bladder afferent C-fibers.

Enkephalins and opioid receptors are known to play a role in the control of micturition in animals with an intact spinal cord (21). Naloxone can also enhance the bladder C-fiber reflex evoked by electrical stimulation of bladder afferents in the pelvic nerve (5, 17). However, this study shows that a large dose of naloxone (1 mg/kg iv) that is known to maximally block the opioid receptors in the spinobulbospinal micturition reflex pathway (18, 21) did not alter the bladder reflex activity in this study, indicating that the opioid receptors play no demonstrable role in the afferent C-fiber-mediated spinal bladder reflex induced by AA irritation (Fig. 2, D–F). Our previous studies in spinal intact cats (15, 23) have also shown that naloxone has no effect on bladder overactivity induced by AA irritation. These results indicate that while opioid receptors are

![Image](http://ajpregu.physiology.org/content/early/2014/06/04/ajpregu.00368.2014/F4-46/F4-46.png)

**Fig. 4.** Inhibition of spinal reflex bladder contractions by pudendal nerve stimulation (PNS) is reduced by propranolol, but not by naloxone. A: CMG tracings before and after propranolol administration. The downward arrow on the CMG tracing indicates where the bladder capacity is measured. B: propranolol (3 mg/kg) significantly reduced PNS inhibition (n = 5 cats). C: administration of naloxone (tracings not shown) had no effect on bladder capacity or PNS inhibition (n = 5 cats). *Significant (P < 0.05) difference from control (one-way ANOVA). #Significant (P < 0.05) difference before and after the drug treatment (two-way ANOVA).
involved in the regulation of the bladder C-fiber reflex elicited by electrical stimulation of bladder afferent nerves, their function is very limited in irritation-induced reflex bladder overactivity. In contrast, opioid receptors are very important in the supraspinal micturition reflex pathways mediated by nonnociceptive bladder afferent Aδ-fibers because naloxone intravenously, intracerebroventricularly, or intrathecally significantly enhances the micturition reflex in spinally intact cats (13, 18, 21). In addition, after chronic SCT, spinal reflex bladder activity can also be enhanced by naloxone (24), indicating a significantly different role of opioid receptors in bladder spinal reflexes after acute and chronic SCT.

On the other hand, β1/β2-adrenergic receptors appear to play a role in the modulation of the nociceptive afferent C-fiber-mediated spinal bladder reflex (Fig. 2. A–C). Propranolol increased the amplitude of spinal reflex bladder contractions (Fig. 2, A and B), indicating that tonic activation of β1/β2-adrenergic receptors presumably mediated by sympathetic efferent pathways in the hypogastic nerves or sympathetic chain can suppress the reflex detrusor contractions by acting at the sites in the bladder smooth muscle (6, 9, 10). Meanwhile, propranolol did not change bladder capacity (Fig. 2, A and C), which indicates that β1/β2-adrenergic receptors are not involved in controlling the afferent limb or the spinal pathways underlying the C-fiber-mediated spinal reflex.

Previous studies have shown that TNS can inhibit bladder overactivity induced by AA irritation in spinal intact cats (23). However, in this study, TNS failed to inhibit the AA-induced
reflex bladder activity after acute SCT (Fig. 5). This result indicates that TNS inhibition of the bladder reflex requires intact supraspinal pathways. However, this does not necessarily mean that TNS inhibition only occurs in the brain because the afferent input to the brain activated by TNS could drive a descending spinal pathway to inhibit bladder reflexes in the spinal cord. SCT would eliminate the descending spinal pathway and the TNS inhibition. It is also possible that TNS inhibition only occurs on the supraspinal reflex activated by nonnociceptive Aδ bladder afferent. On the basis of this assumption, TNS inhibition of bladder overactivity induced by AA irritation in spinal intact cats (23) will be mainly due to inhibition of Aδ-fiber-mediated supraspinal reflex instead of the C-fiber-mediated spinal reflex. It seems clear that additional investigations are needed to determine the site of action for TNS inhibition. However, the result from this study supports that afferent input from the tibial nerve cannot directly inhibit the nociceptive afferent C-fiber-mediated spinal reflex bladder activity in the lumbosacral spinal cord. On the other hand, PNS is still able to inhibit the spinal reflex bladder activity after acute SCT (Fig. 4), indicating that significantly different mechanisms underlie PNS and TNS inhibition.

Propranolol significantly reduced PNS inhibition of the spinal reflex bladder contractions (Fig. 4), indicating that pudendal afferent input must drive the sympathetic efferent output in the hypogastric nerves to inhibit the bladder spinal reflex via β-adrenergic transmitter mechanisms. However, after propranolol treatment, PNS can still significantly inhibit spinal reflex bladder activity and increase bladder capacity (Fig. 4, A and B). This residual PNS inhibition could be due to sympathetic inhibition in bladder ganglia or muscle mediated by α2/β3-adrenergic receptors, or it might occur in the spinal cord where the pudendal afferent input can activate a group of inhibitory interneurons that have synaptic connections to the neurons in bladder spinal reflex pathway. These inhibitory spinal interneurons could be GABAergic (25) but not enkephalinergic since naloxone has no effect on PNS inhibition of the spinal reflex bladder activity (Fig. 4C). A previous study in spinal intact cats (15) also showed that opioid receptors play no role in PNS inhibition of bladder overactivity induced by AA irritation. Additional studies are needed to further determine the possible neurotransmitter mechanisms utilized by the spinal inhibitory interneurons during PNS inhibition.

**Perspectives and Significance**

This study in an acute SCT cat model revealed significantly different mechanisms between PNS and TNS inhibition of reflex bladder activity mediated by nociceptive afferent C-fibers. Sympathetic efferent activity and β1/β2-adrenergic receptors play an important role in modulating C-fiber-mediated spinal reflex bladder activity and in PNS inhibition of this spinal reflex activity, while opioid receptors play no role. Understanding neurotransmitter mechanisms underlying PNS or TNS inhibition is important for clinical application of pudendal or tibial neuromodulation in the treatment of bladder disorders.

**GRANTS**

This study is supported by the National Institutes of Diabetes and Digestive and Kidney Diseases under Grants DK-102427, DK-094905, DK-090006, and DK-091253.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


