Differential role of opioid receptors in tibial nerve inhibition of nociceptive and nonnociceptive bladder reflexes in cats

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Tai C, Larson JA, Ogagan PD, Chen G, Shen B, Wang J, Roppolo JR, de Groat WC. Differential role of opioid receptors in tibial nerve inhibition of nociceptive and nonnociceptive bladder reflexes in cats. Am J Physiol Renal Physiol 302: F1090–F1097, 2012. First published January 11, 2012; doi:10.1152/ajprenal.00609.2011.—Naloxone (an opioid receptor antagonist) was used to examine the role of opioid mechanisms in bladder reflexes and in somatic afferent inhibition of these reflexes by tibial nerve stimulation (TNS). Experiments were conducted in α-chloralose-anesthetized cats when the bladder was infused with saline or 0.25% acetic acid (AA). The bladder volume was measured at the first large-amplitude (>30 cmH2O) contraction during a cystometrogram and termed “estimated bladder capacity” (EBC). AA irritated the bladder, induced bladder overactivity, and significantly (P < 0.0001) reduced EBC to 14.3 ± 1.9% of the saline control. TNS (5 Hz, 0.2 ms) at 4 and 8 times the threshold (T) intensity for inducing an observable toe movement suppressed AA-induced bladder overactivity and significantly increased EBC to 41.5 ± 9.9% (4T, P < 0.05) and 46.1 ± 7.9% (8T, P < 0.01) of the saline control. Naloxone (1 mg/kg iv) completely eliminated TNS inhibition of bladder overactivity. Naloxone (0.001–1 mg/kg iv) did not change EBC during AA irritation. However, during saline infusion naloxone (1 mg/kg iv) significantly (P < 0.01) reduced EBC to 66.5 ± 8.1% of the control EBC. During saline infusion, TNS induced an acute increase in EBC and an increase that persisted following the stimulation. Naloxone (1 mg/kg) did not alter either type of inhibition. However, naloxone administered during the poststimulation inhibition decreased EBC. These results indicate that opioid receptors have different roles in modulation of nociceptive and nonnociceptive bladder reflexes and in somatic afferent inhibition of these reflexes, raising the possibility that opioid receptors may be a target for pharmacological treatment of lower urinary tract disorders.

urinary bladder; neuromodulation; neurotransmitter; naloxone

OPIOD RECEPTORS PLAY A ROLE in tonic inhibition of reflex bladder activity of the cat when saline (nonnociceptive) is used to distend the bladder (15, 21). Enkephalins and other opioid receptor agonists administered intravenously, intrathecally, or intracerebroventricularly can inhibit nonnociceptive bladder reflexes (12a, 15, 19), whereas naloxone (an opioid receptor antagonist) blocks these inhibitory responses and can excite the bladder (3, 15, 21, 24). However, whether opioid receptors also play a role in regulating bladder overactivity induced by nociceptive chemical irritation or contribute to the neuromodulation of bladder activity induced by somatic afferent nerve stimulation is still unknown.

Our recent study in cats (5) revealed that opioid peptides are involved in one component of the somatic afferent inhibition of nonnociceptive bladder reflexes induced by stimulation of the pudendal nerve. Whether opioid receptor mechanisms are also involved in somatic afferent inhibition of nociceptive bladder reflexes was not determined. In humans overactive bladder is a symptom complex of urinary urgency, frequency, and incontinence (27). Nociceptive bladder C-fiber afferents play a very important role in bladder overactivity (4, 13). Clinical application of neuromodulation (tibial, pudendal, or sacral spinal nerve root stimulation) to treat lower urinary tract symptoms (16, 20, 26) has also identified an anti-nociceptive effect of neuromodulation. Therefore, it is important for understanding the pathophysiology underlying overactive bladder and the mechanisms of neuromodulation to determine the contribution of opioid peptides, which have a well-known role in controlling pain mechanisms, to somatic afferent inhibition of nociceptive and nonnociceptive bladder reflexes.

This study revealed that opioid peptide mechanisms play a different role in nociceptive and nonnociceptive bladder reflexes and in somatic afferent inhibition of these reflexes in anesthetized cats. Intravesical infusion of dilute acetic acid (AA) was used as the nociceptive stimulus to induce bladder overactivity. Tibial nerve stimulation (TNS) was used to induce somatic inhibition of bladder reflexes (22, 23) and model the clinical use of tibial neuromodulation in treating overactive bladder symptoms (16). We hypothesized that inhibition of irritation-induced bladder overactivity by TNS could be due to activation of opioid receptors at the synapses in the spinal cord or brain. Naloxone was used to block the opioid receptors and test our hypothesis. Understanding the neurotransmitter mechanisms involved in neuromodulation may promote the development of new pharmacological treatments or improve the clinical outcome by combining neuromodulation with pharmacological therapy.

MATERIALS AND METHODS

All protocols used in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh. Experimental setup. Experiments were conducted in a total of 20 cats (11 males, 9 females, 2.6- to 3.7-kg, 6- to 12-mo-old domestic shorthairs, Liberty Research, Waverly, NY) anesthetized initially with α-chloralose (65 mg/kg iv with supplementation as necessary). Heart rate and blood oxygen level were monitored by a pulse oximeter (9847 V, NONIN Medical, Plymouth, MN) with the sensor attached to the tongue. Systemic blood pressure was monitored via a catheter in the carotid artery. Heart rate, blood pressure, and oxygen level were not significantly changed during the experiment. Drug and fluid were admin-
istered via the ulnar vein, and airway access was secured with a tracheostomy tube.

The ureters were isolated via an abdominal incision, cut, and drained externally. The bladder was cannulated through the urethra with a double lumen catheter. One lumen was used to infuse saline (0.9% sodium chloride) or 0.25% AA at a rate of 0.5–2 ml/min, and the other lumen was attached to a pressure transducer to record the bladder pressure. A ligature was tied around the urethra to prevent leakage. The tibial nerve was exposed on the medial side of left hindlimb above the ankle. A tripolar cuff electrode (NC223pt, Micro-Probe, Gaithersburg, MD) was applied around the nerve and connected to a stimulator (S88, Grass Medical Instruments, Quincy, MA). The skin was then closed by suture.

**Stimulation protocol.** Initially, a cystometrogram (CMG) was performed with saline infusion to determine the estimated bladder capacity (EBC) that was defined as the bladder volume threshold to induce a large-amplitude (>30 cmH2O) and long-duration (>20 s) bladder contraction. Then, multiple saline CMGs were repeated to evaluate the reproducibility. Once the EBC was determined during saline infusion, pharmacological studies were performed in three experimental groups. In the first experimental group, 0.25% AA was infused into the bladder during repeated CMGs to activate nociceptive bladder C-fiber afferents and induce an overactive bladder reflex. In the second and third experimental groups, saline was infused into the bladder during repeated CMGs to initiate reflex bladder activity by nonnociceptive bladder afferent Aδ-fibers. The bladder was emptied after each CMG, and a 3- to 5-min rest period was inserted between successive CMGs to allow the distended detrusor to recover. Uniphasic rectangular pulses (5-Hz frequency, 0.2-ms pulse width) were used to stimulate the tibial nerve. The intensity threshold (T) for inducing observable toe movement was determined by gradually increasing the stimulation intensity. Then, multiples (4T, 8T, or 16–20T) of the threshold intensity were used during the experiments.

In the first experimental group ($n = 6$ cats), before administering naloxone (Sigma, St. Louis, MO) the EBC was first determined during AA infusion under the following three conditions: 1) control condition: no stimulation was applied during the CMG, 2) 4T condition: TNS at 4T intensity was applied during the CMG, and 3) 8T condition: TNS at 8T intensity was applied during the CMG. Then, increasing cumulative doses of naloxone (0.001, 0.01, 0.1, and 1 mg/kg iv) were administered to the animal at ∼40- to 60-min intervals between each dose. The last naloxone dosage (1 mg/kg iv) is large enough to maximally facilitate the micturition reflex under nonnociceptive conditions (3). About 5 min after administering each dose of naloxone, three CMGs were performed under the three different conditions (i.e., control, 4T, and 8T) to determine the drug effect on EBC. At the end of the experiment, TNS at a higher intensity (16–20T) was applied during the CMG to confirm that no additional inhibition could be induced by increasing the stimulation intensity. The half-life of naloxone is ∼60 min (2, 7); and our previous studies in cats (3, 24) indicated a constant effect of naloxone on the micturition reflex that persisted for 60 min. To accommodate for naloxone’s relatively short half-life, the last CMG test in this study was always started within 45 min after each dose of naloxone.

In the second experimental group ($n = 7$ cats), ∼5 min after administering naloxone (1 mg/kg iv) the EBC was measured again during a saline CMG. The 5-min interval after naloxone treatment is chosen based on our previous studies (3, 5), which indicated that the facilitatory effect of naloxone on EBC reached a maximum after this time delay. At the end of the CMG without emptying the bladder, TNS at 4T intensity was applied for 30 min, after which TNS was terminated and the bladder was emptied and another CMG was performed without stimulation to determine the poststimulation effect on EBC. Our previous study (23) showed that TNS of 30-min duration can induce a long-lasting (>2 h) poststimulation inhibition of bladder reflex activity that significantly increases EBC during subsequent saline CMGs. This group of experiments was designed to test whether naloxone can block the TNS-induced poststimulation inhibition.

In the third experimental group ($n = 7$ cats), the poststimulation inhibitory effect on EBC induced by 30-min TNS was first determined without administering naloxone. The acute inhibitory effect on EBC induced by applying TNS (4T intensity) during a saline CMG was also determined before naloxone treatment. Then, naloxone (1 mg/kg iv) was administered and ∼5 min later, the EBC was measured during saline infusion under the three conditions: 1) control, 2) 4T TNS, and 3) 8T TNS as studied in the first experimental group.

Our previous studies (22, 23) showed that TNS intensity of 2–4 times T for inducing toe movement is sufficient to inhibit both nociceptive and nonnociceptive bladder reflexes. At this intensity range, the stimulation probably only activated the large afferent nerve fibers rather than the small Aδ- and C-fiber afferents that can induce painful sensations. In the present study, a higher-stimulation intensity of 8–20 times the T for inducing toe movement was also tested to activate the small nociceptive afferent fibers and to examine the possibility that large and small afferent fibers might activate different inhibitory mechanisms.

**Data analysis.** For the repeated CMG recordings, EBCs were measured and normalized in the same animal so that the results from different animals could be compared. The EBC was normalized to the measurement of the first saline control CMG, the first AA control CMG, or the CMG post-30-min TNS depending on the experiment. Repeated measurements under the same conditions in the same animal were averaged. The results from different animals were averaged and reported as means ± SE. Statistical significance ($P < 0.05$) was detected by Student’s $t$-test (2-tailed) or ANOVA followed by Bonferroni posttests using Prism (GraphPad Software, La Jolla, CA).

**RESULTS**

Inhibitory effect of TNS on the nociceptive bladder reflex induced by AA irritation. Bladder infusion with 0.25% AA irritated the bladder, induced bladder overactivity, and significantly ($P < 0.0001$) reduced EBC to $14.3 ± 1.9%$ (1.9 ± 0.5 ml, $n = 6$) of the control EBC ($13.0 ± 2.3$ ml, $n = 6$) measured during saline CMG (Fig. 1). TNS at the intensity of 4T or 8T suppressed the bladder overactivity caused by activation of the nociceptive bladder C-fiber afferents (Fig. 1A) and significantly increased the EBC to $41.5 ± 9.9%$ ($P < 0.05$, $n = 6$) and $46.1 ± 7.9%$ ($P < 0.01$, $n = 6$), respectively, of the control EBC (Fig. 1B).

Dose-dependent effect of naloxone on TNS inhibition of the nociceptive bladder reflex. The effect of naloxone on TNS inhibition of bladder overactivity is dependent on the drug dosage (Figs. 2 and 3). Administering cumulative doses of naloxone (0.001, 0.01, 0.1, and 1 mg/kg iv) did not change the EBC in the absence of stimulation (Figs. 2A and 3), but progressively reduced the inhibitory effect of TNS (Figs. 2, B–C, and 3). At both low (4T)- and high (8T)-stimulation intensities, the significant increase in EBC induced by TNS was lost when naloxone dosage was 0.01 mg/kg or larger (Fig. 3) and the highest dose (1 mg/kg) of naloxone completely blocked the TNS inhibition (Figs. 2 and 3). After 1 mg/kg naloxone, increasing stimulation intensity to 16–20T also failed to induce any inhibition (Fig. 4, $n = 4$ cats).

Effect of naloxone on the nonnociceptive bladder reflex and on TNS-induced poststimulation inhibition. As reported in our previous study (23), TNS applied continuously for 30 min at 4T intensity during distension of the bladder with saline inhibited reflex bladder activity during the stimulation and also
induced a poststimulation inhibition that significantly \((P < 0.05)\) increased EBC to 131.3 ± 8.8% \((n = 7)\) of the control EBC (Fig. 5). The effect of pretreatment with a high dose of naloxone \((1 \text{ mg/kg iv})\) on this poststimulation inhibition was tested in seven cats. Administration of this dose of naloxone during repeated saline infusion CMGs significantly \((P < 0.01)\) reduced the EBC to 66.5 ± 8.1% \((n = 7)\) of the control EBC (Fig. 6). TNS at 4T intensity applied for 30 min after the naloxone treatment still induced a poststimulation inhibitory effect that significantly \((P < 0.01)\) increased the EBC by ~45% (Fig. 6). The EBC after combined naloxone and TNS inhibition was 94.6 ± 10.6% \((n = 7)\) of the control EBC measured before naloxone (Fig. 6), indicating that TNS inhibition reversed the facilitatory effect of naloxone but did not increase EBC above control levels as it did in the absence of naloxone (Fig. 5).

In another group of animals, the sequence of treatments was reversed. After eliciting the TNS poststimulation inhibition by 30 min of stimulation and performing several CMGs, the single high dose \((1 \text{ mg/kg iv})\) of naloxone was administered and significantly \((P < 0.01)\) reduced EBC to 62.8 ± 17.2% \((n = 7)\) of the control EBC measured before any treatment (Fig. 5). This reduced EBC was similar to the reduced EBC induced by naloxone in control preparations before TNS \((66.5 ± 8.1\% , n = 7; \text{Fig. 6B})\).

**Effect of naloxone on TNS-induced acute inhibition of the nonnociceptive bladder reflex.** After applying TNS for 30 min at 4T intensity which elicited a persistent increase in EBC as reported in our previous study (23) (also see Fig. 5), TNS \((4T \text{ intensity})\) was applied again during saline CMGs to induce an acute inhibitory effect. This stimulation elicited an additional reversible increase in EBC \((26.8 ± 4.9\% , P < 0.001, n = 7, \text{see the first 2 traces in Fig. 7A and the first 2 columns in Fig. 7B})\). A single dose \((1 \text{ mg/kg iv})\) of naloxone reduced by ~63% the persistent \((i.e., \text{poststimulation})\) increase in EBC and blunted but did not abolish the acute reversible inhibition induced by TNS (Fig. 7A), which significantly increased \((P < 0.0005)\) EBC from 47.5 ± 12.7% \((n = 7)\) of the control EBC measured after 30 min of TNS to 74.2 ± 12% \((n = 7)\) of the control (Fig. 7B). This percentage change is approximately the same as that induced by TNS before the administration of naloxone (Fig. 7B). TNS at a higher intensity \((8T)\) elicited a larger increase in EBC to 107.7 ± 20.2% \((P < 0.05, n = 7)\) of control (Fig. 7B).

**DISCUSSION**

This study revealed that intravenous administration of naloxone, an opioid receptor antagonist, has different effects on bladder reflexes and on the inhibition of these reflexes by TNS under different experimental conditions. When tested on normal, nonnociceptive bladder reflexes elicited by filling the bladder with saline, naloxone did not alter acute TNS inhibition but reduced EBC, indicating that the micturition reflex pathway was inhibited by tonic activation of opioid receptors presumably by endogenous enkephalins acting in the central nervous system. On the other hand, following irritation of the
bladder with AA, naloxone suppressed acute TNS inhibition but did not alter EBC, indicating that the tonic enkephalinergic inhibition was inactive during AA-induced bladder overactivity, but was activated by TNS. These data are consistent with the view that different peripheral and central neural pathways mediate nonnociceptive and nociceptive reflex bladder activity (see Fig. 8). Saline distention of bladder primarily activates nonnociceptive, mechanosensitive, Aβ bladder afferents that trigger a spinobulbospi nal bladder reflex transmitted through the spinal cord to synapses in the periaqueductal gray (PAG) and the pontine micturition center (PMC; Fig. 8) (13). On the other hand, AA irritation of the bladder activates nociceptive, C-fiber bladder afferents that facilitate the supraspinal bladder reflex and/or activate a spinal bladder reflex (Fig. 8) (4, 5, 10, 11, 13). Previous studies using saline distension of the bladder (12a, 15, 19) indicated that enkephalinergic mechanisms in the brain regulate bladder capacity, whereas those in the spinal cord control the amplitude of bladder contractions (Fig. 8). Our current study further indicates that enkephalinergic mechanisms are not involved in the tonic control of the C-fiber-mediated spinal reflex but are involved in the inhibitory modulation of this reflex induced by TNS (Fig. 8).

Enkephalinergic inhibitory control of the micturition reflexes by TNS could occur at several sites in the spinal cord as well as in the brain PAG/PMC complex (Fig. 8). In the spinal cord, activation of enkephalinergic inhibitory interneurons (#1 in Fig. 8) by tibial nerve afferent input could inhibit I) spinal tract neurons (#2 in Fig. 8) that form the ascending limb of the
Aơ afferent-evoked spinobulbospinal micturition pathway, 2) excitatory spinal interneurons (#3 in Fig. 8) that are part of the C-fiber-evoked spinal micturition reflex pathway, or 3) the bladder preganglionic neurons (#4 in Fig. 8). Because an enkephalinergic inhibitory action on the preganglionic neurons (#4) would suppress both nonnociceptive and nociceptive reflexes and because only the TNS inhibition of the nociceptive reflex that is not tonic regulatory enkephalins (Fig. 8). In the cat mechano-insensitive C-fiber bladder afferents can be activated by nocuous stimuli (13) and can as mentioned above eliciting a micturition reflex organized in the spinal cord (10). This spinal C-fiber afferent-evoked micturition reflex is enhanced by naltrexone in acute (10) and chronic spinal cord-transected animals (24, 25) but seems insensitive to naltrexone in spinal cord-intact animals that were used in the present experiments. Thus, the emergence of tonic enkephalinergic inhibitory control of the micturition. This regulation could occur within the PAG/PMC switching circuit in the brain stem or by modulating the afferent input to that circuit by inhibition of sensory pathways in the spinal cord. In the cat this excitatory effect of naltrexone occurs after intravenous as well as intracerebral injections but not intrathecal injection (3, 15), suggesting that endogenous opioid peptides tonically regulate the spinobulbospinal micturition switching circuit at the level of the PAG/PMC (Fig. 8) but not at the level of spinal interneurons (site #2) on the ascending limb of the micturition pathway.

On the other hand, intravenous naltrexone did not alter the EBC of the overactive bladder irritated by AA (Figs. 2–3). This lack of effect might be due to maximal activation of the reflex pathway after AA irritation thereby negating any further stimulating effect of naltrexone or it may be related to the emergence of the spinal micturition reflex pathway after AA irritation that is not tonically regulated by enkephalins (Fig. 8). In the cat mechano-insensitive C-fiber bladder afferents can be activated by nocuous stimuli (13) and can as mentioned above eliciting a micturition reflex organized in the spinal cord (10). This spinal C-fiber afferent-evoked micturition reflex is enhanced by naltrexone in acute (10) and chronic spinal cord-transected animals (24, 25) but seems insensitive to naltrexone in spinal cord-intact animals that were used in the present experiments. Thus, the emergence of tonic enkephalinergic inhibitory control of the micturition.

The effect of naltrexone to reduce EBC during saline CMGs clearly indicates that enkephalins act as inhibitory transmitters to regulate the bladder volume threshold for triggering micturition. This regulation could occur within the PAG/PMC switching circuit in the brain stem or by modulating the afferent input to that circuit by inhibition of sensory pathways in the spinal cord. In the cat this excitatory effect of naltrexone occurs after intravenous as well as intracerebral injections but not intrathecal injection (3, 15), suggesting that endogenous opioid peptides tonically regulate the spinobulbospinal micturition switching circuit at the level of the PAG/PMC (Fig. 8) but not at the level of spinal interneurons (site #2) on the ascending limb of the micturition pathway.

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The contribution of enkephalins to TNS-evoked inhibition of reflex bladder activity clearly depends on the experimental conditions. The acute increase in EBC elicited during TNS in AA-irritated bladders was completely abolished by naloxone (Figs. 2–4), while this type of acute inhibition was not affected by naloxone in nonirritated bladders infused with saline (Fig. 7). These observations suggest that TNS produces an inhibition of reflex bladder activity by at least two different mechanisms: 1) enkephalinergic and 2) nonenkephalinergic, the former occurring when the bladder is irritated and the latter when it is not irritated. The existence of these two distinct inhibitory mechanisms raises the possibility as mentioned above that these mechanisms target separate pathways mediating nociceptive and nonnociceptive bladder reflexes. Our previous study (5) also identified a component of pudendal nerve inhibition of the nonnociceptive bladder reflex in the cat that is naloxone sensitive, whereas another group reported that this inhibition was completely naloxone resistant, while in the same study recurrent inhibition of bladder reflexes mediated by preganglionic neuron axon collaterals was naloxone sensitive (17). Thus, our finding that naloxone completely blocked TNS inhibition of the nociceptive bladder reflex was unexpected. It is clear that acute inhibition of reflex bladder activity by stimulation of somatic afferent nerves is complex and may involve multiple neurotransmitter mechanisms depending on the experimental conditions.

The role of enkephalins in the TNS-induced poststimulation inhibition of the nonnociceptive bladder reflex was evaluated in two different types of experiments. In one experiment, treatment with a large dose (1 mg/kg) of naloxone before 30 min of TNS did not prevent the 30–40% poststimulation increase in EBC (Fig. 6), indicating that enkephalins and activation of opioid receptors are not essential for eliciting the persistent modulatory effect. This dose of naloxone also reduced EBC by 34% (to 66.5% of control; Fig. 6) demonstrating that it was sufficient to block the opioid receptors involved in the tonic inhibition of the supraspinal micturition reflex. The...
inhibitory effect of TNS was superimposed on the facilitatory effect of naloxone and increased EBC back to control levels but did not increase EBC above the control before naloxone treatment. This latter change might reflect a blunting of the TNS inhibition due to the ongoing naloxone facilitatory effect or a selective block of the inhibition. In the second type of experiment, naloxone was administered after inducing the poststimulation inhibition in an attempt to reverse the inhibition (Fig. 5). In this experiment, naloxone produced a greater decrease in EBC than in the first set of experiments (68 vs. 34%) and reduced capacity to the same absolute level (62.8% of the control capacity) as it did in the first experiments when administered before TNS (Fig. 6B). This observation raises the possibility that naloxone blocked not only the tonic enkephalinergic inhibition but also reversed the poststimulation inhibition elicited by TNS. Unfortunately, these experiments are difficult to interpret and have not yielded a clear answer regarding the role of enkephalins in TNS-induced poststimulation inhibition.

The facilitatory effect of naloxone on reflex bladder activity caused a problem in accurately measuring bladder capacity. Administration of large doses of naloxone (0.1–1 mg/kg) in AA-treated animals changed the characteristics of bladder activity during a CMG to short-lasting (<20 s) spike-like contractions (Fig. 2, lower 2 rows of traces) and eliminated the obvious, long-duration micturition contractions occurring before the treatment or at lower doses of naloxone (Fig. 2, upper 3 rows of traces). After low doses of naloxone (0.001–0.01 mg/kg), TNS inhibited the spike-like contractions that occurred during bladder filling, indicating that they were mediated by central reflex mechanisms. In these recordings, a micturition contraction was easily identified as the first large-amplitude contraction after a quiescent filling period (Fig. 2, B or C, middle row). Larger doses of naloxone that suppressed this inhibitory effect eliminated the abrupt onset of a micturition contraction and generated contractions that gradually increased in amplitude during bladder filling (Fig. 2, bottom 2 rows of traces). It was more difficult to precisely distinguish a micturition reflex and estimate bladder capacity after large doses of naloxone. Under these conditions, bladder contraction amplitude >30 cmH2O was simply used to detect the micturition contraction. Although this could influence the accuracy of our capacity measurements, TNS was clearly ineffective after the higher doses (0.1–1 mg/kg) of naloxone in altering reflex contractile activity (see Figs. 2–3), indicating that opioid receptors are involved in TNS inhibition under AA irritation conditions.

Some information about the properties of the afferent limb of the TNS-evoked inhibitory reflex has been obtained by examining stimulus intensity-response relationships. A higher TNS intensity (8T vs. 4T) did not further enhance the inhibition of the nociceptive bladder reflex (Fig. 1) and only slightly and not significantly improved the inhibition of nonnociceptive bladder reflex (Fig. 7). It also did not alter the dose-dependent naloxone antagonism of the inhibition (Fig. 3). Further increasing the intensity to 16–20T did not generate any additional inhibition once the effect induced by the 4–8T intensity was fully antagonized (Fig. 4). These results indicate that the inhibitory effect is mainly induced by activating the large rather than the small afferent fibers in the tibial nerve.

The differential role of opioid receptors under nociceptive and nonnociceptive conditions revealed in this study suggests a more general concept regarding the role of opioid mechanisms in the control of the lower urinary tract. It is clear that opioid receptors are activated tonically under normal (i.e., nonnociceptive) physiological conditions (Fig. 6) to suppress the spinobulbospinal bladder reflex and maintain a normal bladder storage function (Fig. 8). Therefore, somatic afferent inhibition of normal bladder activity by TNS has to utilize nonopioid mechanisms (Figs. 6 and 7). Under pathological conditions such as bladder irritation, the tonic opioid mechanism does not regulate the nociceptive C-fiber afferent-mediated spinal reflex (Figs. 2 and 3) so that bladder overactivity can be initiated as a defense mechanism to eliminate potentially harmful substances or infectious agents from the bladder. Therefore, opioid inhibitory mechanisms can be recruited by TNS to induce an anti-nociceptive effect (Figs. 2–4) and correct the abnormal bladder activity under pathological conditions.

Understanding the neurotransmitter mechanisms involved in tibial neuromodulation could identify pharmacological targets for development of new therapies to treat patients suffering from overactive bladder symptoms (1). Furthermore, neuromodulation therapies currently used in clinical applications could also be improved to achieve better clinical outcomes when combined with pharmacological treatments that enhance synaptic transmission in the inhibitory reflex pathways. This study indicates that the opioid receptor system might be a useful target to achieve better clinical outcomes in the treatment of overactive bladder symptoms.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

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