Role of $\mu$, $\kappa$, and $\delta$ Opioid Receptors in Tibial Inhibition of Bladder Overactivity in Cats

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Abbreviations: AA, acetic acid; CMG, cystometrogram; FDA, Food and Drug Administration; OAB, overactive bladder; OR, opioid receptor; PAG, periaqueductal gray; PMC, pontine micturition center; TNS, tibial nerve stimulation; T, threshold

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ABSTRACT

In α-chloralose anesthetized cats we examined the role of opioid receptor (OR) subtypes (μ, κ, and δ) in tibial nerve stimulation (TNS) induced inhibition of bladder overactivity elicited by intravesical infusion of 0.25% acetic acid (AA). The sensitivity of TNS inhibition to cumulative intravenous doses of selective OR antagonists (cyprodime for μ, nor-binaltorphimine for κ, or naltrindole for δ ORs) was tested. Naloxone (1 mg/kg, i.v., an antagonist for μ, κ, and δ ORs) was administered at the end of each experiment. AA caused bladder overactivity and significantly (P < 0.01) reduced bladder capacity to 21.1±2.6% of the saline control. TNS at 2 or 4 times threshold (T) intensity for inducing toe movement significantly (P < 0.01) restored bladder capacity to 52.9±3.6% or 57.4±4.6% of control, respectively. Cyprodime (0.3-1.0 mg/kg) completely removed TNS inhibition without changing AA control capacity. Nor-binaltorphimine (3-10 mg/kg) also completely reversed TNS inhibition and significantly (P < 0.05) increased AA control capacity. Naltrindole (1-10 mg/kg) reduced (P<0.05) TNS inhibition but significantly (P < 0.05) increased AA control capacity. Naloxone (1 mg/kg) had no effect in cyprodime pre-treated cats, but reversed the nor-binaltorphimine induced increase in bladder capacity and eliminated the TNS inhibition remaining in naltrindole pre-treated cats. These results indicate a major role of μ and κ ORs in TNS inhibition while δ ORs play a minor role. Meanwhile, κ and δ ORs also have an excitatory role in irritation-induced bladder overactivity.
INTRODUCTION

Overactive bladder (OAB) is a syndrome characterized by urinary urgency usually accompanied by frequency with/without incontinence, which affects about 16-27% of men and 33-43% of women in the United States (Abrams et al., 2003; Coyne et al., 2011). OAB has a significant impact on quality of life (Coyne et al., 2008). Medications such as anticholinergic drugs are often unsatisfactory for OAB treatment due to their limited efficacy and/or undesirable side effects (Andersson and Pehrson, 2003; Andersson and Wein, 2004; Chapple et al., 2008). Therefore, tibial neuromodulation therapy which is currently approved by the US Food and Drug Administration (FDA) for OAB treatment becomes an attractive option for drug-refractory patients (Peters et al., 2009). However, the mechanisms underlying tibial neuromodulation therapy are not fully understood.

Our previous study (Tai et al., 2012) in cats revealed that intravenous administration of naloxone (an opioid receptor antagonist) completely reverses the inhibition of bladder overactivity elicited by tibial nerve stimulation (TNS) indicating that opioid receptors (ORs) play a major role in the inhibition. However, it is not known which of the three OR subtypes (μ, κ, and δ) (Wollemann, 1990) are involved. It is also known that TNS inhibition can be greatly enhanced (Zhang et al., 2012) when combined with a low i.v. dose of tramadol, an OR agonist (Pandita et al., 2003), raising the possibility that combinations of opioid drugs with TNS might be useful clinically to enhance the efficacy of TNS therapy. However, opioid drugs such as tramadol can produce significant adverse effects (Safarinejad and Hosseini, 2006). Therefore, more detailed information about the types of OR involved in TNS inhibition might lead to
selective targeting of one subtype of OR and lead to the development of more effective combinations of TNS and opioid drugs with fewer adverse effects.

This study in cats was undertaken to determine which subtypes of ORs are involved in TNS inhibition of bladder overactivity induced by 0.25% acetic acid (AA) irritation. Three selective OR antagonists, cyprodime (μ), nor-binaltorphimine (κ), and naltrindole (δ), were administered intravenously in different groups of cats to determine the role of each subtype receptor in TNS inhibition. Our results may provide insights into neurotransmitter mechanisms contributing to the clinical efficacy of tibial neuromodulation and may promote the development of new treatments for OAB that combine tibial neuromodulation and drug therapy.

METHOD

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

Experimental Setup

A total of 22 cats (14 female and 8 male, 3.0-4.2 kg, Liberty Research Inc., Waverly, NY, USA) were used in this study. The animals were anesthetized by isoflurane (2-5% in oxygen) during surgery and maintained with α-chloralose anesthesia (65 mg/kg i.v. with supplementation as needed) during data collection. A pulse oximeter (9847V, NONIN Medical, Inc., Plymouth, MN, USA) was attached on the tongue to monitor the heart rate and blood oxygen level. A tracheotomy was performed and a tube was inserted to maintain an open airway. A catheter was
inserted into right carotid artery to monitor systemic blood pressure. Another catheter was inserted into the left cephalic vein for saline and drug administration. Through an abdominal incision, the ureters were isolated, cut and drained externally. A double-lumen catheter was inserted into the bladder via a small cut in the proximal urethra. One lumen of the catheter was connected to a pump to slowly (1-2 ml/min) infuse saline or 0.25% AA into the bladder and the other lumen was connected with a pressure transducer to measure intravesical pressure. The tibial nerve was exposed in the left leg above the ankle and a tripolar cuff electrode (NC223pt, MicroProbe, Gaithersburg, MD, USA) was implanted for stimulation. All incisions were closed by sutures at the end of surgery.

Stimulation Protocol and Drug Administration

Uniphasic rectangular pulses (5-Hz frequency, 0.2-ms pulse width) were used to stimulate the tibial nerve via the cuff electrode. The intensity threshold (T) for inducing observable toe movement was determined by gradually increasing the stimulation intensity. Based on our previous studies (Matsuta et al, 2013; Tai et al, 2012), intensities of 2T or 4T were used in this study to suppress the bladder overactivity induced by 0.25% AA irritation.

At the beginning of each experiment, multiple cystometrograms (CMGs) were performed with saline infusion to determine the bladder capacity that was defined as the bladder volume threshold to induce a bladder contraction of large amplitude (>30 cmH₂O) and long duration (>20 seconds). Once stable bladder capacity was obtained, 0.25% AA was infused into the bladder to irritate nociceptive C-fiber bladder afferents and induce an overactive bladder reflex. Repeated
CMGs were performed with AA infusion until the bladder capacity stabilized, followed by an additional four AA CMGs: (1) control CMG without TNS, (2) CMG during 2T TNS, (3) CMG during 4T TNS, (4) control CMG without TNS. Then, the animals were divided into three groups for pharmacological studies.

In the first group (N=6 cats), cumulative doses (0.003, 0.01, 0.03, 0.1, 0.3 and 1 mg/kg) of cyprodime (a selective µ OR antagonist, Tocris Bioscience, UK) were administered intravenously. Ten minutes after administering each dose, four AA CMGs were performed: (1) control CMG without TNS, (2) CMG during 2T TNS, (3) CMG during 4T TNS, (4) control CMG without TNS. A 5-minute rest period was inserted between the CMGs to allow the bladder to recover from the previous reflex. The same protocol was also used in the second group of cats (N=6 cats) in which nor-binaltorphimine (a selective κ OR antagonist, Tocris Bioscience, UK) was administered in cumulative doses (0.03, 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg, i.v.) and in the third group (N=10 cats) in which naltrindole (a selective δ OR antagonist, Tocris Bioscience, UK) was administered in cumulative doses (0.03, 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg, i.v.). At the end of each experiment, naloxone (1 mg/kg, i.v.) was administered and then followed by the four repeated CMGs (control, 2T, 4T and control). Time control experiments in our previous study (Schwen et al., 2013) in which vehicle (saline) was injected using a similar drug testing protocol and experimental duration showed that the bladder capacity was not changed during repeated vehicle control CMGs.
Data analysis

Bladder capacity was measured during each CMG and normalized to the saline control CMG in each experiment so that the results from different animals could be compared. Repeated measurements in the same animal under the same experimental conditions were averaged. The results from different animals are reported as mean ± SE. Statistical significance (P<0.05) was detected by a paired t-test or repeated-measures ANOVA followed by Dunnett (one-way) or Bonferroni (two-way) multiple comparison. Two-way ANOVA was performed between TNS and control groups for different drug dosages (Fig.3, Fig.5, and Fig.7). One-way ANOVA was performed in untreated cats for different CMG conditions (saline, AA, 2T, 4T, see Fig.1), or in drug-treated cats for different drug dosages at each CMG condition (2T TNS, 4T TNS, or AA control, see Fig.3, Fig.5, and Fig.7).

RESULT

TNS Inhibition of Bladder Overactivity

The bladder irritated by 0.25% AA became overactive and exhibited significantly (P < 0.01) reduced capacity to 21.1±2.6% of the saline control capacity (10.0±1.0 ml) required to elicit a micturition reflex (see Fig.1). TNS at 2T or 4T intensity suppressed the bladder overactivity and significantly (P < 0.01) increased bladder capacity to 52.9±3.6% or 57.4±4.6%, respectively, of the saline control capacity. TNS did not significantly suppress the amplitude of reflex bladder contractions. Thus, in this paper TNS inhibition represents only the increase in bladder capacity. After TNS the bladder capacity returned to pre-stimulation volume, indicating that there was no
post-stimulation inhibition (Fig.1).

**Effects of Selective OR Antagonists on Bladder Overactivity and TNS Inhibition**

Selectively blocking μ ORs by increasing doses of cyprodime did not significantly change the control bladder capacity during repeated AA CMGs (Fig.2A and Fig.3); but significantly (P < 0.05) reduced TNS inhibition starting from the 0.1 mg/kg dose and completely eliminated the inhibition induced by both 2T and 4T TNS at doses of 0.3-1 mg/kg (Fig.3). Nor-binaltorphimine (a selective κ OR antagonist) also significantly (P < 0.05) reduced TNS inhibition starting from 1 mg/kg dose and completely eliminated the inhibition induced by both 2T and 4T TNS at doses of 3-10 mg/kg (Figs.4-5). However, different from cyprodime, nor-binaltorphimine (1-10 mg/kg, significantly (P < 0.05) increased the control bladder capacity during repeated AA CMGs (Fig.4A and Fig.5). Naltrindole (a selective δ OR antagonist) significantly (P < 0.05) increased AA control capacity (Fig.6A and Fig.7) and significantly (P < 0.05) reduced (about 50%) but did not completely block TNS inhibition at doses of 1-10 mg/kg (Fig.7).

**Effect of Naloxone after Blocking a Subtype of OR**

At the end of the experiments in cyprodime pre-treated cats, naloxone (1 mg/kg, i.v.) did not alter bladder capacity prior to or during TNS (Fig.8A). However, in nor-binaltorphimine pre-treated cats naloxone significantly (P < 0.05) reduced bladder capacity and reversed the nor-binaltorphimine induced increase in bladder capacity (Fig.5 and Fig.8B). In naltrindole pre-treated cats, naloxone did not change the control bladder capacity but eliminated the
remaining TNS inhibition (Fig. 8C).

**DISCUSSION**

This study in cats indicates that µ, κ, and δ OR subtypes have different roles in the neural mechanisms controlling AA-induced bladder overactivity and in the mechanisms of TNS inhibition of bladder overactivity. Activation of µ and κ ORs is essential for producing TNS inhibition (Figs. 2-3 and Figs. 4-5, respectively), but δ ORs play only a minor role (Figs. 6-7). Both κ and δ ORs must have a tonic excitatory influence on AA irritation-induced bladder overactivity because blocking these receptors increases bladder capacity (Figs. 4-7). On the other hand, blocking µ ORs did not change bladder capacity indicating that these ORs do not have a tonic modulatory influence on this type of overactivity (Figs. 2-3). These results suggest that the neural mechanisms contributing to bladder overactivity and modulation of those mechanisms by TNS depends on a complex interaction between multiple endogenous opioid transmitters with multiple ORs.

The analyses of the inhibitory actions of TNS on the micturition reflex are complicated by the fact that endogenous opioid peptides that have a role in the TNS inhibition of the micturition reflex also have a role in the tonic control of the reflex. Thus as shown in Figs. 4-7, antagonists for κ and δ ORs at doses that influence TNS modulation of bladder capacity also change baseline bladder capacity prior to TNS. Drugs that alter baseline capacity could potentially alter the TNS effect indirectly by changing the level of activity in the bladder reflex pathway in addition to directly changing neurotransmission in the TNS inhibitory pathway. For example,
nor-binaltorphimine, the κ OR antagonist, prominently increased baseline bladder capacity at doses that reduced TNS inhibition. The increased capacity induced by the drug presumably reflects a decreased excitability at some sites in the micturition reflex pathway leading to an increase in the set point for initiating the reflex. This change might: (1) increase sensitivity to TNS inhibition or (2) occlude the TNS inhibitory response if the drug and TNS target the same synapse on the micturition reflex pathway. Naltrindole, the δ OR antagonist, produced similar changes albeit of smaller magnitude. On the other hand, cyprodime, the μ OR antagonist, which markedly reduced TNS inhibition, slightly but not significantly reduced bladder capacity suggesting that it reduced tonic inhibition of the reflex mediated by μ OR and increased reflex excitability. Cyprodime also changed the pattern of bladder contractions during filling to shorter duration contractions especially when compared to the long duration contractions that occurred at the end of the CMGs during TNS inhibition (Figs. 2B-C). These changes could also influence indirectly the magnitude of TNS inhibition. Although it is impossible to assess the potential impact of these indirect influences on the results, it is still clear that activation of ORs is the major mechanism underlying TNS inhibition of bladder overactivity and that there is a considerable difference in the relative contribution of different OR subtypes to the inhibition.

The prominent effects of cyprodime on the TNS-induced increase in bladder capacity indicate that activation of μ ORs is essential for eliciting the inhibition. The effect of cyprodime is similar to the effect of naloxone (Tai et al., 2012) which exhibits a 7-fold selectivity for μ ORs over κ ORs and 12-fold selectivity for μ ORs over δ ORs (Goodman et al., 2007; Schmidhammer et al., 1989). Cyprodime has a much higher μ receptor selectivity than naloxone (μ/κ=28,
µ/δ=110) (Schmidhammer et al., 1989) but like naloxone has a similar low nM affinity for binding to ORs in rat brain homogenates (Márki et al., 1999). The dose response curve of cyprodime showing suppression of TNS inhibition (Fig.3) is very similar to that of naloxone (0.001-1 mg/kg) reported in our previous study (Tai et al., 2012). In addition, both agents dose-dependently reduced TNS inhibition without markedly changing AA control capacity. This similarity indicates that the removal of TNS inhibition by naloxone (1 mg/kg) in our previous study (Tai et al., 2012) might be due primarily to the block of µ ORs instead of κ and/or δ ORs. The failure of naloxone administered after cyprodime to change either bladder capacity or the response to TNS (Fig.8A), is consistent with the view that naloxone acts primarily by blocking µ ORs.

In contrast to the small effect of naloxone or cyprodime on bladder capacity in AA irritated bladders, naloxone elicits a marked reduction in capacity in normal bladders infused with saline (Booth et al., 1985; Roppolo et al., 1983; Tai et al., 2012). Assuming that this effect is also due to block of tonic inhibition mediated by µ ORs, it is tempting to speculate that activation of bladder nociceptive afferents with AA down-regulates the tonic µ OR inhibition (Fig.9) or activates an alternative reflex pathway that is insensitive to the inhibition. However, the present results show that the µ OR inhibition, while not tonically active, can still be activated by TNS in AA irritated bladders.

Our recent study indicates that naloxone-sensitive TNS inhibition occurs at the level of the brain stem (Ferroni et al., 2015) (Fig.9), which can be mimicked by application of µ OR agonists (fentanyl or morphine) injected intracerebroventricularly or into the pontine micturition center of
cats (Hisamitsu and de Groat, 1984; Noto et al., 1991) and rats (Dray and Metsch, 1984a,b). Considerably higher doses of morphine and naloxone are required to modulate the micturition reflex when administered intrathecally as compared to intracerebroventricular administration consistent with the view that µ OR inhibitory mechanisms in the micturition reflex pathway are more prominent in supraspinal than spinal circuitry.

Blocking κ ORs by the highly selective nor-binaltorphimine (κ/µ=170, κ/δ =150) (Takemori et al., 1988) increased AA control capacity at the doses effective in reducing TNS inhibition (Fig.5), suggesting that κ ORs act in concert with µ ORs to induce TNS inhibition (Fig.9), but unlike µ ORs they also have a facilitatory role in the tonic control of bladder capacity. It is well known that µ and κ ORs can act in concert to suppress pain (Miaskowski et al., 1992). Naloxone administered at the end of the experiments after nor-binaltorphimine prominently reduced bladder capacity (Fig. 8B) suggesting that: (1) in AA irritated bladders tonic activation of κ ORs suppresses tonic µ OR mediated inhibition of the micturition reflex and (2) block of κ ORs removes the suppression and unmasks tonic µ OR inhibition leading to an increase in bladder capacity (Fig.9). The subsequent administration of naloxone eliminates the unmasked tonic µ OR inhibition and reduces bladder capacity. These observations indicate that the doses of naloxone used in the present and previous study (Tai et al., 2012) act by blocking µ OR rather than κ ORs. This proposed inhibitory interaction between κ and µ ORs is consistent with other reports that activation of κ ORs can antagonize many physiological actions produced by activation of µ ORs (Pan, 1998). This type of interaction between κ and µ ORs is also observed in the micturition reflex pathway in rats (Sheldon et al., 1987, 1988, 1989), where i.c.v. or i.t. injection of a κ OR
agonist antagonized the inhibitory effect on bladder activity induced by i.c.v. or i.t. injection of a μ OR agonist. It is interesting to observe in our study that μ and κ ORs act synergistically to produce TNS inhibition and that activation of both receptors is necessary to produce inhibition of the micturition reflex pathway. On the other hand, in regard to tonic control of the micturition reflex pathway it appears that the two receptors have opposing effects and that activation of κ ORs tonically suppresses μ OR inhibition of the reflex pathway (Fig.9).

Naltrindole, the selective δ OR antagonist (δ/κ=80, δ/μ=300) (Spetea et al., 1998) had only a minimal effect on TNS inhibition at the largest dose (Fig.7). Subsequent administration of naloxone (1 mg/kg) completely eliminated the remaining TNS inhibition without changing bladder capacity (Fig.8C), similar to the cyprodime effect (Fig.3) but different from the nor-binaltorphimine effect (Fig.5) further indicating that the 1 mg/kg dose of naloxone primarily targets μ ORs. Recently, an important role of naloxone sensitive μ ORs was also identified in sacral neuromodulation of isovolumetric bladder contractions in rats (Su et al., 2013). In these experiments the effect of sacral neuromodulation was not altered by nor-binaltorphimine (2.0 mg/kg i.v.) or naltrindole (5.0 mg/kg i.v.).

This study in cats revealed several important roles of different OR subtypes (μ, κ, and δ) in TNS inhibition and AA irritation-induced bladder overactivity. These results provide significant insights into the possible mechanisms underlying the FDA-approved tibial neuromodulation therapy for OAB. Understanding neurotransmitter mechanisms of bladder neuromodulation might identify additional molecular targets to develop new OAB treatments or improve neuromodulation therapy in combination with drug treatments.
AUTHORSHIP CONTRIBUTIONS

Participated in research design: Zhang, Slater, Ferroni, Kadow, Lyon, Shen, Xiao, Wang, Kang, Roppolo, de Groat, and Tai.

Conducted experiments: Zhang, Slater, Ferroni, Kadow, Lyon, Shen, Xiao, Wang, Kang, Roppolo, de Groat, and Tai.

Contributed new reagents or analytic tools: Zhang, Slater, Ferroni, Kadow, Lyon, Shen, Xiao, Wang, Kang, Roppolo, de Groat, and Tai.

Performed data analysis: Zhang, Slater, Ferroni, Kadow, Lyon, Shen, Xiao, Wang, Kang, Roppolo, de Groat, and Tai.

Wrote or contributed to the writing of the manuscript: Zhang, Slater, Ferroni, Kadow, Lyon, Shen, Xiao, Wang, Kang, Roppolo, de Groat, and Tai.
REFERENCES


bladder overactivity by tibial nerve stimulation. Am J Physiol Regul Integr Comp Physiol 305: 126-133.


FOOTNOTES

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FIGURE LEGENDS

Fig. 1. Inhibition of bladder overactivity by tibial nerve stimulation (TNS). A. Repeated CMGs during saline or 0.25 % acetic acid (AA) infusion with/without TNS. Black bars under the bladder pressure traces indicate TNS duration. TNS: 5 Hz, 0.2 ms, T = 1.2 V. Short arrows indicate the start and stop of bladder infusion. Infusion rate = 2 ml/min. B. Summarized results of TNS inhibitory effect on bladder capacity (N = 22 cats). * indicates significantly different from AA control (one-way ANOVA).

Fig. 2. Dose dependent effect of cyprodime on tibial nerve stimulation (TNS) inhibition of bladder overactivity induced by 0.25 % acetic acid (AA). The CMGs at increasing cumulative doses of cyprodime were performed in sequence from left to right in A-C and from top to bottom in each figure. A. The AA CMGs without TNS. B. The AA CMGs during 2T TNS. C. The AA CMGs during 4T TNS. The black bars under the pressure traces indicate TNS duration. TNS: 5 Hz, 0.2 ms, intensity threshold T = 1.2 V. Infusion rate = 2 ml/min.

Fig. 3. Summarized results of cyprodime effect on tibial nerve stimulation (TNS) inhibition of bladder overactivity induced by 0.25% acetic acid (AA) irritation (N = 6 cats). # indicates significantly different from AA control at each dosage (two-way ANOVA). * indicates significantly different from the bladder capacity measured during TNS before cyprodime treatment (one-way ANOVA). TNS: 5 Hz, 0.2 ms, T = 0.5-2.4 V.

Fig. 4. Dose dependent effect of nor-binaltorphimine on tibial nerve stimulation (TNS) inhibition of bladder overactivity induced by 0.25 % acetic acid (AA). The CMGs at increasing cumulative
doses of nor-binaltorphimine were performed in sequence from left to right in A-C and from top to bottom in each figure. A. The AA CMGs without TNS. B. The AA CMGs during 2T TNS. C. The AA CMGs during 4T TNS. The black bars under the pressure traces indicate TNS duration. TNS: 5 Hz, 0.2 ms, intensity threshold T = 0.75 V. Infusion rate = 2 ml/min.

Fig. 5. Summarized results of nor-binaltorphimine effect on tibial nerve stimulation (TNS) inhibition of bladder overactivity induced by 0.25% AA irritation (N = 6 cats). # indicates significantly different from AA control at each dosage (two-way ANOVA). * indicates significantly different from the bladder capacity measured during TNS before nor-binaltorphimine treatment (one-way ANOVA). TNS: 5 Hz, 0.2 ms, T = 0.5-2.4 V.

Fig. 6. Dose dependent effect of naltrindole on tibial nerve stimulation (TNS) inhibition of bladder overactivity induced by 0.25% acetic acid (AA). The CMGs at increasing cumulative doses of naltrindole were performed in sequence from left to right in A-C and from top to bottom in each figure. A. The AA CMGs without TNS. B. The AA CMGs during 2T TNS. C. The AA CMGs during 4T TNS. The black bars under the pressure traces indicate TNS duration. TNS: 5 Hz, 0.2 ms, intensity threshold T = 1 V. Infusion rate = 2 ml/min.

Fig. 7. Summarized results of naltrindole effect on tibial nerve stimulation (TNS) inhibition of bladder overactivity induced by 0.25% AA irritation (N = 10 cats). # indicates significantly different from AA control at each dosage (two-way ANOVA). * indicates significantly different from the bladder capacity measured during TNS before naltrindole treatment (one-way ANOVA). TNS: 5 Hz, 0.2 ms, T = 0.5-2.9 V.

Fig. 8. Naloxone effect on bladder capacity and tibial nerve stimulation (TNS) inhibition in cats.
pretreated with selective opioid antagonists. A. Cyprodime (1 mg/kg) pretreated (N = 4 cats). B. nor-binaltorphimine (10 mg/kg) pretreated (N = 6 cats). C. Naltrindole (10 mg/kg) pretreated (N = 9 cats). * indicates significantly different (paired t-test). TNS: 5 Hz, 0.2 ms, T = 0.5-2.9 V.

Fig. 9. Hypothetical role of μ and κ opioid receptors in tibial nerve stimulation (TNS) inhibition of acetic acid (AA) irritation-induced bladder overactivity. TNS stimulates an inhibitory pathway that may inhibit the spinobulbospinal micturition reflex pathway at multiple sites in the spinal cord or the brain stem PMC/PAG circuitry by activating μ or κ opioid receptors. In addition, a tonically active (~) inhibitory mechanism mediated by μ opioid receptors regulates bladder capacity by modulating micturition switching circuitry in the PMC/PAG. AA-irritation of the bladder suppresses the tonic μ opioid receptor inhibition by activating a κ opioid receptor mechanism and in turn decreases bladder capacity.
Figure 1
Figure 2
Figure 3

Normalized Capacity (%) vs. Cyprodime (mg/kg) for 4T TNS, 2T TNS, and AA Control.
Figure 4

A  AA control  B  2T TNS  C  4T TNS

Before nor-binaltophimine

0.03 mg/kg nor-binaltophimine

0.1 mg/kg nor-binaltophimine

0.3 mg/kg nor-binaltophimine

1 mg/kg nor-binaltophimine

3 mg/kg nor-binaltophimine

10 mg/kg nor-binaltophimine

Start Infusion

Stop Infusion

100 cmH2O

100 sec

Start Infusion

Stop Infusion

Stop Infusion

Stop Infusion
Figure 5
Figure 6

Before Naltrindole

0.03 mg/kg Naltrindole

0.1 mg/kg Naltrindole

0.3 mg/kg Naltrindole

1 mg/kg Naltrindole

3 mg/kg Naltrindole

10 mg/kg Naltrindole

Start Infusion

Stop Infusion

A AA control

2T TNS

4T TNS

Start Infusion

Stop Infusion

75 cmH2O

100 sec
Figure 7
Figure 8

A

[Graph showing normalized capacity (%)]

B

[Graph showing normalized capacity (%)]

C

[Graph showing normalized capacity (%)]
Figure 9

PMC = Pontine micturition center
PAG = Periaqueductal gray
μ = μ opioid receptors
κ = κ opioid receptors

- Excitatory neurons
- Inhibitory neurons
- Excitatory synapses
- Inhibitory synapses

Bladder
primary afferent

PMC/PAG

spinal ascending tract neuron

TNS

μ + κ

μ + κ

μ

κ

~ tonically activated

AA-irritated afferent

sacral parasympathetic neuron

Bladder