Role of μ , κ , and δ Opioid Receptors in Tibial Inhibition of Bladder Overactivity in Cats

Zhaocun Zhang, Richard C. Slater, Matthew C. Ferroni, Brian T. Kadow, Timothy D. Lyon, Bing Shen, Zhiying Xiao, Jicheng Wang, Audry Kang, James R. Roppolo, William C. de Groat, and Changfeng Tai

Department of Urology, Qilu Hospital, Shandong University, Jinan, P.R. China (Z.Z.); Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania (Z.Z., R.C.S., M.C.F., B.K., T.D.L., B.S., Z.X., J.W., A.K., C.T.); Department of Urology, The Second Hospital, Shandong University, Jinan, P.R. China (Z.X.); and the Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania (J.R.R., W.C.D., C.T.)

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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 i.v. 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 $21.1\% \pm 2.6\%$ 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 $52.9\% \pm 3.6\%$ or $57.4\% \pm 4.6\%$ 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 pretreated cats, but it reversed the nor-binaltorphimine-induced increase in bladder capacity and eliminated the TNS inhibition remaining in naltrindole pretreated cats. These results indicate a major role of μ and κ ORs in TNS inhibition, whereas 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 or without incontinence; OAB 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 because of 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 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 i.v. 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. It is not known, however, 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 (Beakley et al., 2015). 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 i.v. in different groups of cats to determine the role of each subtype receptor in TNS inhibition. Our results may provide insights into

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ABBREVIATIONS: AA, acetic acid; ANOVA, analysis of variance; CMG, cystometrogram; OAB, overactive bladder; OR, opioid receptor; TNS, tibial nerve stimulation; T, threshold.

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.

Materials and Methods

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) 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 (NONIN Medical, Inc., Plymouth, MN) 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 the 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

(MicroProbe, Gaithersburg, MD) 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-m 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 (Tai et al., 2012; Matsuta et al., 2013), intensities of 2 T or 4 T 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 cm H₂O) and long duration (>20 s). Once stable bladder capacity was obtained, 0.25% AA was infused into the bladder to irritate nociceptive C-fiber bladder afferents and induce bladder overactivity. 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 2 T TNS, (3) CMG during 4 T TNS, and (4) control CMG without TNS. Then the animals were divided into three groups for pharmacologic 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, Bristol, UK) were administered intravenously. Ten minutes after administering each dose, four AA CMGs were performed: (1) control CMG without TNS, (2) CMG during 2 T TNS, (3) CMG during 4 T TNS, (4) control CMG without TNS. A 5-minute rest period was inserted between the CMGs to allow the bladder to recover

Fig. 1. Inhibition of bladder overactivity by TNS. (A) Repeated CMGs during saline or 0.25% AA infusion with or 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). *Significantly different from AA control (one-way ANOVA).

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) 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) 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, 2 T, 4 T, 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 from the same animal under the same experimental conditions were averaged. The results from different animals are reported as mean $\pm$ S.E. Statistical significance ($P < 0.05$) was detected by a paired t test or repeated-measures analysis of variance (ANOVA) followed by Dunnett's (one-way) or Bonferroni's (two-way) multiple comparison. Two-way ANOVA was performed between TNS and control groups for different drug dosages (Figs. 3, 5, and 7). One-way ANOVA was performed in untreated cats for different CMG conditions (saline, AA, 2 T, 4 T; see Fig. 1), or in drug-treated cats for different drug dosages at each CMG condition (2 T TNS, 4 T TNS, or AA control; see Figs. 3, 5, and 7).

Fig. 3. Summarized results of cyprodimine effect on TNS inhibition of bladder overactivity induced by 0.25% AA irritation ($n = 6$ cats). *Significantly different from AA control at each dosage (two-way ANOVA). *wSignificantly different from the bladder capacity measured during TNS before cyprodimine treatment (one-way ANOVA). TNS: 5 Hz, 0.2 ms, T = 0.5–2.4 V.

Result

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

Effects of Selective OR Antagonists on Bladder Overactivity and TNS Inhibition. Selectively blocking μ ORs by increasing doses of cyprodimine did not significantly change the control bladder capacity during repeated AA CMGs (Figs. 2A and 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 2 T and 4 T 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 2 T and 4 T TNS at doses of 3–10 mg/kg (Figs. 4 and 5). Unlike cyprodimine, however, nor-binaltorphimine (1–10 mg/kg) significantly ($P < 0.05$) increased control bladder capacity during repeated AA CMGs (Figs. 4A and 5). Naltrindole (a selective δ OR antagonist) significantly ($P < 0.05$) increased AA control capacity (Figs. 6A and 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 cyprodimine pretreated cats, naloxone (1 mg/kg, i.v.) did not alter bladder capacity either before or during TNS (Fig. 8A); however, in nor-binaltorphimine pretreated cats, naloxone significantly ($P < 0.05$) reduced bladder capacity and reversed the nor-binaltorphimine-induced

Fig. 2. Dose-dependent effect of cyprodimine on TNS inhibition of bladder overactivity induced by 0.25% AA. The CMGs at increasing cumulative doses of cyprodimine were performed in sequence from left to right (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 4 T 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. 4. Dose-dependent effect of nor-binaltorphimine on TNS inhibition of bladder overactivity induced by 0.25% 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 2 T TNS. (C) The AA CMGs during 4 T 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.

increase in bladder capacity (Figs. 5 and 8B). In naltrindole pretreated 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. 2–5) but δ ORs play only a minor role (Figs. 6 and 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 and 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 before 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 short-duration contractions, especially compared with the long-duration contractions that occurred at the end of the CMGs during TNS inhibition (Figs. 2, B and 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

Fig. 5. Summarized results of nor-binaltorphimine effect on TNS inhibition of bladder overactivity induced by 0.25% AA irritation ($n = 6$ cats). #Significantly different from AA control at each dosage (two-way ANOVA). *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 TNS inhibition of bladder overactivity induced by 0.25% AA. The CMGs at increasing cumulative doses of naltrindole were performed in sequence from left to right (A–C) and from top to bottom in each figure. (A) The AA CMGs without TNS. (B) The AA CMGs during 2 T TNS. (C) The AA CMGs during 4 T 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.

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 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 (Schmidhammer et al., 1989; Goodman et al., 2007). Cyprodime has a much higher μ receptor selectivity than naloxone ($\mu/\kappa = 28$, $\mu/\delta = 110$) (Schmidhammer et al., 1989), but like naloxone it has a similar low nanomolar 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 quite 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 (Roppolo et al., 1983; Booth et al., 1985; Tai et al., 2012). Assuming that this effect is also due to blocking 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. The present results show, however, that the μ OR inhibition, although 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 brainstem (Ferroni et al., 2015) (Fig. 9), which can be mimicked by application of μ OR agonists (fentanyl or morphine) injected i.c.v. 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 i.t. compared with i.c.v. administration, which is 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 ($\kappa/\mu = 170$, $\kappa/\delta = 150$) (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 notably reduced bladder capacity (Fig. 8B), suggesting that (1) in animals with AA-irritated bladders, tonic activation of κ ORs suppresses tonic μ OR-mediated inhibition of the micturition reflex and (2) that blocking 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

Fig. 7. Summarized results of naltrindole effect on TNS inhibition of bladder overactivity induced by 0.25% AA irritation ($n = 10$ cats). #Significantly different from AA control at each dosage (two-way ANOVA). *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 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). *Significantly different (paired t test). TNS: 5 Hz, 0.2 ms, T = 0.5–2.9 V.

doses of naloxone used in the present and in the 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 physiologic 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 ($\delta/\kappa = 80$, $\delta/\mu = 300$) (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.

Fig. 9. Hypothetical role of μ and κ opioid receptors in TNS inhibition of 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 brainstem pontine micturition center (PMC)/periaqueductal gray (PAG) circuitry by activating μ or κ opioid receptors. In addition, a tonically active ($\sim$) inhibitory mechanism mediated by μ opioid receptors regulates bladder capacity by modulating micturition switching circuitry in the pontine micturition center (PMC)/periaqueductal gray (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.

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 Food and Drug Administration–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 through combination with drug treatments.

Authorship Contributions

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

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

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

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

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

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Address correspondence to: Dr. Changfeng Tai, Department of Urology, University of Pittsburgh, 700 Kaufmann Building, Pittsburgh, PA 15213. E-mail: cftai@pitt.edu