Contribution of opioid and metabotropic glutamate receptor mechanisms to inhibition of bladder overactivity by tibial nerve stimulation

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Matsuta Y, Mally AD, Zhang F, Shen B, Wang J, Roppolo JR, de Groat WC, Tai C. Contribution of opioid and metabotropic glutamate receptor mechanisms to inhibition of bladder overactivity by tibial nerve stimulation. Am J Physiol Regul Integr Comp Physiol 304: R126–R133, 2013. First published April 10, 2013; doi:10.1152/ajpregu.00572.2012.—The contribution of metabotropic glutamate receptors (mGluR) and opioid receptors to inhibition of bladder overactivity by tibial nerve stimulation (TNS) was investigated in cats under α-chloralose anesthesia using LY341495 (a group II mGluR antagonist) and naloxone (an opioid receptor antagonist). Slow infusion cystometry was used to measure the volume threshold (i.e., bladder capacity) for inducing a large bladder contraction. After measuring the bladder capacity during saline infusion, 0.25% acetic acid (AA) was infused to irritate the bladder, activate the nociceptive C-fiber bladder afferents, and induce bladder overactivity. AA significantly (P < 0.0001) reduced bladder capacity to 26.6 ± 4.7% of saline control capacity. TNS (5 Hz, 0.2 ms) at 2 and 4 times the threshold (T) intensity for inducing an observable toe movement significantly increased bladder capacity to 62.2 ± 8.3% at 2T (P < 0.01) and 80.8 ± 9.2% at 4T (P = 0.0001) of saline control capacity. LY341495 (0.1–5 mg/kg iv) did not change bladder overactivity, but completely suppressed the inhibition induced by TNS at a low stimulus intensity (2T) and partially suppressed the inhibition at high intensity (4T). Following administration of LY341495, naloxone (0.01 mg/kg iv) completely eliminated the high-intensity TNS-induced inhibition. However, without LY341495 treatment a 10 times higher dose (0.1 mg/kg) of naloxone was required to completely block TNS inhibition. These results indicate that interactions between group II mGluR and opioid receptor mechanisms contribute to TNS inhibition of AA-induced bladder overactivity. Understanding neurotransmitter mechanisms underlying TNS inhibition of bladder overactivity is important for the development of new treatments for bladder disorders.

GLUTAMATERGIC NEUROTRANSMISSION involves activation of both ionotropic glutamate receptors (iGluR) and metabotropic glutamate receptors (mGluR) that consist of eight subtypes classified into group I (mGluR 1 and 5), group II (mGluR 2 and 3), and group III (mGluR 4, 6, 7, and 8). An interaction between iGluR and opioid receptor mechanisms is believed to be important in pain pathways, because block of ionotropic N-methyl-D-aspartate (NMDA) receptors enhances the antinociceptive effect of morphine (an opioid receptor agonist) (14, 28, 36, 37). Recently, the interaction between mGluR and opioid receptors has also been identified in studies of opioid-induced antinociception (15, 16, 29, 45). Opioid-induced antinociceptive effects were enhanced by antagonists (15, 16, 45) and agonists (29) of group II mGluR in different types of somatic nociception. Currently, it is unknown whether this interaction between group II mGluR and opioid receptors occurs in visceral nociceptive mechanisms.

Recent results from this laboratory (38) revealed that tibial nerve stimulation (TNS) inhibits bladder overactivity elicited by intravesical infusion of acetic acid (AA), which stimulates nociceptive bladder afferent nerves. This TNS-induced antinociceptive effect is completely eliminated by naloxone (an opioid receptor antagonist), indicating a role for opioid receptor activation in TNS-induced antinociception (39). Tramadol, which produces an active metabolite with opioid receptor agonist activity, enhances the TNS-induced inhibition of AA-induced bladder overactivity (47). The current study examined the contribution of group II mGluR and the possible interaction between these receptors and opioid receptor mechanisms in TNS-induced inhibition of AA-induced bladder overactivity in cats anesthetized with α-chloralose. Intravesical infusion of dilute AA (0.25%) was used to irritate the bladder and activate nociceptive C-fiber bladder afferents. LY341495 (a group II mGluR antagonist) and naloxone were administered to evaluate the role of glutamate and opioid receptors. The effects of these agents on TNS inhibition may provide insights into the mechanisms underlying the clinical efficacy of tibial neuromodulation for the treatment of overactive bladder (OAB) symptoms (30, 31).

MATERIALS AND METHODS

The Animal Care and Use Committee at the University of Pittsburgh approved all protocols involving the use of animals in this study.

Experimental setup. Experiments were conducted in 18 cats (10 male, 8 female, 2.7–3.9 kg, 6- to 12-mo-old domestic shorthairs; Liberty Research, Waverly, NY) anesthetized initially with isoflurane (2–5% in oxygen) and maintained 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 right carotid artery. Drug and fluid were administered via the right cephalic vein, and airway access was secured with a tracheotomy tube.

The ureters were isolated via an abdominal incision, cut, and drained externally using penrose drainage tubing. The bladder was cannulated through the urethra with a double-lumen catheter. One lumen was used to infuse saline 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 proximal urethra ~2 cm from the bladder to prevent leakage. The tibial nerve...
was exposed on the medial side of right hindlimb above the ankle. A tripod cuff electrode (NC223pt; MicroProbe, Gaithersburg, MD) was applied around the nerve and connected to a stimulator (S88; Grass Medical Instruments, Quincy, MA).

**Stimulation protocol and drug administration.** Uniphasic rectangular pulses (5 Hz frequency, 0.2-ms pulse width) were delivered to the tibial nerve via the cuff electrode. The intensity threshold (T) for inducing toe movement was determined by gradually increasing the stimulation intensity. Because our previous study indicated that a 2T stimulus intensity was required to inhibit reflex bladder contractions (40), intensities of 2T or 4T were used in this study to suppress nociceptive bladder overactivity induced by 0.25% AA irritation.

A cystometrogram (CMG) was performed with saline infusion to measure bladder capacity, which was defined as the bladder volume threshold to induce a large amplitude (>30 cmH₂O) and long duration (>20 sec) bladder contraction. Then, multiple (3–5) saline CMG were repeated to evaluate the reproducibility in a 30- to 90-min period. Once the bladder capacity was determined during saline infusion, 0.25% AA was infused into the bladder during repeated CMG to activate nociceptive C-fiber bladder afferents and induce an OAB reflex. After 15–40 min of AA infusion, CMG for bladder capacity to be stabilized, four CMG were performed during AA infusion: 1) control without TNS; 2) during 2T TNS; 3) during 4T TNS; and 4) control without TNS (Fig. 1A). Then, pharmacological studies were performed.

Cumulative doses (0.1, 0.3, 1, 3, and 5 mg/kg iv) of LY341495 (12S)-2-Amino-2[(1S,2S)-2-carboxycyclopropyl-1-yl]-3-(xanth-9-yl) propanoic acid; Abcam, Cambridge, MA) were given in 10 cats. LY341495 is an antagonist for both mGluR2 and mGluR3 receptors that are classified as group II mGluR. Starting 15 min after administering each dose of LY341495, four CMG were performed during AA infusion: 1) control without TNS; 2) during 2T TNS; 3) during 4T TNS; and 4) control without TNS. In 9 cats following the testing of LY341495, cumulative doses (0.001, 0.01, and 0.1 mg/kg iv) of naloxone (Sigma-Aldrich, St. Louis, MO) were injected to block opioid receptors prior to repeated testing of TNS. Naloxone is a nonselective antagonist for μ, κ, and δ opioid receptors. Starting 5 min after administering each dose of naloxone, four CMG were performed during AA infusion: 1) control without TNS; 2) during 2T TNS; 3) during 4T TNS; and 4) control without TNS. A 5-min rest period was inserted between the repeated CMG to allow the bladder to recover from previous contractions.

In another experimental group (n = 8 cats), the animals were not treated with LY341495, but the same protocol for repeated CMG tests (control, 2T, 4T, and control) was also used during AA infusion. Naloxone alone was administered in cumulative doses (0.001, 0.01, and 0.1 mg/kg iv) to evaluate the effect of varying levels of opioid receptor blockade on TNS-induced inhibition in the absence of LY341495.

**Data analysis.** For the repeated CMG recordings, bladder capacities were measured and normalized to the measurement of the first saline control CMG or the AA control CMG before the naloxone test in the same animal 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 means ± SE. Statistical significance (P < 0.05) was detected by one-way ANOVA followed by Dunnett’s multiple comparison, or two-way ANOVA followed by Bonferroni multiple comparison.

**RESULTS**

**TNS inhibition of bladder overactivity induced by AA irritation.** Intravesical infusion of 0.25% AA irritated the bladder, activated nociceptive C-fiber bladder afferents, and significantly (P < 0.0001) reduced bladder capacity to 26.6 ± 4.7% of the saline control capacity (8.5 ± 1.3 ml, n = 10 cats) (Fig. 1). TNS at 2T and 4T intensity suppressed AA-induced bladder overactivity and significantly increased bladder capacity to 62.2 ± 8.3% (P < 0.01) and 80.8 ± 9.2% (P = 0.0001) of the saline control capacity, respectively (Fig. 1B). After the 2T and 4T TNS, bladder capacity returned to 26.7 ± 5.7% of the saline control capacity (Fig. 1B).

**Dose-dependent effect of LY341495 on TNS inhibition of bladder overactivity.** The effect of LY341495 on TNS inhibition of bladder overactivity was dependent on drug dosage and TNS intensity (Fig. 2 and Fig. 3). Administering cumulative doses of LY341495 (0.1, 0.3, 1, 3 and 5 mg/kg iv) did not significantly (P > 0.05) change the control bladder capacity in the absence of stimulation (Fig. 2A and Fig. 3). However, LY341495 completely blocked the inhibition induced by low-intensity (2T) TNS at doses of 1–5 mg/kg (Fig. 2A and B; Fig. 3), and significantly (P < 0.001) reduced the inhibition induced by high-intensity (4T) TNS at doses of 3–5 mg/kg (Fig. 2C; Fig. 3). Compared with AA control capacity, high-inten-
sity (4T) TNS could still significantly increase the bladder capacity at doses of 3–5 mg/kg. In these experiments following 2T and 4T TNS, bladder capacity returned to the control level prior to stimulation (i.e., a poststimulation effect did not occur). Poststimulation inhibition following TNS has been observed during saline CMG (40) but not during AA irritation of the bladder (38).

The number of small, uninhibited detrusor contractions that occurred before the micturition contraction was not changed significantly \((P > 0.05)\) by LY341495 treatment or stimulation. On average from the 10 cat experiments, the number of uninhibited detrusor contractions was 1.8 ± 0.5 under AA control conditions before LY341495 treatment, and 2.3 ± 0.5 after the last dose (5 mg/kg) of LY341495 treatment. There were 1.7 ± 0.8 uninhibited detrusor contractions during 4T TNS before LY341495 treatment and 1.4 ± 0.5 contractions after the last dose of LY341495 treatment.

**Dose-dependent effect of naloxone on TNS inhibition of bladder overactivity.** After the last dose (5 mg/kg) of LY341495 was administered, the effect of cumulative doses of naloxone (0.001–0.1 mg/kg iv) on residual TNS inhibition elicited by 4T stimulation was examined in 9 cats. Naloxone did not change the AA control bladder capacity in the absence of TNS, but a dose of 0.01 mg/kg completely blocked residual TNS inhibition (Fig. 4A and Fig. 5A).

In another group of experiments \((n = 8\) cats) without LY341495 pretreatment, cumulative doses of naloxone (0.001–0.1 mg/kg iv) also did not change the AA control
bladder capacity prior to TNS (Fig. 4B and Fig. 5B) but dose dependently reduced the magnitude of TNS inhibition. However, compared with the results in animals pretreated with LY341495 (5 mg/kg), a 10 times higher naloxone dose (0.1 mg/kg) was needed to completely block the 4T TNS inhibition (Fig. 4 and Fig. 5). In these animals the 0.01 mg/kg naloxone dose eliminated the inhibition induced by 2T TNS (Fig. 5B). Inhibition at this intensity was also eliminated by 3 mg/kg and 5 mg/kg doses of LY341495 (Fig. 3 and Fig. 5A).

**DISCUSSION**

This study revealed that LY341495 (a group II mGluR antagonist) administered intravenously in a range of doses did not change the bladder overactivity induced by AA irritation, but partially suppressed in a dose-dependent manner TNS inhibition of bladder overactivity (Fig. 2 and Fig. 3). After the maximal dose of LY341495 (5 mg/kg), naloxone (an opioid receptor antagonist) completely eliminated the remaining TNS inhibition (Fig. 4 and Fig. 5) at a dose (0.01 mg/kg) 10 times smaller than the dose (0.1 mg/kg) required to completely block the inhibition in animals that were not treated with LY341495.
LY341495 (5 mg/kg) pretreated

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Without LY341495

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**Fig. 5.** LY341495 pretreatment significantly reduces the naloxone dosage required to eliminate TNS inhibition of bladder overactivity induced by 0.25% AA infusion. A: naloxone effect in LY341495 (5 mg/kg) pretreated animals (n = 9 cats). B: naloxone effect without LY341495 pretreatment (n = 8 cats). Bladder capacities were normalized to the capacity measured during AA control CMG before naloxone treatment (i.e., at 0 mg/kg naloxone). Stimulation: 5 Hz, 0.2 ms, T = 0.35–2.4 V. *Significantly different from control capacity at each dosage (two-way ANOVA).

These results indicate that neurotransmitter mechanisms involving group II mGluR and opioid receptors are essential for TNS inhibition of reflex bladder overactivity and that there may be a significant synergistic interaction between these two mechanisms.

Various studies have implicated group II mGluR in peripheral and central nociceptive mechanisms. Group II mGluR have been identified with immunohistochemical methods in the spinal cord and brain, and in small to medium size dorsal root ganglia neurons, and in cutaneous sensory nerves that coexpress TRPV1 receptors (5, 7, 27). Group II mGluR antagonists enhance nociceptive behavior and primary afferent firing induced by intraplantar injection of capsaicin; and a group II mGluR agonist suppressed this enhancement (9). Group II mGluR agonists are also believed to act in the spinal cord to produce analgesia (13, 34) by suppressing the release of glycine, GABA, or glutamate (35, 48), whereas knockdown of group II mGluR enhances nociceptive responses (46). These results indicate that somatic nociceptive pathways are controlled by tonically active group II mGluR inhibitory mechanisms. However, our studies did not detect similar tonically active inhibitory mechanisms in the control of nociceptive bladder reflexes. Bladder overactivity was not altered after administration of LY341495, which induces hyperalgesia and enhances nociceptive behavior in somatic pain models (9, 34). However, the drug did suppress TNS-induced inhibition of bladder overactivity. This suggests that the group II mGluR inhibitory mechanism in bladder reflex pathways was not effective except during TNS.

Pharmacological studies of somatic nociceptive mechanisms have provided evidence for a linkage between group II mGluR and opioid receptors. Group II mGluR antagonists (15, 16, 45) enhance the inhibitory effect of opioid receptor agonists on somatic nociceptive responses induced by thermal stimuli or formalin injection. On the other hand, a group II mGluR agonist also enhances the opioid-induced antinociceptive effect on neuropathic pain (29). These results indicate that mGluR-opioid interaction could be complex depending on different types of nociception. Our study showing that LY341495 enhances the potency of naloxone in blocking TNS inhibition (Figs. 4 and 5) raises the possibility that group II mGluR also facilitate endogenous opioid inhibitory mechanisms in the TNS inhibitory pathway and that removal of the facilitation by LY341495 reduces the opioid inhibition and makes it more susceptible to blockade by naloxone.

The inhibition of reflex bladder activity by TNS is believed to occur by modulation of the micturition reflex pathway at a site in the central nervous system. As shown in Fig. 6, both spinal and supraspinal pathways can mediate reflex bladder activity. Therefore, TNS inhibition could occur in the brain or spinal cord. Because naloxone and LY341495 were both administered systemically, it is impossible to determine their site of action. However, it is likely that these drugs interact at least in part at synapses in the lumbosacral spinal cord on the basis of the following observations: 1) group II mGluR and opioid receptors are expressed in the spinal dorsal horn (6, 8, 21, 22, 32, 41); 2) intrathecal administration of group II mGluR agonists suppress nociceptive behavior (13, 23, 24, 34); 3) AA-induced bladder overactivity is mediated by a reflex pathway organized in the spinal cord (17); 4) our previous study (39) suggested that TNS inhibition of AA-induced bladder overactivity could be mediated by suppression of transmission at a spinal interneuronal synapse prior to the sacral parasympathetic preganglionic neurons; and 5) spinal opioid receptors have a prominent inhibitory effect on micturition (12, 20, 42, 43).

Figure 6 shows a hypothetical spinal mechanism for TNS inhibition, which is intended to facilitate the discussion about possible sites of interaction between naloxone and LY341495. Alternative sites of action in the brain are also indicated but detailed pathways are not shown. In the diagram, TNS inhibition mediated by inhibitory interneuron-1 targets excitatory interneuron-3 on the spinal micturition pathway interposed between the bladder C-fiber afferent and a bladder preganglionic neuron (neuron-4). Electrophysiological evidence for this type of interneuronal inhibition by activation of somatic afferent nerves has been obtained in the cat spinal cord (11). Because TNS inhibition is blocked by naloxone, we propose...
that inhibitory interneuron-1 is enkephalinergic and releases an opioid peptide. Opioid peptides may be released as cotransmitters with glutamic acid because they are contained in subpopulations of glutamatergic neurons in the spinal dorsal horn (26, 44). Thus these two transmitters may interact to elicit a synergistic postsynaptic inhibitory effect at the synapse between interneuron-1 and interneuron-3. Group II mGluR and opioid receptors share a common intracellular signaling mechanism that inhibits adenylyl cyclase activity and reduces cAMP levels (4, 10). This may account for the synergism and for the enhancement of opioid-induced analgesia by exogenously administered group II mGluR agonists. In addition, activation of group II mGluR can inhibit voltage-gated Ca\(^{2+}\) channels, activate potassium channels to decrease neuronal excitability, and suppress synaptic transmission (4, 10, 18, 49). Elimination of the synergism following block of group II mGluR with LY341495 may explain the reduction in TNS inhibition after LY341495 and the increased potency of naloxone to block TNS inhibition.

Group II mGluR mechanisms could also indirectly affect TNS opioid inhibitory mechanisms via changes in NMDA iGluR. Activation of group II mGluR inhibits NMDA receptor mechanisms (1, 25, 33), and inhibition of NMDA receptors enhances opioid-induce antinociception (14, 28, 36, 37). Therefore, it is possible in our experiments that LY341495 reduced a tonic inhibition of NMDA receptors mediated by group II mGluR, thereby weakening an opioid antinociceptive effect and partially suppressing the TNS inhibition. This could be tested in future experiments by evaluating the sensitivity of TNS inhibition to other drugs that modulate NMDA receptors. Additionally, nonspecific effects of the tested drugs could also play a role in the drug interaction such as changes in drug metabolism, which could alter the potency of the drug.

An alternate hypothesis for the effect of LY341495 on TNS inhibition is that the drug acts at more proximal sites on the TNS reflex pathway to downregulate the firing of inhibitory interneuron-1. This mechanism would require a collateral inhibitory pathway that modulates TNS inhibition (interneuron-5; Fig. 6). It is known that group II mGluR are located presynaptically on glutamatergic (10, 33) and GABAergic/ glycinergic nerve terminals (35, 48) where they suppress transmitter release. Block of group II mGluR with LY341495 would increase the release of the transmitters, enhance transmission in the collateral inhibitory pathway, and therefore decrease the firing of inhibitory interneuron-1.

**Perspectives and Significance**

Our recent studies showing that tramadol, which produces an active metabolite with opioid receptor agonist activity, can significantly enhance TNS inhibition of AA-induced bladder overactivity (47) have provided additional support for the involvement of opioid receptor mechanisms in TNS inhibition of bladder nociception. Our current results raise the possibility that group II mGluR agonists might interact with tramadol to further enhance TNS inhibition of bladder nociception. However, whether these results obtained in anesthetized animals can be translated into clinical applications in human subjects still needs to be validated. Understanding neurotransmitter mechanisms underlying TNS inhibition of nociceptive bladder overactivity is important for development of new therapies to treat OAB or painful bladder syndrome/interstitial cystitis (2, 3, 19) and reduce adverse drug effects by combining drug therapy with neuromodulation.

**GRANTS**

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

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