Role of spinal metabotropic glutamate receptor 5 in pudendal inhibition of the nociceptive bladder reflex in cats

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Reese JN, Rogers MJ, Xiao Z, Shen B, Wang J, Schwen Z, Roppolo JR, de Groat WC, Tai C. Role of spinal metabotropic glutamate receptor 5 in pudendal inhibition of the nociceptive bladder reflex. Am J Physiol Renal Physiol 308: F832–F838, 2015. First published February 11, 2015; doi:10.1152/ajprenal.00623.2014.—This study examined the role of spinal metabotropic glutamate receptor 5 (mGluR5) in the nociceptive C-fiber afferent-mediated spinal bladder reflex and in the inhibition of this reflex by pudendal nervous stimulation (PNS). In α-chloralose-anesthetized cats after spinal cord transaction at the T9/T10 level, intravesical infusion of 0.25% acetic acid irritated the bladder, activated nociceptive C-fiber afferents, and induced spinal reflex bladder contractions of low amplitude (<50 cmH2O) and short duration (<20 s) at a smaller bladder capacity ~80% of saline control capacity. PNS significantly ([P < 0.01]) increased bladder capacity from 85.5 ± 10.1 to 137.3 ± 14.1 or 148.2 ± 11.2% at 2T or 4T stimulation, respectively, where T is the threshold intensity for PNS to induce anal twitch. MTEP {3-[[(2-methyl-4-thiazolyl)ethynyl]pyridine; 3 mg/kg iv, a selective mGluR5 antagonist} completely removed the PNS inhibition and significantly ([P < 0.05]) increased bladder capacity from 71.8 ± 9.9 to 94.0 ± 13.9% of saline control, but it did not change the bladder contraction amplitude. After propranolol (3 mg/kg iv, a β1/β2-adrenergic receptor antagonist) treatment, PNS inhibition remained but MTEP significantly ([P < 0.05]) reduced the bladder contraction amplitude from 18.6 ± 2.1 to 6.6 ± 1.2 cmH2O and eliminated PNS inhibition. At the end of experiments, hexamethonium (10 mg/kg iv, a ganglionic blocker) significantly ([P < 0.05]) reduced the bladder contraction amplitude from 20.9 ± 3.2 to 8.1 ± 1.5 cmH2O on average demonstrating that spinal reflexes were responsible for a major component of the contractions. This study shows that spinal mGluR5 plays an important role in the nociceptive C-fiber afferent-mediated spinal bladder reflex and in pudendal inhibition of this spinal reflex.

OVERACTIVE BLADDER (OAB) is characterized by urinary urgency with or without urge incontinence usually with frequency and nocturia (1). Current therapies for OAB are not satisfactory since they are either invasive or have low efficacy with significant side effects (2, 4, 24). Pudendal neuromodulation has been applied clinically to treat patients with refractory OAB (19, 20), but the mechanisms underlying pudendal inhibition of bladder activity are not fully understood. Revealing the neurotransmitters, their sites of action, and receptors involved in pudendal inhibition could provide molecular targets to develop new therapies for OAB.

Our previous study in cats (16) showed that pudendal nerve stimulation (PNS) inhibits bladder overactivity elicited by bladder irritation with intravesical administration of 0.25% acetic acid (AA) and that intravenous administration of MTEP 3-[[2-(2-methyl-4-thiazolyl)ethyl]thiophen-2-yl]pyridine}, a selective antagonist for metabotropic glutamate receptor 5 (mGluR5), significantly reduces the PNS inhibition. The mGluRs are G protein-coupled receptors consisting of eight subtypes (mGluR1–8) divided into three groups (groups I–III) (6). Group I receptors (mGluR1 and mGluR5) activate phospholipase C, release intracellular Ca2+, and activate protein kinase C. The mGluR5 is widely expressed in the central nervous system (CNS) and previous neuroanatomical and immunocytochemical studies have localized these receptors at postsynaptic sites on the spinal interneurons playing an excitatory role in synaptic transmission (25). mGluR5 are involved in thermal nociception and inflammatory pain (3, 26) as well as irritation-induced bladder overactivity in rats (13). However, the site in the CNS at which mGluR5 is involved in bladder overactivity and pudendal inhibition of this activity is difficult to determine because bladder activity is mediated by both spinal and supraspinal reflex pathways (12). In normal physiological conditions, bladder distention activates the non-nociceptive Aδ afferents that trigger a spinobulbosplinal reflex (10), while nociceptive C-fiber afferents are silent (14). In pathological conditions such as spinal cord injury (7, 9) or bladder infection/irritation that induce bladder overactivity (21), the nociceptive C-fiber afferents are also activated which triggers a spinal micturition reflex (14, 27).

The purpose of this study in cats is to determine the role of spinal mGluR5 in the nociceptive C-fiber afferent-mediated spinal bladder reflex and in PNS inhibition of this reflex. Spinal cord transaction (SCT) was performed at the T9/T10 level to eliminate the Aδ-fiber afferent-mediated supraspinal reflex. Intravesical infusion of 0.25% AA was used to irritate the bladder and activate reflex bladder contractions mediated via a spinal pathway and the C-fiber afferents. Then, the sensitivity of the reflex bladder activity to PNS induced inhibition and MTEP, administered intravenously, was examined to determine the involvement of mGluR5 in the AA-induced spinal reflex and in PNS inhibition.

METHODS

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

Surgical procedure. Twelve cats (4 males and 8 females, 3.0–4.2 kg; Liberty Research, Waverly, NY) were anesthetized with isoflurane (2–3% in oxygen) during surgery and then with α-chloro-
lose (65 mg/kg iv and supplemented as needed) during data collection. Heart rate and blood oxygen saturation levels were monitored by a pulse oximeter (9847V, NONIN Medical, Plymouth, MN) attached to the tongue. Arterial blood pressure was monitored via a catheter in the right carotid artery. Drugs and fluid were given via a catheter in the cephalic vein and airway access was secured using a tracheostomy tube.

The ureters were isolated via a midline abdominal incision, cut, and drained externally. The bladder was cannulated through the urethra with a double lumen catheter that was secured in place with a ligature to prevent urethral leakage. One lumen was used to slowly infuse (1–2 ml/min) saline or 0.25% AA; the other lumen was attached to a pressure transducer to measure bladder pressure. The pudendal nerve was dissected in the region of the right sciatic notch and a tripolar cuff electrode (NC223pt, MicroProbe, Gaithersburg, MD) was applied around the nerve and connected to a stimulator (S88, Grass Medical Instruments, Quincy, MA) via a constant voltage stimulus isolator (SIU5, Grass Medical Instruments). A laminectomy was performed to expose the spinal cord at the thoracic (T9/T10) level for a transection (SIU5, Grass Medical Instruments). A laminectomy was performed to expose the spinal cord at the thoracic (T9/T10) level for a transection.

**Experimental protocol.** Based on previous studies (16, 27), uniphasic rectangular pulses of 0.2-ms pulse width and 5-Hz frequency were used for PNS. The stimulation threshold (T) defined as the minimal intensity for inducing an anal twitch was determined at the beginning of the experiment. Initially before the spinal cord was transected, cystometrograms (CMGs) were performed by slowly infusing the bladder with saline to determine the bladder capacity, defined as the bladder volume threshold required to induce a micturition contraction of large amplitude (>50 cmH2O) and long duration (>20 s). Because the urethra was ligated to prevent bladder emptying, the bladder was emptied manually after each CMG by withdrawing the saline through the catheter. Multiple CMGs were performed to ensure reproducibility of the saline control capacity. Then, the spinal cord was completely transected at the T9/T10 level. Thirty minutes following SCT, a saline CMG was performed to confirm the disappearance of the large-amplitude micturition contraction. Then, bladder infusion was changed from saline to 0.25% AA to irritate the bladder, activate the nociceptive C-fiber bladder afferents, and induce spinal reflex bladder activity. During the AA CMG, bladder capacity was determined by the volume threshold required to induce a bladder contraction of amplitude greater than 10 cmH2O (27). To ensure reproducibility of the AA control capacity, multiple CMGs were performed by filling and manually emptying the bladder. Then, the animals were divided into two groups.

In the first group (n = 6 cats), four CMGs were performed with AA infusion: 1) control CMG without PNS, 2) CMG during 2T PNS, 3) CMG during 4T PNS, and 4) control CMG without PNS to determine any poststimulation effect. At the end of the last CMG, the bladder remained full and isovolumetric bladder contractions were established. Then, MTEP (3 mg/kg iv, a selective mGluR5 antagonist; Abcam, Cambridge, MA) was administered during the isovolumetric bladder contractions. Ten minutes after MTEP treatment (16), the bladder was emptied and the same CMG protocol as above was repeated. In the second group (n = 6 cats), the animals were first treated with propranolol (3 mg/kg iv, a β1/β2-adrenergic receptor antagonist; Sigma, St. Louis, MO). Then, the same CMG protocol as above was used before and 10 min after MTEP (3 mg/kg iv) was administered. The propranolol dosage was chosen to maximally antagonize the β1/β2-adrenergic receptors (11) and the MTEP dosage is known to be effective in suppressing PNS inhibition of bladder overactivity (16). In both groups of cats at the end of the experiment when the bladder was filled with AA and maintained under isovolumetric conditions, hexamethonium (10 mg/kg iv, a ganglionic blocker; Sigma) (27) was administered to suppress the nicotinic acetylcholine receptors located in parasympathetic ganglia in the pelvic efferent neurons.

**Fig. 1.** Effect of spinal cord transection (SCT) at the T9/T10 level on reflex bladder activity. **A:** cystometrograms (CMGs) during saline infusion before and after SCT or during 0.25% acetic acid (AA) infusion after SCT. Infusion rate = 1 ml/min. **B:** summarized results from 12 cats. The downward white arrow in A marks the contraction that is used to determine the bladder capacity after SCT. The bladder capacity is normalized to the measurement during saline infusion before SCT. *Significantly different (paired t-test). Infusion rate = 1–3 ml/min.

**Fig. 2.** Hexamethonium (Hx) reduced the amplitude of spinal reflex bladder contractions. Hx (10 mg/kg iv) was administered during isovolumetric contractions (A) and significantly reduced the bladder contraction amplitude in both MTEP [3-[2-(methyl-4-thiazolyl)ethyl]pyridine]-pretreated and propranolol+MTEP-pretreated cats (B). *Significant (P < 0.05) difference (paired t-test); n = 6 cats in each treatment group.
pathways to the bladder and eliminate the bladder activity mediated by spinal reflexes.

Data analysis. Bladder capacity measured during each CMG was normalized to the initial saline control capacity before SCT in the same animal to reduce the influence of individual differences. The amplitude of maximal bladder contractions was measured before and after a drug treatment to indicate the effect on spinal reflex bladder activity. Measurements were averaged across the animals for the same conditions and reported with SE. Statistical significance ($P < 0.05$) was determined by a paired $t$-test or repeated-measures ANOVA.

Fig. 3. Effect of MTEP on spinal reflex bladder activity induced by AA irritation. A: bladder activity during CMG or under isovolumetric conditions before and after MTEP (3 mg/kg iv) treatment. B: MTEP had no effect on the maximal amplitude of isovolumetric contractions. C: MTEP significantly increased bladder capacity. The downward white arrow on CMG traces in A indicates where the bladder capacity is measured. *Significant ($P < 0.05$) difference (paired $t$-test); $n = 6$ cats.

Fig. 4. Effect of MTEP on spinal reflex bladder activity induced by AA irritation in propranolol (3 mg/kg iv)-pretreated cats. A: bladder activity during CMG or under isovolumetric conditions before and after MTEP (3 mg/kg iv) treatment. B: MTEP significantly reduced maximal amplitude of isovolumetric contractions. C: MTEP significantly increased bladder capacity. The downward white arrow on CMG traces in A indicates where the bladder capacity is measured. *Significant ($P < 0.05$) difference (paired $t$-test); $n = 6$ cats.
followed by Dunnett (1-way) or Bonferroni (2-way) posttests. Two-way ANOVA was performed with two within-subject factors: MTEP (2 levels: MTEP-treated, MTEP-untreated), conditions (4 levels: control, 2T, 4T, postcontrol) to detect the significant difference between MTEP-treated and MTEP-untreated conditions. In each condition, one-way ANOVA was performed to detect significant difference among four levels of treatment (control, 2T, 4T, postcontrol).

RESULTS

Effects of MTEP on spinal reflex bladder contractions. With an intact spinal cord, saline infusion produced large-amplitude (>50 cmH2O) and long-duration (>60 s) reflex bladder contractions during CMGs. Following SCT at T9/T10 level, this large micturition reflex was lost (Fig. 1A). However, AA irritation activated bladder nociceptive C-fiber afferents and induced low-amplitude (<50 cmH2O) and short-duration (<20 s) contractions at a significantly (P < 0.05) reduced the maximal contraction amplitude from 25.3 ± 5.4 to 10.7 ± 2.2 cmH2O for MTEP-pretreated cats and from 16.6 ± 2.7 to 5.6 ± 1.4 cmH2O for propranolol + MTEP-pretreated cats (Fig. 2), showing that the bladder contractions before hexamethonium were mediated in part by spinal reflex mechanisms and that the smaller contractions after hexamethonium treatment were mediated by intrinsic smooth muscle activity.

Without propranolol pretreatment, MTEP (3 mg/kg iv) applied during AA-induced isovolumetric contractions did not change the maximal contraction amplitude (Fig. 3, A and B), but it significantly (P < 0.05) increased the bladder capacity for inducing the spinal reflex contractions from 71.8 ± 9.9 to 94.0 ± 13.9% of saline control (Fig. 3, A and C). However, in propranolol-pretreated cats MTEP significantly (P < 0.05) reduced the maximal contraction amplitude from 18.6 ± 2.1 to 6.6 ± 1.2 cmH2O and increased the bladder capacity from 56.9 ± 10.5 to 78.5 ± 13.0% of saline control (Fig. 4).

Effects of MTEP on PNS inhibition of spinal reflex bladder contractions. In cats without propranolol pretreatment, PNS inhibited reflex bladder activity and significantly (P < 0.01) increased bladder capacity from 85.5 ± 10.1 to 137.3 ± 14.1 or 148.2 ± 11.2% of control capacity at 2T or 4T, respectively (Fig. 5, A and C). MTEP (3 mg/kg iv) completely eliminated PNS inhibition (Fig. 5, B and C).

In cats with propranolol pretreatment, PNS also inhibited reflex bladder activity and significantly (P < 0.05) increased bladder capacity from 62.7 ± 11.3 to 82.9 ± 13.3 or 82.1 ± 13.3% at 2T or 4T, respectively (Fig. 6, A and C). Following propranolol treatment, MTEP (3 mg/kg iv) again completely eliminated PNS inhibition (Fig. 6, B and C).

DISCUSSION

This study in cats showed that after SCT, which eliminated the non-nociceptive A6 afferent-mediated supraspinal reflex bladder contraction (Fig. 1A), AA irritation induced transient, low-amplitude spinal reflex bladder contractions (Figs. 1–2) that are likely mediated through activation of C-fiber nociceptive afferents (Fig. 7) (14). MTEP (a selective mGluR5 antagonist) significantly increased the bladder capacity during
AA infusion without changing the amplitude of bladder contractions under isovolumetric conditions (Fig. 3). MTEP alone completely removed PNS inhibition of the spinal reflex (Fig. 5). After propranolol administration which reduced but did not completely block PNS inhibition (22), MTEP treatment eliminated the remaining PNS inhibition (Fig. 6). In propranolol-treated cats, MTEP also significantly reduced the amplitude of spinal reflex contractions and increased the bladder capacity (Fig. 4).

After SCT at T9/T10 level eliminated the supraspinal reflex bladder contraction during saline distention (Fig. 1A), AA induced bladder contractions of low amplitude (<50 cmH2O) and short duration (<20 s; Figs. 1A, 3A, and 5A). These small bladder contractions are mediated in part by spinal reflexes because they are sensitive to hexamethonium (a ganglionic blocking agent; Fig. 2) that blocks the efferent pathway from the spinal cord to the bladder (Fig. 7). Although propranolol and/or MTEP were administered in this study before hexamethonium, our previous study showed the same sensitivity to...

Fig. 7. Possible sites where MTEP acts to modulate: 1) the spinal micturition reflex activated by C-fiber afferents (shown at right) and 2) inhibition of bladder activity elicited by stimulation of pudendal afferent nerves (shown at left). The supraspinal micturition reflex activated by Aδ afferents and removed by SCT is also shown at right. Hx and propranolol block transmission, respectively, in bladder ganglia and at the sympathetic neuroeffector junction.
hexamethonium in untreated cats (27). In addition, these small bladder contractions can be largely removed by lidocaine injection into the sacral spinal cord or transection of the sacral spinal roots and spinal cord (27). It is also known that the C-fiber afferents that are inactive during saline distention become activated and mechno-sensitive during bladder irritation (14). Previous studies further demonstrated that the spinal C-fiber reflex can be activated in acute (7, 18) and chronic (9) SCT cats by electrical stimulation of bladder afferents and recording of efferent activity on the pelvic nerve and that the Aδ-fiber afferent-mediated bladder reflex is eliminated under both conditions. Therefore, it would be logical to conclude that in this study AA irritation triggers a spinal bladder reflex by activating the nociceptive bladder C-fiber afferents (Fig. 7).

A previous study in spinal-intact rats (13) showed that MTEP significantly inhibited irritation-induced bladder overactivity and increased bladder capacity. In this study with acute SCT cats, MTEP also significantly increased bladder capacity during AA irritation (Figs. 3C and 4C), indicating that mGluR5 in the spinal cord may play an important role in nociceptive C-fiber afferent-mediated bladder overactivity (Fig. 7). However, our previous study in spinal-intact cats (16) showed that MTEP only had a transient inhibitory effect on AA-induced bladder overactivity without increasing bladder capacity, indicating that 1) the role of mGluR5 in bladder overactivity in spinal-intact cats and rats is different and 2) the contribution of spinal mGluR5 mechanisms to bladder overactivity in cats may be suppressed by input from the brain and/or that an effect of MTEP on the brain negates its action on the spinal cord. In addition, MTEP only reduced bladder contraction amplitude after administration of propranolol (Figs. 3B and 4B), a β1/β2-adrenergic receptor antagonist that in cats blocks the tonic sympathetic inhibitory input to the bladder passing through the hypogastric nerves (Fig. 7) (8, 11). Propranolol significantly increases the bladder contraction amplitude in the acute SCT cat model (22). Therefore, in animals without propranolol pretreatment MTEP must have suppressed the parasympathetic excitatory input to the bladder thereby reducing the contraction amplitude (Fig. 4, A and B), but at the same time must also have suppressed the sympathetic inhibitory input thereby counteracting the reduction in parasympathetic control resulting in no change in contraction amplitude (Figs. 3, A and B, and 7).

In acute SCT cats, MTEP (3 mg/kg) completely eliminated the PNS inhibition of AA-induced spinal reflex bladder activity (Fig. 5), indicating a critical role of spinal mGluR5 in PNS inhibition. It is likely that at least two mechanisms are involved in the PNS inhibition (Fig. 7): 1) suppression of the spinal parasympathetic excitatory reflex pathway to the bladder and 2) activation of the spinal sympathetic inhibitory reflex pathway to the bladder. A previous study (17) showed that PNS can drive the sympathetic pathway to inhibit the micturition reflex; and our previous study (22) showed that PNS inhibition is significantly reduced after propranolol treatment. As discussed above, it is also possible that MTEP may block the sympathetic inhibitory input to the bladder thereby reducing PNS inhibition (Fig. 7). Our current study further shows that the residual PNS inhibition after propranolol treatment can be completely removed by MTEP (Fig. 6), indicating that mGluR5 in the spinal cord is involved in the synaptic transmission underlying the PNS inhibition of the parasympathetic excitatory pathway to the bladder (Fig. 7). However, in spinal-intact cats MTEP (~50 mg/kg) blocked the inhibition induced by 2T PNS but not the inhibition by 4T PNS (16). The different results in spinal-intact and acute SCT cats raise the possibility that 4T PNS activates a third inhibitory mechanism that does not involve mGluR5 and occurs at a supraspinal site.

To determine whether neuromodulation of bladder activity acts in part by altering lumbosacral spinal circuitry, the present experiments were conducted in acute SCT cats in which neural mechanisms rostral to T9–T10 were eliminated and spinal bladder reflexes were activated by sensitizing bladder C-fiber afferents with AA (Fig. 7). The chronic SCT cat can also be used as a model to study the spinal mechanisms of PNS inhibition. However, this model is more complicated because it involves neuroplasticity and reorganization of parasympathetic reflex circuits in the spinal cord (7, 9, 12); and therefore may be less relevant for understanding the mechanisms of action of PNS in treating idiopathic OAB symptoms in patients who have no detectable spinal cord pathology. Furthermore, neuromodulation is not commonly used to treat neurogenic detrusor overactivity in people with chronic spinal cord injury (5, 15); although a recent clinical study indicated that sacral neuromodulation may produce beneficial effects if it is applied during the early phase of spinal cord injury when it may affect neuroplasticity in the spinal cord (23). Thus, the acute SCT model used in the present experiments in which sensitization of bladder afferent nerves causes the rapid unmasking of a spinal micturition reflex may be the most useful in vivo preparation for identifying the spinal neurotransmitter mechanisms involved in neuromodulation and for evaluating new strategies for the treatment of OAB.

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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