Involvement of metabotropic glutamate receptor 5 in pudendal inhibition of nociceptive bladder activity in cats

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Non-technical summary Lower urinary tract disorders including painful and overactive bladder conditions are very difficult to treat. Neuromodulation which is one of the successful therapies for lower urinary tract disorders, stimulates afferent nerves to modulate the neural pathway and achieve a therapeutic effect. We show that the metabotropic glutamate receptor 5 is activated in the central nervous system during pudendal neuromodulation. Understanding the neurotransmitter mechanisms involved in neuromodulation therapy may promote the development of new pharmacological treatments or improve the clinical outcome by combining neuromodulation with pharmacological therapy.

Abstract This study used MTEP, a metabotropic glutamate receptor 5 (mGluR5) antagonist, to examine the role of mGluR5 in the neural control of the urinary bladder and in the inhibition of the micturition reflex by pudendal nerve stimulation (PNS). Experiments were conducted in 11 female cats under α-chloralose anaesthesia when the bladder was infused with either saline or 0.25% acetic acid (AA). AA irritated the bladder, induced bladder overactivity and significantly ($P < 0.001$) reduced bladder capacity to $14.9 \pm 10.3\%$ of the saline control capacity. MTEP ($0.1–50 \text{ mg kg}^{-1}$, I.V.) significantly ($P < 0.05$) increased bladder capacity during saline distension but not during AA irritation. However, MTEP induced a transient inhibition of isovolumetric bladder contractions under both conditions. PNS (5 Hz), which was tested at the threshold (T) intensity for inducing a complete inhibition of isovolumetric bladder contractions and at an intensity of $3–4T$, suppressed AA-induced bladder overactivity and significantly increased bladder capacity to $68.0 \pm 31.3\%$ at $1T$ ($P < 0.05$) and $98.5 \pm 55.3\%$ at $3–4T$ ($P < 0.01$) of the saline control capacity. MTEP dose dependently ($0.1–50 \text{ mg kg}^{-1}$, I.V.) suppressed PNS inhibition of bladder overactivity at low intensity ($1T$) but not at high intensity ($3–4T$). During saline infusion PNS significantly ($P < 0.05$) increased bladder capacity to $167.7 \pm 27.1\%$ at $1T$ and $196.0 \pm 37.4\%$ at $3–4T$. These inhibitory effects were not observed after MTEP ($0.1–50 \text{ mg kg}^{-1}$, I.V.) which also increased bladder capacity. These results indicate that glutamic acid has a transmitter function in bladder and somato-bladder reflex mechanisms and raise the possibility that mGluR5 may be a target for pharmacological treatment of lower urinary tract disorders.

Introduction

Glutamate activates two classes of receptors, ionotropic (iGluRs) and metabotropic glutamatergic receptors (mGluRs). Previous studies (Maggi *et al.* 1990; Matsumoto *et al.* 1995; Yoshiyama *et al.* 1993*a*,*b*, 1995; Kakizaki *et al.* 1998) revealed that iGluRs are involved in central nervous system (CNS) control of the lower urinary tract (LUT). However, iGluR antagonists are not used clinically to treat LUT symptoms due to their CNS side effects (Bruno *et al.* 2001; Andersson & Wein, 2004). The mGluRs are G-protein-coupled receptors consisting of eight subtypes (mGluR1–8) in three groups (groups I–III) (De Blasi *et al.* 2001). Group I receptors (mGluR1 and mGluR5) activate phospholipase C, release intracellular Ca^{2+} and activate protein kinase C. The mGluR5s are widely expressed in CNS and primarily function as postsynaptic receptors that increase neuronal excitability and potentiate the effect of glutamate on iGluR (Jia *et al.* 1999; Carlton *et al.* 2001; Kew, 2004).

The mGluRs play a critical role in inflammation-induced hyperexcitability of spinal cord neurons (Neugebauer *et al.* 1999; Kew, 2004). Pathological conditions such as nerve injury can increase mGluR5 expression and induce plasticity in glutamatergic synaptic transmission that may play a role in neuropathic pain (Hudson *et al.* 2002; Dolan *et al.* 2003). Intrathecal administration of group I mGluR agonists induces hyperalgesia and nociceptive behaviours (Fisher & Coderre, 1996, 1998). Conversely, mGluR5 antagonists attenuate peripheral thermal nociception and inflammatory pain (Bhave *et al.* 2001; Walker *et al.* 2001) as well as inhibit the irritation-induced bladder overactivity (Guarneri *et al.* 2008). However, mGluR5 antagonists also significantly reduce the antinociceptive/antihyperalgesic effects of cannabinoids or capsaicin injected into the periaqueductal grey matter or the spinal cord (Palazzo *et al.* 2001, 2002;

Hama & Uran, 2004). Thus the involvement of mGluR5 in nociceptive mechanisms is complex and seems to vary at different sites in the CNS.

In this study we investigated the role of mGluR5 in nociceptive and antinociceptive mechanisms affecting urinary bladder reflexes in anaesthetized cats. Intravesical infusion of dilute acetic acid was used as the nociceptive stimulus to induce bladder overactivity. PNS was used as the antinociceptive stimulus to model the clinical use of neuromodulation in treating painful and overactive bladder conditions. In humans interstitial cystitis/bladder pain syndrome (IC/BPS) is a symptom complex of urinary urgency, frequency and bladder pain that can be treated effectively by either PNS or sacral root stimulation (Peters, 2002; van Kerrebroeck *et al.* 2007). Pudendal neuromodulation has been reported to be superior to sacral neuromodulation in patients with intractable LUT symptoms or IC/BPS (Peters *et al.* 2002, 2005). We hypothesized that pudendal inhibition of irritation-induced bladder overactivity could be due to activation of mGluR5s by the pudendal afferent firing at the synapses in the spinal cord or brain. MTEP (a potent and selective mGluR5 antagonist) was used to block the mGluR5s and test our hypothesis. Understanding the neurotransmitter mechanisms involved in pudendal neuromodulation may promote the development of new pharmacological treatments or improve the clinical outcome by combining neuromodulation with pharmacological therapy.

Methods

All protocols used in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh. Due to the physiological and anatomical similarities between cat and human in the lower urinary
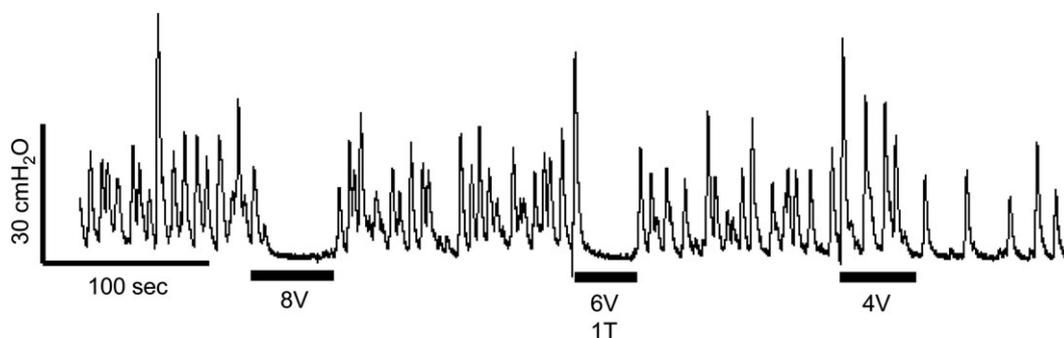
Figure 1. Intensity-dependent inhibitory effects of PNS on isovolumetric bladder contractions induced by distension of the bladder by 0.25% AA
Threshold intensity (*T*) for eliciting inhibition was 6 V. The black bars under the bladder pressure trace indicate stimulation duration. Stimulation: 5 Hz frequency, 0.2 ms pulse width.

tract, cats were used in this study and humanely killed at the end of the experiments by intravenous injection of 5 ml (2 mmol ml^{-1}) KCl under deep anaesthesia.

Experimental setup

Experiments were conducted in a total of 11 female cats (2.8–3.2 kg) anaesthetized initially with isoflurane (2–5% in oxygen) and maintained with α-chloralose (65 mg kg^{-1}
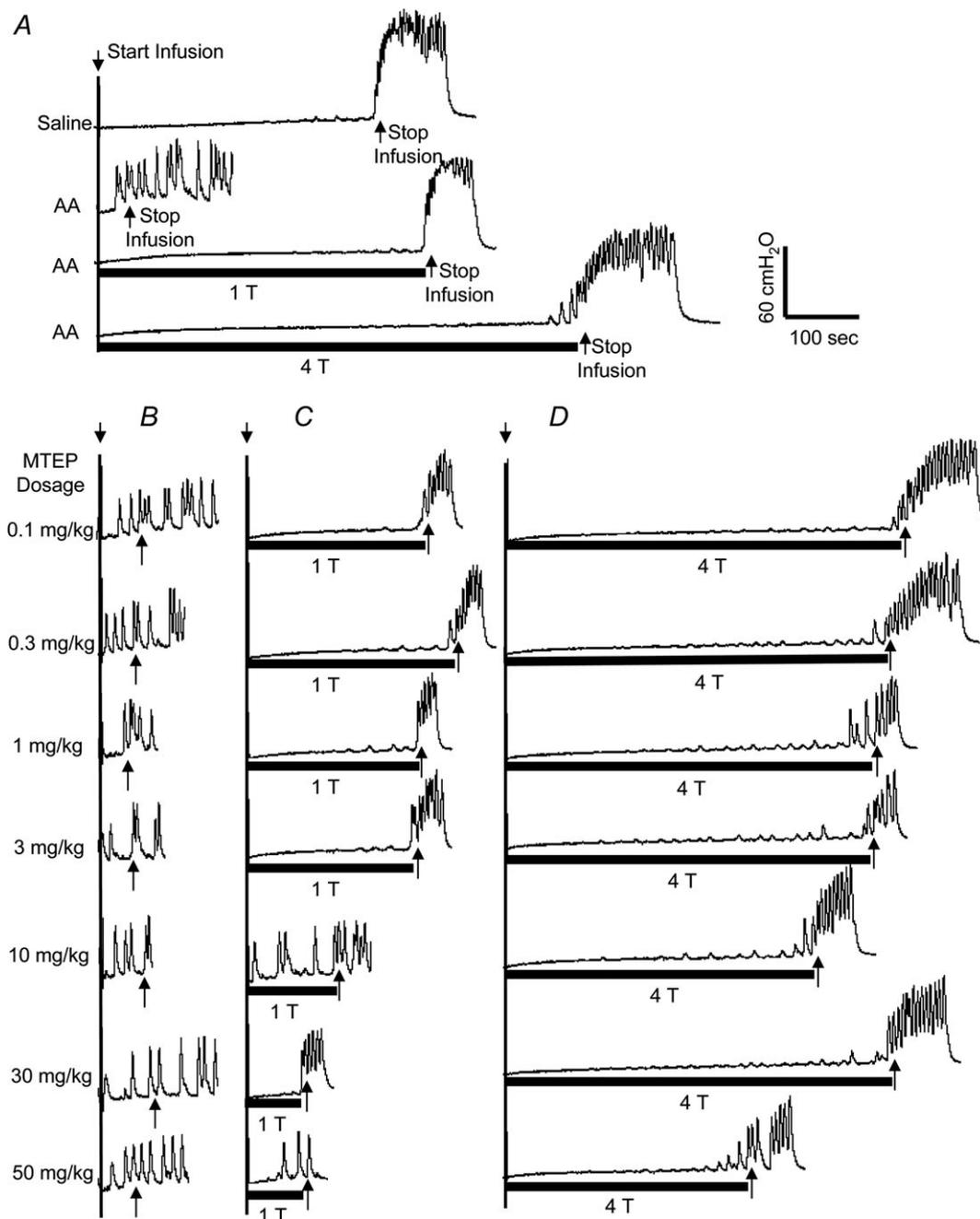
Figure 2. Dose-dependent effect of MTEP on pudendal inhibition and micturition reflex during CMGs
A, control CMGs without MTEP during saline or 0.25% AA infusion with/without stimulation. *B–D*, the MTEP dosage marked on the left is for panels *B*, *C* and *D*. The CMGs at increasing cumulative doses of MTEP were performed in sequence from left to right and from top to bottom in each panel. *B*, the AA CMGs without stimulation. *C*, the AA CMGs during 1*T* stimulation. *D*, the AA CMGs during 4*T* stimulation. The black bars under the pressure trace indicate stimulation duration. Stimulation: 5 Hz, 0.2 ms, inhibition threshold *T* = 1.5 V. Short arrows indicate the start and stop of bladder infusion.

i.v. with supplementation as necessary). Heart rate and blood oxygen level were measured by a pulse oximeter (9847 V, NONIN Medical, Inc., Playmouth, MN, USA) with the sensor attached to the tongue. Systemic blood pressure was measured via a catheter in the carotid artery. Drug and fluid were administered via the ulnar vein, and airway access was secured with a tracheostomy tube.

The ureters were isolated via an abdominal incision, cut, and drained externally. The bladder was cannulated through the urethra with a double lumen catheter. One lumen was used to infuse saline or 0.25% acetic acid (AA) at a rate of $0.5–2\ ml\ min^{-1}$, and the other lumen was attached to a pressure transducer to record the bladder pressure. A ligature was tied around the urethra to prevent leakage. The pudendal nerve was dissected from the left

Figure 3. Summarized results of the effects of MTEP on the micturition reflex and on inhibition of the micturition reflex during PNS after irritation of the bladder with 0.25% AA
A, effect of 0.25% AA irritation and PNS inhibition on bladder capacity. *Significantly different from 0.25% AA data. *B*, dose-dependent effect of MTEP on bladder capacity with or without PNS during AA infusion. *Significantly different from the bladder capacity measured at 1*T* without MTEP treatment (i.e. at 1*T* and 0 mg kg^{-1}). #Significantly different from the AA control data. Stimulation: 5 Hz, 0.2 ms. *T*, threshold intensity for inducing complete inhibition of isovolumetric rhythmic bladder contractions. *n* = 6 cats.

side via a 3–4 cm incision between the tail and the sciatic notch. A tripolar cuff electrode (NC223pt, MicroProbe, Inc., Gaithersburg, MD, USA) was applied around the nerve and connected to a stimulator (S88, Grass Medical Instruments, Quincy, MA, USA).

Stimulation protocol

Initially a cystometrogram (CMG) was performed with saline infusion to determine the bladder capacity that was defined as the bladder volume threshold to induce a large amplitude ($>30\ cm\ H_2O$) and long duration ($>20\ s$) bladder contraction. Then, multiple saline CMGs were repeated to evaluate the reproducibility. Once the bladder capacity was determined during saline infusion, pharmacological studies were performed in two experimental groups. In the AA group ($n = 6$ cats) 0.25% AA was infused into the bladder during repeated CMGs in order to activate nociceptive bladder C-fibre afferents and induce overactive bladder reflexes. In the saline experimental group ($n = 5$ cats) repeated CMGs were performed to initiate reflex bladder activity by non-nociceptive bladder afferent Aδ-fibres. In both experimental groups the bladder was first infused to a volume about 100–110% of the bladder capacity to induce isovolumetric rhythmic bladder contractions. Based on our previous studies (Tai *et al.* 2006, 2008, 2011; Chen *et al.* 2010), 5 Hz PNS was used to determine the intensity threshold (T) for completely inhibiting the isovolumetric rhythmic bladder contractions. Then, multiples (1T or 3–4T) of the threshold intensity were used in the pharmacological experiments. PNS at these intensities (1–4T) induced clearly observable contractions of anal sphincter.

Before administering MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine), the bladder capacity was first determined in both experimental groups under the following three conditions: (1) control condition, no stimulation was applied during the CMG; (2) 1T condition, PNS at 1T intensity was applied during the CMG; (3) 3–4T condition, PNS at 3–4T intensity was applied during the CMG. Then, increasing cumulative doses of MTEP (0.1, 0.3, 1, 3, 10, 30, or 50 mg kg^{-1}, i.v.) were administered to the animal at about 50–70 min intervals between each dose. After each dose of MTEP, three CMGs were performed under the three different conditions (i.e. control, 1T and 3–4T) to determine the drug effect on bladder capacity. Before administering each dose of MTEP, an additional control CMG was performed to infuse the bladder to a volume about 100–110% of the micturition threshold volume to induce rhythmic bladder contractions. Individual doses of MTEP were administered during the isovolumetric rhythmic bladder contractions, and the acute MTEP effect on isovolumetric rhythmic bladder contractions was monitored in the

subsequent 10–15 min period. The bladder was emptied after each CMG, and a 5–10 min rest period was inserted between successive CMGs to allow the distended detrusor to recover.

Data analysis

For the repeated CMG recordings, bladder capacities were measured and normalized to the measurement of the first saline control CMG in the same animal so that the results from different animals could be compared. The transient inhibitory effect of MTEP on the isovolumetric rhythmic bladder contractions was measured by the duration of the complete inhibition of the bladder activity. Repeated measurements under the same conditions in the same animal were averaged. The results from different animals were averaged and reported as mean ± standard error of the mean. Statistical significance ($P < 0.05$) was detected by Student's t test or ANOVA followed by Bonferroni post tests.

Results

Intensity-dependent inhibitory effect of PNS on isovolumetric bladder activity

The inhibition of isovolumetric rhythmic bladder contractions during AA irritation was dependent on the intensity

of PNS (see Fig. 1). The intensity threshold (T) to induce a complete inhibition was determined to be 6 V in the experiment shown in Fig. 1. The same protocol was used to determine the inhibition threshold during saline distension. Complete inhibition of isovolumetric rhythmic bladder contractions as shown in Fig. 1 was elicited in all animals. The inhibition thresholds (T) were not significantly different during AA irritation (3.2 ± 1.3 V) or during saline distension (2.3 ± 0.3 V).

Dose-dependent effect of MTEP on pudendal inhibition of bladder overactivity

CMGs revealed that bladder capacity which averaged 7.9 ± 1.8 ml during saline infusion was reduced by more than 80% to 1.2 ± 0.5 ml during infusion of 0.25% AA (first 2 traces in Fig. 2A, and Fig. 3A). PNS at intensities of $1T$ and $3–4T$ significantly increased bladder capacity (third and fourth traces in Fig. 2A, and Fig. 3A).

The effect of MTEP on pudendal inhibition of bladder overactivity is dependent on the drug dosage and the intensity of PNS (Fig. 2C and D). Administering cumulative doses of MTEP (0.1, 0.3, 1, 3, 10, 30 and 50 mg kg^{-1}, I.V.) did not significantly change the bladder capacity in the absence of stimulation (Fig. 2B), but progressively reduced the effect of PNS (Fig. 2C and
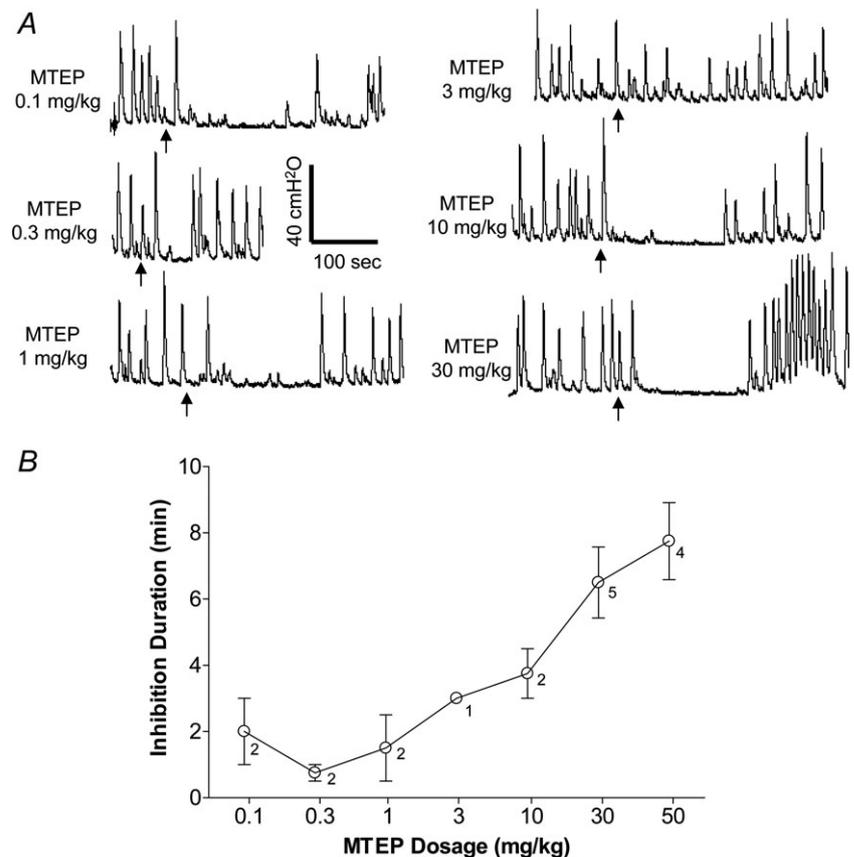
Figure 4. Transient inhibitory effect of MTEP on isovolumetric rhythmic bladder contractions during AA distension
A, recordings showing examples of complete inhibition of reflex bladder contractions during a short period after administration of MTEP (indicated by arrows). *B*, average duration of the complete inhibition increases with cumulative MTEP doses. $n = 6$ cats. Note: results in *B* exclude the data from tests where the MTEP-elicited transient inhibition was absent (i.e. duration = 0 min, see 3 mg kg^{-1} dose in *A*). The number beside each data point indicates the number of animals that exhibited the transient inhibition.

D). At a stimulation intensity of 1*T*, the highest doses of MTEP completely blocked the pudendal inhibition (Fig. 2*B* and *C*). However, at the higher stimulation intensity (4*T*) even the highest doses of MTEP only partially reduced the inhibitory effect of PNS on the bladder capacity (Fig. 2*D*). The summarized data from six cats is shown in Fig. 3. Bladder irritation by infusing 0.25% AA significantly ($P < 0.001$) reduced bladder capacity to $14.9 \pm 10.3\%$ of saline control capacity (Fig. 3*A*). PNS at intensities of 1*T* and 3–4*T* significantly increased the capacity to $68.0 \pm 31.3\%$ ($P < 0.05$) and $98.5 \pm 55.3\%$ ($P < 0.01$), respectively, of the saline control capacity (Fig. 3*A*). Cumulative doses of MTEP did not significantly change the small bladder capacity caused by AA irritation

(Fig. 3*B*). However, at doses greater than 1 mg kg^{-1} MTEP significantly ($P < 0.05$) reduced the increase in bladder capacity induced by PNS at 1*T* (Fig. 3*B*). Cumulative doses of MTEP only partially ($P > 0.05$) reduced the inhibitory effect of higher PNS intensities (3–4*T*) (Fig. 3*B*).

Transient effect of MTEP on isovolumetric rhythmic bladder contractions during AA irritation

Although cumulative doses of MTEP did not change the small bladder capacity induced by AA irritation (Fig. 3*B*), they did cause a transient complete inhibition of the isovolumetric rhythmic bladder contractions (Fig. 4*A*). This transient effect was observed in all animals during
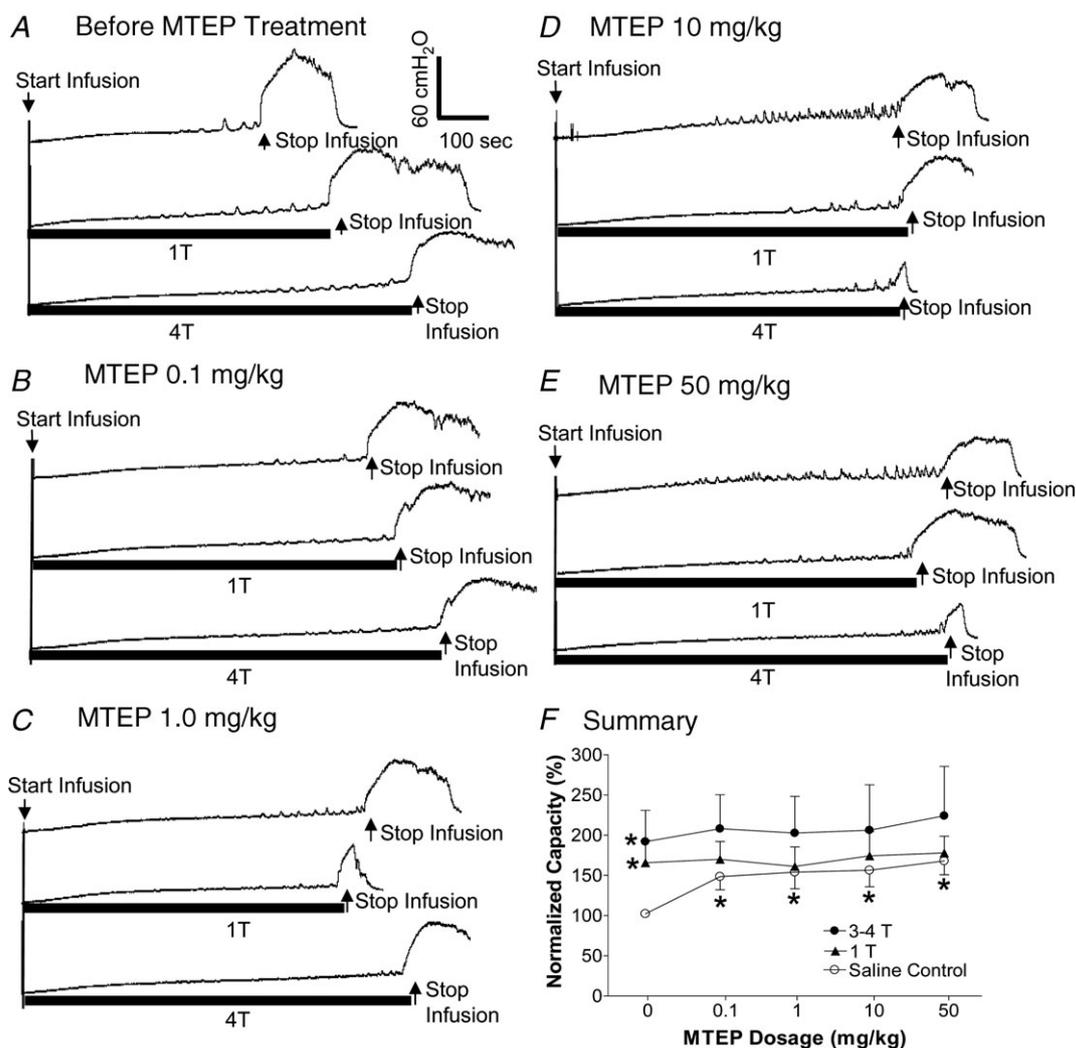
Figure 5. Dose-dependent effect of MTEP on pudendal inhibition and micturition reflex during saline infusion CMGs
A–E, CMG traces with or without PNS at different dosage of MTEP. Stimulation: frequency 5 Hz, pulse width 0.2 ms, and inhibition threshold $T = 1.5$ V. The black bars under bladder pressure traces mark the stimulation duration. Arrows indicate the start and stop of saline infusion. *F*, bladder capacity measured at different dosage of MTEP ($n = 5$ cats). *Significantly different from the bladder capacity measured during saline control before MTEP treatment (i.e. saline control at 0 mg kg^{-1}).

AA irritation ($n = 6$), but did not occur at every MTEP dose in each animal. The effect was usually detectable within 1–2 min of MTEP administration and lasted 1–10 min. The duration of the observed transient inhibition increased with increasing doses of MTEP as shown in Fig. 4B that excluded the data from tests where the MTEP-elicited transient inhibition was absent (i.e. duration = 0 min).

Effect of MTEP on pudendal inhibition of bladder activity during saline distension

Before administration of MTEP the bladder capacity measured during saline distension was significantly ($P < 0.05$) increased by PNS to $167.7 \pm 27.1\%$ ($1T$) and $196.0 \pm 37.4\%$ (3–$4T$) of control capacity (Fig. 5A and F). Administering cumulative doses of MTEP (0.1, 1, 10 and 50 mg kg^{-1}, I.V.) significantly ($P < 0.05$) increased the bladder capacity in the absence of stimulation to approximately 150% of control capacity (Fig. 5B–F). PNS at $1T$ and 3–$4T$ after treatment with MTEP did not increase bladder capacity further (Fig. 5B–F). Bladder capacities during PNS at $1T$ and 3–$4T$ intensities were not significantly different before or after MTEP administration (Fig. 5F).

Inhibitory effect of MTEP on isovolumetric rhythmic bladder contractions during saline distension

MTEP at every dosage completely inhibited isovolumetric rhythmic bladder contractions during saline distension in all animals ($n = 5$) (Fig. 6). The inhibition was only monitored for 10–15 min in order to follow the same experimental protocol as in the experiments using

AA irritation. Since the inhibition lasted more than 10–15 min, the bladder was emptied and the recording was interrupted (see Fig. 6). However, in two animals at an MTEP dose of 1 mg kg^{-1} the recording was continued until spontaneous bladder contractions returned (second trace in Fig. 6) indicating a period of complete inhibition lasting about 30 min.

Discussion

This study revealed that intravenous administration of MTEP, an mGluR5 antagonist, suppressed reflex bladder activity as well as the PNS-induced inhibition of bladder activity. The magnitude of the effects of MTEP depended on the conditions of the experiment (see Table 1). For example when the bladder was filled with saline the MTEP inhibition of isovolumetric bladder contractions and the increase in bladder capacity was prominent, while the MTEP suppression of PNS inhibition was modest. However, in AA-irritated bladders MTEP had a weaker effect on isovolumetric bladder contractions, did not change bladder capacity but significantly blunted the increase in bladder capacity induced by PNS. These results indicate that glutamic acid is an excitatory transmitter in the micturition reflex pathway and also a transmitter in the PNS-evoked central inhibitory mechanisms that modulate bladder capacity (Fig. 7).

MTEP could act at multiple sites to suppress micturition reflexes. Saline distension of bladder primarily activates non-nociceptive, mechano-sensitive, Aδ bladder afferents that trigger a spinobulbospinal micturition reflex transmitted through the spinal cord to synapses in the periaqueductal grey (PAG) and the pontine micturition centre (PMC) (Fig. 7) (Fowler *et al.* 2008). On the other hand
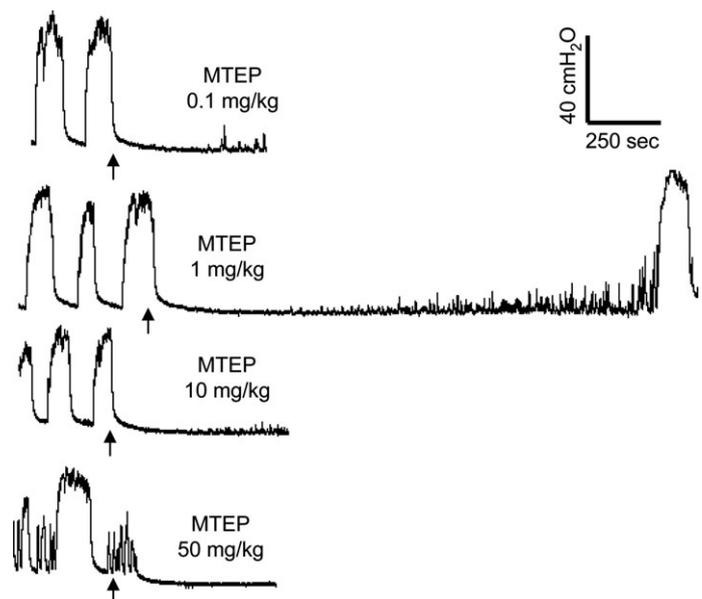
Figure 6. Transient inhibitory effect of MTEP on isovolumetric rhythmic bladder contractions during saline distension
After the MTEP is given (indicated by arrow) a complete inhibition of bladder contractions is observed in all cats at different doses. The inhibition lasted longer than the 10–15 min observation period ($n = 5$). In two cats the inhibition was monitored until contractions returned indicating a recovery period of about 30 min (see the second trace).

Table 1. Changes in bladder capacity under different experimental conditions

	PNS		
	Untreated	MTEP treated	MTEP
Saline CMG	Increase	No effect	Increase
AA CMG	Increase	No effect	No effect

AA irritation of the bladder activates nociceptive, C-fibre bladder afferents that facilitate the supraspinal micturition reflex and/or activate a spinal micturition reflex (Fig. 7) (Chancellor & de Groat, 1999; Fowler *et al.* 2008). Although glutamic acid and iGluRs, such as AMPA and NMDA receptors, are known to be important at spinal and supraspinal synapses in the micturition reflex pathways (Yoshiyama *et al.* 1993*a*,*b*; Yoshiyama & de Groat, 2005) the sites where mGluR5s play a role are still uncertain.

mGluRs are widely expressed in both the central and peripheral nervous systems and are known to modulate neuronal excitability and synaptic transmission (Shigemoto *et al.* 1993; Valerio *et al.* 1997; Hama & Urban, 2004). Neuroanatomical and immunocytochemical studies (Vidnyanszky *et al.* 1994; Alvarez *et al.* 2000; Cartmell & Schoepp, 2000) suggest that group I mGluRs are excitatory and primarily localized postsynaptically. Previous studies in rats (Yoshiyama & de Groat, 2007) showed that intrathecal injection of MCPG (a group I/II mGluR antagonist) did not alter the micturition reflex induced by saline distension of the bladder; while intrathecal injection of ACPD (a group I/II mGluR agonist) inhibited the micturition reflex induced by saline distension (Tanaka *et al.* 2003). More recent studies in rats demonstrated that intravenous administration of MPEP, a less selective and less potent mGluR5 antagonist than MTEP (Lea & Faden, 2006), dose dependently increased bladder capacity and inhibited the micturition reflex induced by saline distension of the bladder (Guarneri *et al.* 2008; Hu *et al.* 2009) and also increased bladder capacity during AA irritation (Guarneri *et al.* 2008). MPEP did not change bladder Aδ afferent firing in response to saline distension indicating that a central rather than a peripheral action must mediate the effect of the drug (Hu *et al.* 2009).

Our present study in cats showed that MTEP suppresses reflex bladder contractions recorded under isovolumetric conditions during both saline distension and AA irritation of the bladder, but significantly increases bladder capacity only during saline infusion. These data suggest that MTEP raises the volume threshold for triggering the spino-bulbospinal micturition reflex but does not alter the volume threshold for the spinal C-fibre reflex and/or the facilitated spinobulbospinal reflex. The suppression of the spinobulbospinal micturition reflex by MTEP suggests that mGluR5s expressed on interneurons in the spinal cord or brain stem are essential for excitatory transmission in the reflex pathway (Fig. 7). Conversely, mGluR5s seem to be less important at spinal synapses involved in the micturition reflex activated by nociceptive C-fibre afferents.

Our recent study in cats (Chen *et al.* 2010) showed that enkephalins and opioid receptors can account for only part of the PNS inhibition of the spinobulbospinal micturition reflex activated by Aδ afferents, indicating that other neurotransmitters/receptors must be involved in the inhibition. The present study indicates that the mGluR5 mechanisms might play a role in the non-opioid PNS inhibition (Fig. 5) and in PNS inhibition of the bladder overactivity initiated by AA activation of nociceptive C-fibre bladder afferents (Figs 2 and 3). Whether opioid receptors play a role in PNS inhibition of nociceptive bladder activity still needs to be investigated. However, based on these findings it seems reasonable to hypothesize that PNS inhibition of bladder activity must involve multiple neurotransmitters/receptors and that these neurotransmitters might be different for the inhibitory mechanisms modulating the spinobulbospinal and spinal micturition reflex pathways.
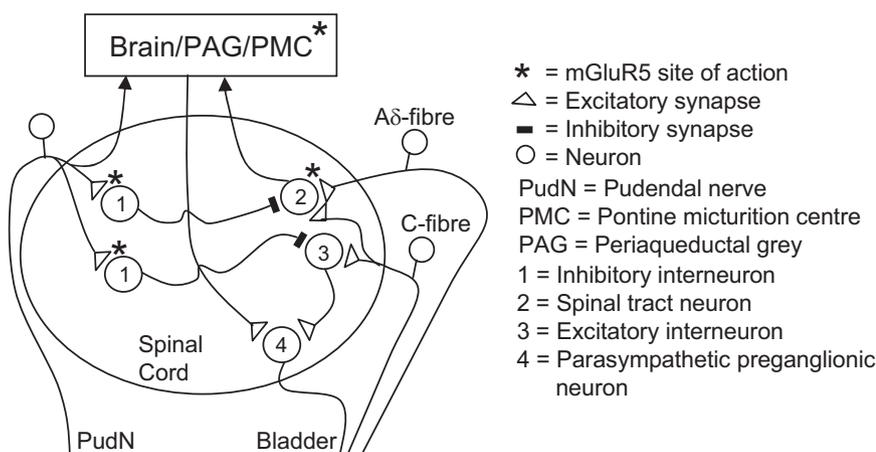
Figure 7

The possible locations (*) in the sacral spinal cord and brain stem of mGluR5s that participate in: (1) the spinobulbospinal and spinal micturition reflex pathways, and (2) the micturition inhibitory mechanism evoked by PNS. * also indicates possible sites of action of the mGluR5 antagonist.

* = mGluR5 site of action
△ = Excitatory synapse
■ = Inhibitory synapse
○ = Neuron
PudN = Pudendal nerve
PMC = Pontine micturition centre
PAG = Periaqueductal grey
1 = Inhibitory interneuron
2 = Spinal tract neuron
3 = Excitatory interneuron
4 = Parasympathetic preganglionic neuron

The site at which MTEP affects pudendal nerve-mediated inhibition is uncertain. It is known that group I mGluRs are expressed on spinal inhibitory interneurons and that activation of these receptors enhances firing, and thereby, increases downstream inhibition (Vidnyanszky et al. 1994). Therefore, pudendal nerve inhibition of bladder activity may depend on glutamatergic excitatory transmission via mGluR5s on spinal interneurons that release inhibitory neuro-transmitters (such as enkephalins, GABA and glycine) which have been shown to be involved in the inhibitory mechanisms controlling bladder reflexes (de Groat & Ryall, 1968; Booth et al. 1985; Mallory et al. 1991; Noto et al. 1991; de Groat et al. 1993; Miyazato et al. 2004). Because electrophysiological experiments have revealed that stimulation of pudendal afferents evokes short latency, presumably disynaptic, inhibitory responses in bladder preganglionic neurons (de Groat & Ryall, 1969; de Groat et al. 1982) it seems reasonable to hypothesize that pudendal primary afferent terminals make direct glutamatergic (mGluR5 mediated) excitatory synaptic connections with inhibitory interneurons that in turn suppress excitatory transmission in spinal and/or supraspinal components of micturition reflexes (Fig. 7). A suppression by MTEP of excitatory transmission at these sites would be expected to significantly reduce the pudendal inhibition of bladder activity induced by either $A\delta$- or C-fibre afferents. The dose-dependent decrease in PNS inhibition during AA irritation (Figs 2 and 3) supports this idea. The failure of PNS to increase bladder capacity during saline infusion following administration of a range of MTEP doses (0.1–50 mg kg^{-1}, I.V.) is also consistent with this hypothesis. However, the experiments using saline distension are difficult to interpret because MTEP even in the lowest dose significantly increased bladder capacity to 150% of control. This inhibitory effect of MTEP may have shifted bladder capacity to a maximum level thereby occluding rather than blocking the inhibitory effect of PNS. This interpretation is consistent with the demonstration prior to MTEP treatment that increasing the PNS intensity from $1T$ to 3–$4T$ did not significantly increase the inhibition during saline infusion suggesting that it was maximal.

It is worth noting that immunohistochemical studies have indicated the localization of mGluR5 receptors in cat brain (Reid et al. 1995; Goodwin et al. 1996), but studies regarding the localization in cat spinal cord are currently not available. However, distribution of mGluR5 receptors in the spinal cord has been shown in many other mammalian species including mice, rats and sheep (Anwyl, 1999; Karim et al. 2001; Dolan et al. 2003; Pitcher et al. 2007; Li et al. 2010). Therefore, it is highly likely that mGluR5 receptors are also located in the cat spinal cord. However, an immunohistochemical study of the cat spinal cord is warranted to localize the mGluR5 receptors.

In this study PNS induced observable contractions of the anal sphincter. Although 5 Hz PNS was used, the frequency of sphincter afferent activity induced by contractions might be different from 5 Hz. Both the PNS-induced and the sphincter contraction-induced afferent activity in the pudendal nerve could contribute to the inhibitory effect of PNS on reflex bladder activity.

Understanding the neurotransmitter mechanisms involved in pudendal neuromodulation could identify pharmacological targets for development of new therapies to treat patients suffering from IC/BPS, pelvic pain or LUT symptoms. Furthermore, neuromodulation therapies currently used in clinical applications could also be improved to achieve better clinical outcomes when combined with pharmacological treatments that enhance synaptic transmission in the inhibitory reflex pathways.

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Author contributions

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