Published in final edited form as:

J Urol. 2012 September ; 188(3): 1012–1016. doi:10.1016/j.juro.2012.04.096.

Involvement of Opioid Receptors in Inhibition of Bladder Overactivity Induced by Foot Stimulation in Cats

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Abstract

Purpose—We examined the role of opioid receptors in the inhibition of bladder overactivity induced by electrical stimulation of the foot.

Materials and Methods—Experiments were done in 6 cats under α -chloralose anesthesia when the bladder was infused with saline or 0.25% acetic acid. Naloxone (1 mg/kg intravenously) was administered to block opioid receptors. To modulate reflex bladder activity electrical stimulation (5 Hz, 0.2 millisecond pulse width) was applied to the foot via skin surface electrodes at intensities of multiple times the threshold needed to induce observable toe movement.

Results—Acetic acid irritated the bladder, induced bladder overactivity and significantly decreased bladder capacity to a mean $\pm$ SE 25.3% $\pm$ 5.9% that of saline control capacity ($p = 0.0001$). Foot stimulation at 4T suppressed acetic acid induced bladder overactivity and significantly increased bladder capacity to 47.1% $\pm$ 5.9% of control ($p = 0.0007$). Naloxone did not significantly change bladder capacity during acetic acid irritation but it completely eliminated the inhibition of bladder overactivity induced by foot stimulation.

Conclusions—Results indicate that opioid receptors have an important role in foot afferent inhibition of bladder overactivity. This raises the possibility that opioid receptors might be used as a pharmacological target to enhance the efficacy of foot stimulation for inhibiting bladder overactivity.

Keywords

urinary bladder; overactive; electric stimulation; receptors; opioid; foot; cats

Overactive bladder symptoms, including urgency, frequency and incontinence, are difficult to manage with medication.¹ Currently tibial, pudendal and sacral neuromodulation^{2–5} are used to treat these conditions when they are refractory to other therapy. However, sacral and pudendal neuromodulation requires surgery to implant an InterStim® stimulator and electrodes.^{6–9} Tibial neuro-modulation requires 30-minute stimulation once weekly for 12 consecutive weeks via a percutaneously inserted Urgent PC® needle electrode.¹⁰ A skilled medical staff inserts the needle electrode close to the tibial nerve during each treatment. After the initial 12-week therapy maintenance treatments are usually required once every 2

to 3 weeks.¹¹ An effective, noninvasive neuromodulation therapy that is easily manageable by the patient is currently needed.

Previous research efforts were directed toward developing noninvasive neuromodulation approaches for OAB, including intravaginal¹² or intra-anal¹³ stimulation with ring electrodes on a vaginal/anal plug or dorsal penile/clitoral nerve stimulation using transcutaneous electrical stimulation applied to the penis or perigenital skin area.^{14–17} However, these approaches are not used clinically since they target inconvenient locations and cause discomfort and difficulty in maintaining the electrodes in place for an extended period.

Our recent studies in cats identified what is to our knowledge a new approach to suppress bladder over-activity by activating somatic afferent nerves in the foot using skin surface electrodes.^{18,19} Foot stimulation, which is noninvasive and convenient, could be developed as OAB treatment. Our studies in cats also indicated that foot stimulation¹⁸ is similar to tibial neuromodulation² since it did not completely suppress experimentally induced bladder overactivity. However, if the neurotransmitters involved in the inhibition induced by foot stimulation could be identified, pharmacological agents targeting these neurotransmitters might be used to enhance the effect of stimulation.

Thus, in this study we investigated the role of opioid peptide transmitters and opioid receptor activation in the inhibition of irritation induced bladder overactivity using electrical stimulation of afferent nerves in the foot. Naloxone was used to block the opioid receptors. Understanding the neurotransmitter mechanisms involved in foot neuromodulation may promote the development of new pharmacological treatments or improve the clinical outcome by combining neuromodulation with pharmacological therapy.

MATERIALS AND METHODS

All protocols used in this study were approved by the University of Pittsburgh animal care and use committee.

Experimental Setup

Experiments were done in 4 male and 2 female cats weighing 2.5 to 3.2 kg. Anesthesia was initiated with isoflurane (2% to 5% in oxygen) and maintained with α -chloralose (65 mg/kg intravenously with supplementation as needed). Heart rate and blood oxygen level were monitored by a 9847V handheld pulse oximeter (NONIN®) with the sensor attached to the tongue. Systemic blood pressure was monitored by a catheter in the carotid artery. Drug and fluid were administered via the cephalic 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% AA at 0.5 to 2 ml per minute. The other lumen was attached to a pressure transducer to record bladder pressure. A ligature was tied around the urethra to prevent leakage. After removing the fur surface self-adhesive F-E10ND pad electrodes 1 cm in diameter were attached to the skin area on the left hind foot and connected to an S88 stimulator (Grass Medical Instruments, Quincy, Massachusetts) (fig. 1).

Stimulation and Drug Test Protocol

Initially CMG was performed with saline infusion to determine control bladder capacity, defined as the bladder volume threshold needed to induce a micturition reflex contraction greater than 30 cm H₂O in amplitude. Multiple saline CMGs were repeated to evaluate

reproducibility. After bladder capacity was determined during saline infusion 0.25% AA was infused in the bladder during repeat CMGs to activate nociceptive bladder C-fiber afferents and induce bladder overactivity. The bladder was emptied after each CMG and a 3 to 5-minute rest period was allowed between successive CMGs to enable the distended detrusor to recover. Uniphasic rectangular pulses at 5 Hz frequency and 0.2 millisecond pulse width were used to stimulate the foot. The T needed to induce observable toe movement was determined by gradually increasing stimulation intensity. Multiples of T (4 to 8T) were used during the experiments.

Before administering naloxone bladder capacity was determined during AA infusion under 2 conditions. 1) As the control, no stimulation was applied during CMG. 2) For the 4T condition foot stimulation at 4T intensity was applied during CMG.

After emptying the bladder a single dose of naloxone (1 mg/kg intravenously) was administered, which was large enough to maximally facilitate the micturition reflex during saline infusion.²⁰ Beginning 5 minutes after naloxone administration an additional 2 AA CMGs were done under control or 4T conditions to determine the drug effect on bladder capacity. At the end of the experiments foot stimulation at higher intensity (6 to 8T) was applied during AA CMG to determine whether inhibition could be enhanced by increasing stimulation intensity.

Data Analysis

For repeat CMG recording bladder capacity was measured and normalized to the measurement of the first saline control CMG in the same cat so that results in different cats could be compared. Repeat measurements under the same conditions in the same cat were averaged. Results in different cats were averaged and are shown as the mean $\pm$ SE. Statistical significance was determined by the paired Student t test at $p < 0.05$.

RESULTS

Bladder infusion with 0.25% AA irritated the bladder and significantly decreased mean capacity to $25.3\% \pm 5.9\%$ (3.1 ± 0.5 ml) of the 13.4 ± 1.2 ml measured during saline CMG ($p = 0.0001$, figs. 2 and 3). Foot stimulation at 4T intensity significantly increased bladder capacity to a mean of $47.1\% \pm 5.9\%$ of control capacity ($p = 0.0007$, see table and figs. 2 and 3).

Intravenous administration of naloxone (1 mg/kg) markedly changed CMG and complicated bladder capacity measurement. After naloxone the large amplitude (greater than 30 cm H₂O), long duration (greater than 30 seconds) bladder contractions that were used to estimate bladder capacity during control CMGs were eliminated. They were replaced by short duration (15 to 19 seconds), frequent (1 to 1.4 per minute) contractions that appeared at small bladder volumes near the beginning of CMG. In 6 cats contractions gradually increased in amplitude as the bladder filled (fig. 2). In these recordings bladder capacity was measured using the same criterion as for control CMGs, ie the bladder volume at which contractions exceeded 30 cm H₂O. Using this method bladder capacity did not significantly change after naloxone (see table and figs. 2 and 3). However, after naloxone 4T foot stimulation failed to inhibit bladder activity and did not increase bladder capacity (figs. 2 and 3). Further increasing stimulation intensity to 6 to 8T inhibited the small amplitude bladder contractions in 2 of the 3 tested cats but still failed to increase bladder capacity (see table and figs. 2 and 3).

DISCUSSION

This study revealed that opioid receptors are involved in the inhibition of bladder overactivity induced by foot stimulation in cats. Thus, agonists targeting opioid receptors might be useful in combination with foot stimulation to enhance the inhibitory effect on bladder overactivity. Further studies of opioid receptor agonists combined with foot stimulation are warranted.

Naloxone treatment changed the characteristics of bladder activity during AA CMG (fig. 2), causing frequent bladder contractions of small amplitude before inducing a large amplitude (greater than 30 cm H₂O) micturition reflex contraction. The small amplitude pre-micturition contractions also emerged after naloxone treatment during saline CMGs in our previous study in chloralose anesthetized cats but not in cats under ketamine anesthesia.²⁰ This effect of naloxone suggests that the small amplitude contractions were tonically inhibited by an opioid receptor mechanism and emerged after opioid receptors were blocked. These small amplitude pre-micturition contractions could be inhibited by foot stimulation at higher intensity but the bladder volume threshold (bladder capacity) needed to induce a large amplitude micturition contraction was not increased by low or high intensity stimulation. Results indicate that the small amplitude pre-micturition contractions unmasked by naloxone treatment must be mediated by a different neural mechanism than the large amplitude micturition contractions. Also, nonopioid mechanisms must be involved in the foot inhibition of the small amplitude pre-micturition contractions.

Our previous studies showed that foot stimulation at an intensity 2 to 4 times the threshold needed to induce toe movement was strong enough to inhibit the bladder overactivity induced by bladder irritation.^{18,19} At this intensity range the stimulation probably only activated large afferent nerve fibers^{21,22} rather than small Aδ and C-fiber afferents, which can induce painful sensations. In this study higher stimulation intensity (6 to 8T) did not generate an additional increase in bladder capacity after the inhibitory effect induced by low (4T) intensity stimulation was fully antagonized by naloxone (figs. 2 and 3). This indicates that increasing stimulation intensity to recruit more afferent nerve fibers in the foot and activate smaller diameter fibers does not evoke additional nonopioid inhibitory mechanisms, such as those mediated by GABA or glycine. Thus, lower intensity (2 to 4T) foot stimulation might be optimal for clinical applications for OAB.

Tibial nerve innervates the plantar side of the foot. Electrical stimulation applied to the plantar surface of the foot activates the same afferent nerves as tibial nerve stimulation at the ankle site. Tibial and pudendal afferent nerves overlap at the sacral spinal cord segments. However, foot stimulation by skin surface electrodes has several advantages over other transcutaneous stimulation methods. It targets a more convenient location than anal/genital, ie pudendal, stimulation with less discomfort and difficulty in maintaining the electrodes in place for an extended period. The nerves innervating the foot skin/muscle are close to the skin surface and easily activated by skin surface electrodes. Thus, patients could self-administer stimulation without requiring precise placement of the electrodes on the foot. In contrast, tibial neuromodulation requires insertion of a needle electrode by trained medical personnel to stimulate the tibial nerve at the ankle site.^{10,11}

Although OAB pathology is currently not fully understood,²³ the C-fiber bladder afferent pathway seems to have a role.^{24,25} We used AA to irritate the bladder, activate C-fiber bladder afferents and induce bladder overactivity. Although this model in chloralose anesthetized cats does not necessarily reflect the pathological conditions of OAB, it is useful to examine the possible neurotransmitter mechanisms underlying foot inhibition of C-fiber afferent mediated bladder overactivity in cats.

A clinical study to confirm our results is feasible since naloxone can be administered to humans at relatively high doses and electrical stimulation of the foot served as a sham control in a recent clinical study of the efficacy of tibial neuromodulation for OAB.²⁶ Although that study demonstrated the effectiveness of foot stimulation for OAB, the effect was weak and the decrease in OAB symptoms was limited. This clinical result is similar to our current result showing that foot stimulation in cats only partially suppressed the bladder overactivity induced by bladder irritation (figs. 2 and 3).

Due to the apparent similarity in the inhibitory effect of foot stimulation on bladder overactivity in humans and cats further pharmacological studies of foot stimulation in cats are warranted. They might help in designing clinical experiments aimed at identifying molecular targets for new pharmacotherapies for OAB or new drugs that might be used as combination therapy to enhance the efficacy of tibial, pudendal or sacral neuromodulation.

CONCLUSIONS

Opioid receptors are involved in the foot inhibition of chemical irritation induced bladder overactivity in cats. Foot stimulation, which is noninvasive and convenient, could potentially be an effective OAB treatment if pharmacological agents targeting opioid mechanisms can be used to enhance the inhibitory effect of foot stimulation.

Acknowledgments

Supported by National Institutes of Health Grants DK-068566, DK-090006 and DK-091253.

Abbreviations and Acronyms

AA	acetic acid
CMG	cystometrogram
OAB	overactive bladder
T	intensity threshold

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Figure 1.
Electrode placement for electrical stimulation of cat hind foot. One electrode each is placed at front of foot pad and at heel.

Figure 2.

Naloxone eliminated bladder capacity increase induced by 4 and 8T electrical stimulation of hind foot. Small amplitude pre-micturition contractions unmasked by naloxone were inhibited by 8 but not 4T electrical stimulation. CMG was repeated from infusion start to stop sequentially in same cat at 3 to 5-minute intervals. Stimulation was done at 5 Hz, 0.2 millisecond pulse width at T of 4 V and 1 ml per minute infusion rate. Bold horizontal bars indicate stimulation duration. Asterisk indicates contraction amplitude greater than 30 cm H₂O.

Figure 3.

Naloxone (*Nx*) effect on foot inhibition of bladder overactivity. Stimulation was done at 5 Hz, 0.2 millisecond pulse width at T of 4 to 14 V. Six cats were in saline, AA, AA plus 4T, AA plus naloxone and AA plus naloxone plus 4T groups, and 3 were in 6 to 8T group. Asterisk indicates statistically significant difference ($p < 0.05$).

Bladder capacity in each cat during different CMG conditions

Cat No.	% Saline (ml)	% AA (ml)	% AA Combined (ml)			
			4T	Naloxone	Naloxone + 4T	Naloxone + 6-8T
1	99.4 (17.2/17.3)	16.8 (2.9)	33.5 (5.8)	8.1 (1.4)	4.6 (0.8)	
2	101.8 (11.6/11.4)	13.2 (1.5)	47.4 (5.4)	2.6 (0.3)	2.6 (0.3)	1.8 (0.2)
3	94.0 (9.4/10)	50.0 (5.0)	70.0 (7.0)	8.0 (0.8)	18.0 (1.8)	27.0 (2.7)
4	98.7 (14.6/14.8)	18.9 (2.8)	44.6 (6.6)	20.3 (3.0)	9.5 (1.4)	
5	96.3 (10.4/10.8)	35.2 (3.8)	55.6 (6.0)	33.3 (3.6)	32.4 (3.5)	25.9 (2.8)
6	103.8 (16.6/16.0)	17.5 (2.8)	31.3 (5.0)	6.3 (1.0)	5.0 (0.8)	