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Effect of methysergide on pudendal inhibition of micturition reflex in cats

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ABSTRACT

The role of 5-HT_2 and opioid receptors in pudendal inhibition of bladder activity induced by intravesical infusion of saline or 0.25% acetic acid (AA) was investigated in anesthetized cats using methysergide (a 5-HT_2 receptor antagonist) and naloxone (an opioid receptor antagonist). AA irritated the bladder and significantly ($P < 0.0001$) reduced bladder capacity to 27.0 ± 7.4% of saline control capacity. Pudendal nerve stimulation (PNS) at multiples of the threshold (T) intensity for inducing anal sphincter twitching restored bladder capacity to 60.1 ± 8.0% at 1–2T ($P < 0.0001$) and 92.2 ± 14.1% at 3–4T ($P = 0.001$) of the saline control capacity. Methysergide (0.03–1 mg/kg, i.v.) suppressed low intensity (1–2T) PNS inhibition but not high intensity (3–4T) inhibition, and also significantly ($P < 0.05$) increased control bladder capacity at the dosage of 0.3–1 mg/kg. During saline infusion without AA irritation, PNS significantly increased bladder capacity to 150.8 ± 9.9% at 1–2T ($P < 0.01$) and 180.4 ± 16.6% at 3–4T ($P < 0.01$) of the saline control capacity. Methysergide (0.1–1 mg/kg) significantly ($P < 0.05$) increased saline control bladder capacity and suppressed PNS inhibition at the dosage of 0.03–1 mg/kg. After methysergide treatment (1 mg/kg), naloxone significantly ($P < 0.05$) reduced control bladder capacity during AA infusion but had no effect during saline infusion. Naloxone also had no influence on PNS inhibition. These results suggest that 5-HT_2 receptors play a role in PNS inhibition of reflex bladder activity and interact with opioid mechanisms in micturition reflex pathway. Understanding neurotransmitter mechanisms underlying pudendal neuromodulation is important for the development of new treatments for bladder disorders.

Introduction

Overactive bladder (OAB) is a symptom complex of urinary urgency, frequency and incontinence that can be treated by pudendal nerve or sacral neuromodulation (Peters et al., 2005, 2010; van Kerrebroeck et al., 2007). Pudendal neuromodulation has been reported to be superior to sacral neuromodulation in patients with intractable OAB or painful bladder syndrome (PBS) (Peters, 2002; Peters et al., 2005). However, the mechanisms underlying neuromodulation therapies are currently still unknown. Understanding the mechanisms could potentially improve the efficacy of these therapies or develop new treatments for bladder disorders (Andersson, 2004; Andersson and Wein, 2004).

Our studies in cats (Tai et al., 2012; Zhang et al., 2012) have identified an involvement of opioid receptors in tibial nerve stimulation-induced inhibition of nociceptive bladder overactivity caused by intravesical acetic acid (AA) irritation. Activation of opioid receptors also plays a minor role in pudendal nerve stimulation (PNS)-induced inhibition of non-nociceptive reflex bladder activity in cats caused by saline distention of the bladder (Chen et al., 2010); but does not contribute to PNS inhibition of nociceptive bladder overactivity (Mally et al., 2013). Thus, neurotransmitters other than endogenous opioid peptides must mediate PNS inhibition of reflex bladder activity.

Previous studies in various species (cats, rats, guinea pigs) have raised the possibility that 5-hydroxytryptamine (5-HT) may function as an inhibitory or excitatory transmitter that modulates the micturition reflex pathway in the brain and spinal cord (Cheng and de Groat, 2010; de Groat, 2002; Mbaki et al., 2012; Ramage, 2006) and that it also has an important role in the regulation of nociceptive mechanisms in the central nervous system (Basbaum and Fields, 1984). Thus, we have conducted a pharmacological study to examine the contribution of 5-HT to the inhibition of nociceptive and non-nociceptive bladder reflexes induced by PNS. We have used methysergide, a non-selective 5-HT_2 receptor antagonist, which in a rat model of somatic nociception was effective after intrathecal administration in significantly reducing the antihyperalgesic effect of transcutaneous electrical nerve stimulation or mechanical joint manipulation (Radhakrishnan et al., 2003; Skyba et al., 2003). These results indicated that descending serotonin (5-HT) inhibitory mechanisms might be involved in neuromodulation of somatic nociception in the spinal cord.

In addition, intrathecal administration of methysergide to cats enhanced the axonal firing in the lateral funiculus of T11–T12 spinal

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segments elicited by electrical stimulation of bladder afferent axons in the pelvic nerve (Espey et al., 1998). These observations suggested that methysergide-sensitive 5-HT receptors generate a tonic inhibitory modulation of the ascending limb of the spinobulbospinal micturition reflex pathway (Espey et al., 1998). This modulatory input arises in the brain stem raphe nuclei where electrical (McMahon and Spillane, 1982; Morrison and Spillane, 1986) or chemical stimulation (Chen et al., 1993) has been shown to inhibit bladder reflexes. Therefore, the present study was undertaken to determine if methysergide-sensitive 5-HT receptors might be involved in PNS inhibition of nociceptive or non-nociceptive bladder reflexes.

Intravesical infusion of diluted (0.25%) AA was used in this study to irritate the bladder, activate the nociceptive bladder C-fiber afferents, and induce bladder overactivity in α-chloralose anesthetized cats, while saline infusion was used to distend the bladder, activate the non-nociceptive bladder Aδ-fiber afferents, and induce normal reflex bladder activity (Fowler et al., 2008; Häbler et al., 1990). PNS was employed as the antinociceptive stimulus to model the clinical use of pudendal neuromodulation in treating OAB or painful bladder syndrome (PBS) (Peters, 2002; Peters et al., 2005). Methysergide and naloxone (an opioid receptor antagonist) were administered intravenously to determine the role of 5-HT and opioid receptor mechanisms in the neuromodulation. WAY100635 (a 5-HT_{1A} receptor antagonist) was used to exclude the possibility that methysergide affects the bladder activity through activation of 5-HT_{1A} receptors.

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 a total of 20 cats (11 male, 9 female, 3.0–4.4 kg, Liberty Research Inc., Waverly, NY, USA) anesthetized initially with isoflurane (2–3% in oxygen) and maintained with α-chloralose (65 mg/kg, i.v. with supplementation as necessary). Heart rate and blood oxygen level were monitored by a pulse oximeter (9847V, NONIN Medical, Inc., Plymouth, MN, USA) 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 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 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 urethra to prevent leakage. The pudendal nerve was dissected from the right side via a 3–4 cm incision between the tail and the sciatic notch. A tripolar cuff electrode

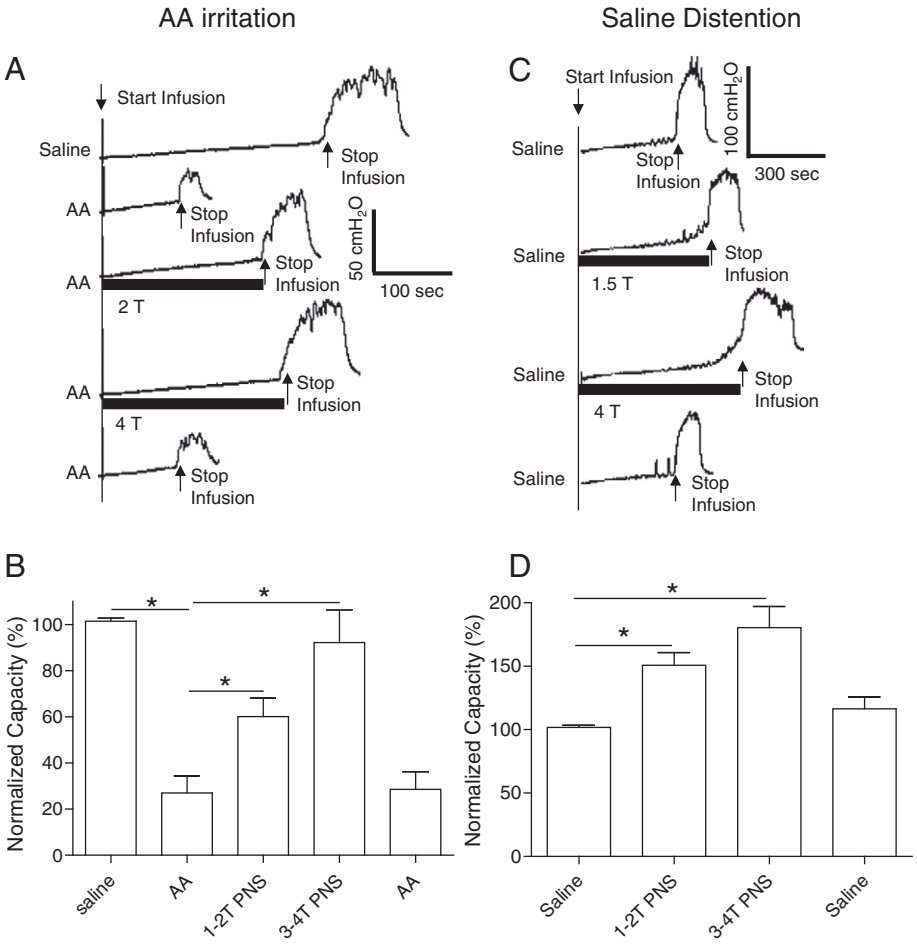

Fig. 1. Pudendal nerve stimulation (PNS) inhibited bladder overactivity caused by acetic acid (AA) irritation (A and B) and normal bladder activity induced by saline distention (C and D). PNS duration is indicated by the black bar under the bladder pressure trace. T-PNS threshold intensity for inducing anal sphincter twitching. A. Repeated CMGs during AA infusion (2 ml/min). PNS: 5 Hz, 0.2 ms, T = 1.2 V. B. Normalized bladder capacity during AA irritation (N = 8 cats). C. Repeated CMGs during saline infusion (2 ml/min). PNS: 5 Hz, 0.2 ms, T = 1.2 V. D. Normalized bladder capacity during saline infusion (N = 8 cats). * indicates a significant difference ($P < 0.05$).