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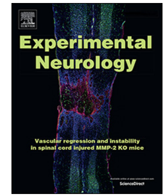

Research Paper

Spinal interneuronal mechanisms underlying pudendal and tibial neuromodulation of bladder function in cats

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ABSTRACT

This study examined the mechanisms underlying pudendal and tibial neuromodulation of bladder function at the single neuron level in the spinal cord. A microelectrode was inserted into the S2 spinal cord of anesthetized cats to record single neuron activity induced by bladder distention over a range of constant intravesical pressures (10–40 cmH_2O). Pudendal nerve stimulation (PNS) or tibial nerve stimulation (TNS) was applied at 5 Hz frequency and 0.2 ms pulse width and at multiples of the threshold (T) intensities for inducing anal or toe twitches. A total of 14 spinal neurons from 11 cats were investigated. Both PNS and TNS at 2 T intensity significantly ($p < .05$) reduced by 40–50% the frequency of firing induced by bladder distention at 20–40 cmH_2O in the same spinal neurons. This reduction was not changed by blocking opioid receptors with naloxone (1 mg/kg, i.v.). Activation of pudendal afferents by repeatedly stroking (3–5 times per second) the genital skin using a cotton swab also inhibited the neuron activity induced by bladder distention. Prolonged (30 min) TNS at 4 T intensity produced a short lasting (10–18 min) post-stimulation inhibition that reduced by 40–50% bladder-related neuron activity at different bladder pressures. These results indicate that PNS and TNS inhibition of reflex bladder activity may be mediated in part by convergence of inhibitory inputs onto the same population of bladder-related interneurons in laminae V-VII of the S2 spinal cord and that an opioid receptor mechanism is not involved in the inhibition.

1. Introduction

Pudendal and tibial neuromodulation are effective therapies to treat overactive bladder (Peters et al. 2009, 2010) – a disorder that is defined by the International Continence Society as a syndrome characterized by urinary urgency with or without urge incontinence, usually with urinary frequency and nocturia (Abrams et al. 2002). However, the mechanisms underlying these neuromodulation therapies are not fully understood. It is commonly assumed that pudendal or tibial neuromodulation stimulates afferent axons that in turn activate inhibitory mechanisms either in the spinal cord or in the brain to suppress the neuron activity in the micturition reflex pathway (de Groat and Tai 2017). Our previous studies showed that: (1) in acute spinal cord transected cats spinal reflex bladder activity is inhibited by pudendal nerve stimulation (PNS) but not by tibial nerve stimulation (TNS) (Xiao et al. 2014b); (2) in spinal intact cats electrical stimulation of the pontine micturition center to activate the descending limb of the micturition reflex pathway elicits bladder activity that is inhibited by PNS but not by TNS (Lyon et al. 2016); (3) intrathecal administration of a $GABA_A$ receptor antagonist in cats suppresses PNS inhibition of bladder overactivity (Xiao et al. 2014a); and (4) application of an opioid receptor antagonist to the pons suppresses TNS inhibition of bladder overactivity (Ferroni et al. 2015). These results indicate that PNS inhibition of bladder reflexes can occur at the spinal level but TNS inhibition may not.

To directly test the hypothesis that pudendal but not tibial neuromodulation acts in the spinal cord to suppress reflex bladder activity, this study was conducted in anesthetized cats to record the effects of PNS or TNS on the firing of bladder-related neurons in the S2 spinal cord. The neurons were identified by their responses to bladder distention. The results of this study indicate that PNS as well as TNS acts in

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the spinal cord to modulate bladder function.

2. Materials and methods

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

2.1. Surgical procedures

A total of 11 cats (4 male and 7 female, 2.9–4.1 kg; Liberty Research, Waverly, NY) were used. The animals were anesthetized initially with isoflurane (2–5% in oxygen) during surgery and then switched to α-chloralose anesthesia (initial dose 65 mg/kg i.v. followed by supplemental doses as needed) during data collection. The left cephalic vein was catheterized for intravenous administration of fluid and drug. A midline anterior cervical incision was used to access the airway, which was kept patent via tracheostomy. The right carotid artery was catheterized for monitoring arterial blood pressure. Oxygen saturation and heart rate were measured via a pulse oximeter (9847VNONIN Medical, Plymouth, MN) attached to the tongue. A midline laparotomy was performed and the ureters were transected, ligated, and then drained externally. A single-lumen urethral catheter (2.3 mm inner diameter) was inserted into the bladder via a small anterior urethrotomy and fixed in place by a suture around the urethra. The single-lumen catheter was attached to a T-adapter to allow for simultaneous bladder pressure recording via a pressure transducer and bladder distention via a saline reservoir. Bladder pressure was maintained at a constant level by adjusting the height of the saline reservoir connected to the urethral catheter. Small incisions were made in the right sciatic notch lateral to the tail and on the medial side of the left hindlimb above the ankle to expose the right pudendal nerve and left tibial nerve, respectively. A tripolar cuff electrode (NC223pt, Microprobes, Gaithersburg, MD) was placed around each intact nerve and connected to a stimulator (S88; Grass Medical Instruments, Quincy, MA). All incisions were then closed by sutures.

The spinal cord and cauda equina were then exposed between the L5 and S3 vertebrae via a dorsal laminectomy. The spinal dura was cut and the S2 spinal cord was exposed. The animal was mounted in a modified Narishige spinal frame in which the hip was supported by metal pins, and the spinous process at the rostral end of the laminectomy was supported by a clamp. The skin, which had been cut posteriorly from L4 to S3 was tied along the margin to form a pool that was filled with warm (35–37 °C) mineral oil.

2.2. Searching for bladder-related neurons in S2 spinal cord

This study focused on the identification of bladder-related neurons in S2 spinal cord because previous tracing studies showed that S2 spinal cord is a major spinal segment for bladder afferent projections (Morgan et al. 1981). A tungsten microelectrode (WE50030.5A10, 0.5 MΩ, Microprobes, Gaithersburg, MD) attached to a microelectrode driver was lowered to the dorsal surface of the spinal cord on the right side in the middle of the S2 spinal cord segment along the dorsal root entry zone. With bladder pressure maintained at a constant pressure of 40 cmH_2O by setting the height of the saline reservoir, the microelectrode was slowly advanced in 5 µm steps into the spinal cord. At each electrode location, neuron activity amplified 20,000 times by an amplifier (P511, Grass Instruments, MA) was displayed on an oscilloscope and sent to an audio monitor. When single neuron firing was detected, the bladder pressure was lowered to 0 cmH_2O to assess the contribution of bladder afferent input to the neuron activity. If the neuron stopped firing at 0 cmH_2O bladder pressure, the microelectrode was kept in place to further investigate PNS or TNS effects on the activity. Otherwise, the microelectrode was advanced further into the cord. The microelectrode was withdrawn once it reached 2 mm depth before entering the ventral horn of the spinal cord. Then, the microelectrode was moved 0.5–1 mm rostral/caudal or medio/lateral to a new location on the spinal cord surface to repeat the search procedure until a bladder-related neuron was identified.

Table 1
The sequence of different tests.

Order	Test
1	Bladder neuron identified
2	Pudendal nerve stimulation
3	Tibial nerve stimulation (TNS)
4	Peri-genital stimulation
5	Post-TNS effect
6	Naloxone treatment

2.3. Stimulation protocol and drug administration

Uniphasic rectangular pulses (5-Hz frequency, 0.2-ms pulse width), which have previously been shown to be effective in inhibiting bladder activity (Larson et al. 2011; Tai et al. 2012), were delivered to the tibial or pudendal nerve. The intensity threshold (T) for inducing toe or anal sphincter twitch was determined at the beginning of the experiment. Based on our prior studies, stimulation intensity of 2 T was used for PNS or TNS of short duration (about 3 min) to acutely suppress bladder-related neuron activity during the stimulation (Fuller et al. 2017; Uy et al. 2017), while 4 T was used for prolonged (30-min duration) TNS to produce a post-stimulation inhibitory effect (Tai et al. 2011b).

Table 1 shows the sequence of different tests as described below in detail. When a bladder neuron was identified, the single unit activity was sampled at 20 kHz by an analog-to-digital converter (PCI-6024E, National Instruments, TX) and saved in a computer running the LabVIEW program (National Instruments, TX). Initially, the pressure response was determined by recording the activity at a range of bladder pressures (10, 20, 30, or 40 cmH_2O) for 20–30 s with a 20–30 s interval of 0 cmH_2O pressure between each pressure increase. To evaluate the effect of PNS on neuron activity ($N = 13$ neurons), the pressure response test was repeated under the following conditions: 1) control without PNS; 2) during 2 T PNS; 3) control after PNS. The same test was then performed using TNS ($N = 10$ neurons). Following the PNS and TNS tests, the effect of peri-genital stimulation (PGS) on neuron activity was determined at 10 and 30 cmH_2O bladder pressures first without PGS (control) then during PGS ($N = 6$ neurons). PGS was applied by repeatedly stroking (3–5 times per second) the genital area with a cotton swab to determine if physiological stimulation of a population of mechanosensitive cutaneous afferents in the pudendal nerve can mimic the effect of electrical stimulation of the whole pudendal nerve.

After testing the acute effects of PNS/TNS/PGS during the stimulation, the post-stimulation inhibitory effect induced by prolonged (30-min duration) TNS as revealed in our previous studies (Tai et al. 2011b) was investigated. The bladder pressure response (10–40 cmH_2O) was first determined without any stimulation to serve as the control condition, and then followed by a period of 4 T TNS for 30 min with the bladder pressure at 0 cmH_2O. Within 0.5–1.5 min after cessation of 30-min TNS, the bladder pressure response (10–40 cmH_2O) was tested again to determine the post-stimulation inhibition. Then, at an interval of 1–11 min the bladder pressure was increased from 0 cmH_2O to 40 cmH_2O for 20 s to assess the recovery from the post-stimulation inhibition. Once the neuron activity at 40 cmH_2O recovered to control level, the full pressure response test (10–40 cmH_2O) was performed again.

At the end of the experiment, naloxone (1 mg/kg) was administered intravenously with the bladder pressure at 0 cmH_2O. Five minutes after naloxone treatment, the acute effect of PNS ($N = 6$ neurons) or TNS ($N = 7$ neurons) on neuron activity was evaluated again using the bladder pressure response test.

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