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Research Paper

Phasic activation of the external urethral sphincter increases voiding efficiency in the rat and the cat

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ABSTRACT

Objective: Electrical stimulation of the pudendal nerve (PN) is a potential therapy for bladder dysfunction, but voiding efficiency (VE) produced by PN stimulation appears limited to 60–70%. We conducted experiments in rats and cats to investigate the hypothesis that introduction of artificial phasic bursting activity of the external urethral sphincter (EUS) would enhance VE under conditions where such activity was absent.
Materials and methods: Cystometry experiments were conducted in 17 urethane anesthetized female Sprague-Dawley rats and 4 α-chloralose anesthetized male cats. The effects of phasic stimulation of the pudendal motor branch on VE were quantified in intact conditions, following bilateral transection of the motor branch of the PN, and following subsequent bilateral transection of the sensory branch of the PN.
Results: Artificial phasic bursting activity in the EUS generated by electrical stimulation of the motor branch of the PN increased VE in both rats and cats. Subsequent transection of the sensory branch of the PN abolished the increased VE elicited by phasic stimulation in both rats and cats.
Conclusions: Artificial phasic EUS bursting restored efficient voiding in rats. Introduction of artificial phasic bursting in cats, which normally exhibit EUS relaxation while voiding, was also effective in promoting efficient voiding. In both species phasic EUS activity increased voiding efficiency via activation of pudendal sensory pathways. These results provide further insight into the function of phasic EUS activity in efficient voiding and highlight a novel approach to increase VE generated by pudendal afferent nerve stimulation.

1. Introduction

Electrical stimulation of the pudendal nerve is under investigation for restoration of bladder emptying in persons with spinal cord injury (Kennelly et al., 2011; Yoo et al., 2009, 2011). Selective electrical stimulation of individual branches of the pudendal nerve evokes sustained bladder contractions and increases voiding efficiency (VE) beyond that of distention-evoked reflex voiding in cats (Woock et al., 2008; Yoo et al., 2008a). However, while pudendal nerve stimulation is a promising approach, VE is limited to 60–70% in anesthetized cats (Boggs et al., 2006; Woock et al., 2008; Yoo et al., 2008a), and larger VE is desirable for clinical translation. The objective of the present study was to test the hypothesis that artificial phasic activation of the external urethral sphincter (EUS), as is observed during voiding in rats and dogs, would enhance VE in cats.

Phasic bursting activity in the EUS, which occurs during voiding in rats (Chen et al., 2011; Chen et al., 2012; Kakizaki et al., 1997; Kruse et al., 1990; Peng et al., 2008; Vera and Nadelhaft, 2001) and dogs (Nishizawa et al., 1984a; Nishizawa et al., 1984b), is observed in the electromyogram (EMG) of the striated EUS and as high frequency oscillations (HFOs) in intravesical pressure. While the origin and function of this activity remain unclear, elimination of phasic EUS activity either by transection of the pudendal nerve innervating the EUS in the rat (Cruz and Downie, 2005; Peng et al., 2006) and dog (Nishizawa et al., 1984a) or by neuromuscular blockade in the rat (Conte et al., 1991; Kruse et al., 1993; Maggi et al., 1986; Peng et al., 2006; Vera and Nadelhaft, 2001; Yoshiyama et al., 2000) causes a significant reduction in VE.

We conducted a series of experiments in rats and cats to investigate the hypothesis that introduction of artificial EUS bursting activity by electrical stimulation could increase VE under conditions where such activity was absent. We first conducted a series of experiments in rats to verify the role of phasic EUS bursting in efficient voiding and to test the hypothesis that re-introduction of phasic EUS activity following motor nerve transection could restore efficient voiding. Subsequently, we quantified the effects of introducing phasic EUS bursting activity on bladder contractions and VE in the cat, which, unlike the rat, normally exhibits relaxation of the EUS while voiding (Fedirchuk and Shefchyk,

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1993). Finally, in both rats and cats, transection of the sensory branches of the pudendal nerves revealed that phasic EUS activity increased voiding efficiency through a sensory pathway, presumably by amplifying the sensory input to the augmenting reflex (Barrington, 1941). Collectively, these results provide further insight into the function of phasic EUS activity in efficient voiding and highlight a novel approach to increase VE generated by pudendal afferent nerve stimulation.

2. Materials and methods

All animal care and experimental procedures were reviewed and approved by the Duke University Institutional Animal Care and Use Committee.

2.1. Rat surgical preparation and procedures

Female Sprague-Dawley rats ($n = 17$) weighing between 256 and 343 g were anesthetized with urethane (1.2 g/kg s.c., and supplemented as necessary). Body temperature was monitored using an esophageal temperature probe and maintained at 36°–38 °C with a recirculating water blanket. Heart rate and arterial blood oxygen saturation levels (Sp02) were monitored using a pulse oximeter (Nonin Medical Inc., 2500A VET).

For cystometrogram (CMG) measurements, the bladder was exposed via a midline abdominal incision. A PE-50 polyethylene catheter, the tip of which was heated to create a collar, was inserted into the bladder lumen through a small incision in the apex of the bladder dome, and a 6-0 silk suture was tied around the collar. The abdominal wall was closed with 4-0 silk suture. The bladder catheter was connected via a 3-way stopcock to an infusion pump (Braintree Scientific Inc., BS-8000) and to a pressure transducer (ArgoTrans, Argon Medical Devices Inc., Plano, TX) connected to a bridge amplifier and filter (13-6615-50, Gould Instruments, Valley View, Ohio) for measuring intravesical pressure (IVP).

Two PFA-coated platinum-iridium wires (0.0055 inch-diameter, A-M Systems, Sequim, WA) were bilaterally inserted percutaneously into the EUS to record EUS EMG. EUS EMG leads were connected through a preamplifier (HIP5, Grass Products, Warwick, RI) to an amplifier (P511, Grass Products). IVP and EUS EMG signals were amplified, filtered, and sampled at 1000 Hz (IVP) or 4000 Hz (EUS EMG) using a PowerLab/16SP acquisition unit (AD Instruments, Colorado Springs, CO) and displayed for off-line analysis using LabChart 7 Pro (v7.3.7, AD Instruments).

After placement of the bladder catheter, abdominal closure, and introduction of the percutaneous EUS electrodes, the rat was flipped to a prone position. The motor and sensory branches of the pudendal nerves were then exposed bilaterally via the ischiorectal fossa (Damaser et al., 2007; McKenna and Nadelhaft, 1986; Pacheco et al., 1989). Using a posterior approach, the gluteus muscles were incised to expose the rostral portion of the iliac crests, the ilium and sacrum were separated, and the pudendal nerves were carefully dissected. At approximately 2.4 cm distal to the S1 plexus at the anastomosis juncture, a pair of bipolar electrodes (AS 631, Cooner Wire Company, Chatsworth, CA) were glued onto each motor branch (left and right sides) with Kwik-Cast sealant (World Precision Instruments, Inc., Sarasota, FL) for electrical stimulation. For the duration of the experiment, the rats remained in the prone position and were not suspended or secured to a frame.

The bladder was continuously filled with physiological saline at room temperature (4–8 ml/h) using an infusion pump with an open urethra for at least 45 min to allow post-surgical recovery. The bladder was subsequently emptied, and CMGs recorded. For each CMG, the bladder was filled until a micturition event was observed, at which time the infusion pump was turned off. Approximately 1 min after the bladder pressure returned to baseline, the bladder was emptied. Voided and residual volumes were recorded and used to calculate bladder capacity and VE. At least three repeated CMG trials were recorded for each experimental condition, and parameters from multiple trials within an animal were averaged.

After control CMGs, the motor branches were transected bilaterally, approximately 2 mm central to the stimulation electrodes. The distal pudendal motor stump was stimulated with regulated current, 0.1 ms per phase, biphasic pulses delivered at a stimulus amplitude which produced a maximal EUS EMG response. During micturition, pudendal efferent nerves in rats have a median burst frequency of 40 Hz with 3 to 4 action potentials per burst (D'Amico and Collins, 2012). Therefore, a pattern of stimulation that produced 3 EUS EMG evoked potentials per burst at a frequency of 40 Hz with 160 ms inter-burst frequency ($\approx$ 6 Hz) was used to mimic this bursting activity (Fig. 1G). For the stimulation trials, stimulation was initiated when the infused volume reached 92% $\pm$ 0.11% (mean $\pm$ SD) of the infused volume necessary to evoke a micturition contraction in trials following bilateral pudendal motor branch transection. The stimulation was turned off when the bladder pressure returned to baseline (Fig. 1E). To determine the impact of surgical placement of the pudendal electrode on the motor branch, pre-surgical control CMGs were recorded prior to pudendal surgery and implantation of the electrodes in $n = 5$ of 13 experiments. Additionally, we investigated the effect of unilateral stimulation of both the left and right motor branches (stimulated independently) on the CMGs in $n = 7$ of 13 experiments.

To determine the mechanisms underlying the effects of EUS burst activity on VE, following the initial experimental protocol, the pudendal sensory branches were bilaterally transected approximately 2.4 cm distal to the S1 plexus at the anastomosis juncture in $n = 6$ of 13 experiments, and subsequent CMGs were performed with and without bilateral motor branch stimulation. For the stimulation trials, stimulation was initiated when the infused volume reached 94% $\pm$ 0.04% (mean $\pm$ SD) of the infused volume necessary to evoke a micturition contraction in the preceding trials following both motor and sensory pudendal nerve transections.

In $n = 4$ experiments, α-bungarotoxin was administered to block muscle activity as an alternative to surgical transection of the pudendal motor branch, and CMG and EUS EMG activities were recorded prior to and after drug administration. The right jugular vein was cannulated with a PE-50 polyethylene catheter for administration of 0.4 mg/kg α-bungarotoxin (R&D Systems, Inc., Minneapolis, MN), and the trachea was cannulated for artificial ventilation using the PhysioSuite Monitor. End tidal CO_2 was measured using a ML206 Gas Analyzer (AD Instruments) and maintained between 3 and 4%.

2.1.1. Data analysis

Voided volume and residual volume were measured after each trial to calculate the VE:

$$\text{VE}\ (\%) = \frac{\text{voided volume}}{\text{voided volume} + \text{residual volume}} \times 100.$$

The area under the curve (AUC) of the bladder contraction as a function of time (the pressure-time integral) was calculated between the start of micturition contraction to the end of the void. The bladder contraction amplitude was defined as the peak bladder pressure during a micturition event. For pre-surgical control vs. electrode placement comparisons of the EUS EMG bursting phase, the smoothed (0.08 s window moving average) rectified EUS EMG activity was used. The area under the EUS EMG bursting phase as a function of time (the voltage-time integral), maximum burst amplitude, burst phase frequency, and burst duration was calculated.

All parameters were analyzed using GraphPad Prism version 6.05 for Windows (GraphPad Software, La Jolla California USA). Comparisons of electrode placement vs. pre-surgical control, left vs. right unilateral stimulation, unilateral vs. bilateral stimulation, and α-bungarotoxin were analyzed using a paired *t*-test. The effect of pudendal motor