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Pelvic floor muscles and the external urethral sphincter have different responses to applied bladder pressure during continence

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Abstract

Objectives—To determine the functional innervation of the pelvic floor muscles (PFM) and if there is PFM activity during an external pressure increase to the bladder in female rats.

Methods—Thirty-one female adult virgin Sprague Dawley rats received an external increase in bladder pressure until urinary leakage was noted while bladder pressure was recorded (leak point pressure; LPP) under urethane anesthesia. Six of the rats underwent repeat LPP testing after bilateral transection of the levator ani nerve. Another 6 rats underwent repeat LPP testing after bilateral transection of the pudendal nerve. Simultaneous recordings of PFM (pubo-/iliococcygeus muscles) electromyogram (EMG) and external urethral sphincter (EUS) EMG were recorded during cystometry and LPP testing.

Results—Thirteen rats (42%) showed tonic PFM EMG activity during filling cystometry. Eighteen rats (58%) showed no tonic PFM EMG activity at baseline, but PFM EMG could be activated by pinching the perineal skin. This activity could be maintained unless voiding occurred. The external increase in bladder pressure caused significantly increased EUS EMG activity as demonstrated by increased amplitude and frequency. However, there was no such response in PFM EMG. LPP was not significantly different after levator ani nerve transection, but was significantly decreased after pudendal nerve transection.

Conclusions—PFM activity was not increased during external pressure increases to the bladder in female rats. Experimental designs using rats should consider this result.

Keywords

Animal model; Electromyography; Pelvic floor; Rat; Reflex; Urinary incontinence; Urodynamics

Introduction

Pelvic floor muscles (PFM) in women, which support the pelvic organs, include the levator ani and the coccygeus muscles, but do not include the external urethral sphincter (EUS) or the external anal sphincter. The levator ani consists of the iliococcygeus, the pubococcygeus, and

the puborectalis muscles. The PFM in female rats has a similar bowl-shaped anatomic organization as humans with some exceptions, such as lack of a recognized puborectalis muscle.^{3,20}

There are conflicting opinions regarding levator ani and pudendal nerve innervation of the PFM.^{1,22,23} Nonetheless, the PFM is considered to be critical to multiple aspects of urogenital and anorectal function and PFM exercise is regarded as a primary choice for treatment of stress urinary incontinence.²⁴ Increased active contraction of the striated muscle of the EUS, involving a bladder-to-EUS continence reflex, can also transiently help maintain continence.⁵

The bladder-to-EUS reflex via the pudendal nerve has been confirmed in female rats.^{12,15} However, it is not clear if there is a similar reflex involving the PFM via the levator ani nerve and/or the pudendal nerve. Although bladder and urethral pressure responses to an external increase in bladder pressure have been recorded,¹² neuromuscular functional testing to directly assess the PFM response to an external increase in bladder pressure has not previously been performed in rats. Such testing could demonstrate if the PFM has a similar reflex for prevention of urinary leakage when an external pressure is applied to the bladder.

The objective of this project was to assess the bladder-to-PFM reflex via both the pudendal and levator ani nerves and determine if it contributes to urinary continence. We tested PFM and EUS electromyogram (EMG) responses to an external increase in bladder pressure to leakage (leak point pressure; LPP) in female rats before and after bilateral transection of the pudendal and levator ani nerves. These results will enable us to begin to differentiate the role of the PFM and the EUS in female rats.

Materials and Methods

Experimental outline

Thirty-one female adult virgin Sprague-Dawley rats (287 ± 7 g) were used to record bladder pressure, PFM (pubo- or iliococcygeus muscle) EMG and EUS EMG simultaneously during filling cystometry and LPP testing (Fig. 1). Twelve of these rats then underwent bilateral transection of either the levator ani nerve ($n = 6$) or the pudendal nerve ($n = 6$) followed by repeat testing. All procedures were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic.

Electrophysiological recordings

All rats were anaesthetized with urethane (1.0 g/kg) intraperitoneally for simultaneous electrophysiological recordings supplemented with isoflurane inhalation (2.5 %) anesthesia for surgical preparation before recordings. Under a microscope, the pubic symphysis was exposed via a vertical midline incision and the overlapped rectus muscles were transected. The bilateral inferior epigastric vessels were ligated and cut. The urethra was exposed after opening the pubic symphysis with mosquito forceps. The pubococcygeus and iliococcygeus muscles were partly exposed by bluntly separating the pubic symphysis from the ischiorectal fossa.

Bipolar parallel platinum electrodes (30-gauge blunt-end needles 2 mm apart) were placed on the outside of the mid-urethra to record EUS EMG. The electrodes were fixed on a manipulator and connected to an amplifier (Model P511 AC Amplifier, Astro-Med, Inc.; band pass frequencies from 3 Hz to 3 kHz). The EMG signal was recorded at 10 kHz by a multiple channel electrophysiological recording system (DASH 8X, Astro-Med, Inc.). For PFM EMG recordings, an identical set of recording electrodes were touched to the iliococcygeus muscle and/or pubococcygeus muscle through the outside surface of the pelvis. The ischiorectal fossa was opened slightly for the approach to the PFM. As above, the electrodes were connected to

an amplifier and the electrophysiological recording system. The isoflurane was turned off and allowed to wear off prior to electrophysiological recordings.

Filling cystometry and leak point pressure testing

A labeled polyethylene catheter (PE-50) was inserted 2.5 cm into the bladder from the urethral orifice, and was connected to both a pressure transducer (model P122; Astro-Med, Inc.) and a syringe pump (model 200; KD Scientific, New Hope, PA). Air pressure at the level of the bladder was calibrated as zero. The bladder was filled with saline at 5 ml/h while bladder pressure and electrophysiological recordings were made. For LPP testing, the bladder was filled to approximately 0.3 ml. An external increase in bladder pressure until urinary leakage was then made by slowly pressing a cotton applicator on the bladder and removing it quickly at the first sign of fluid leakage at the urethral meatus. LPP, a measure of urethral resistance to leakage, is defined as baseline bladder pressure subtracted from peak bladder pressure at leakage.

A mean of 3-4 LPP tests were performed in each animal and a mean of the results was calculated and used for group comparisons. If an active bladder pressure contraction was induced by LPP testing, the results were not collected and analyzed. The bladder was then refilled to 0.3 ml and the test was repeated.

Nerve transection

Rats that showed tonic PFM EMG activity during continence ($n = 13$) were divided into two groups for bilateral transection of the levator ani nerve, which innervates the PFM (the pubococcygeus and iliococcygeus muscles in rat), and bilateral transection of the pudendal nerve, which innervates the EUS. Under microscopy, the levator ani nerve was transected bilaterally where it crosses the internal iliac vessels. It is the second branch of the L6-S1 trunk and travels along with the first branch of the trunk, the pelvic nerve.²⁰ The pudendal nerve motor branch was transected bilaterally in Alcock's canal interior to the ischiorectal fossa. The LPP test was repeated after nerve transection. One rat was eliminated because the pelvic nerve was transected accidentally during levator ani nerve transection. Therefore, there were twelve rats, 6 per group, for repeat LPP testing and electrophysiological recordings after nerve transection.

Data analysis

EUS EMG and PFM EMG activity at baseline and at the peak pressure of the LPP test were identified and archived in 3 or 4 one-second samples for each rat (AstroVIEWX, Astro-Med, Inc.; Fig. 2). Quantitative assessment of EMG activity was performed as done previously, by determining the mean rectified amplitude of the potential and the mean frequency of motor unit firing.¹² Power supply interference (60 Hz and 120 Hz) was filtered with a digital band pass filter (59 - 61 Hz and 119 - 121 Hz; SigmaPoint 2008, Myosotic LLC, Woodinville, WA). A threshold for differentiating noise was set at 0.2 μ V according to the noise amplitude observed during amplifier calibration. Mean values of amplitude and firing frequency above noise were calculated for each animal and were used to calculate a mean and standard error for each group. The Wilcoxon signed rank test was used to compare EUS EMG and PFM EMG data before and during LPP testing (Sigma Stat, Systat, Inc., CA). A paired t-test was used to compare LPP results before and after nerve transection. $P < 0.05$ was used to indicate a statistically significant difference. Data is presented as mean $\pm$ standard error of the mean.

Results

Different effects of EUS & PFM EMG activity during filling cystometry

EUS EMG in all rats demonstrated tonic baseline activity during continence and bursting activity during voiding (Fig. 2, 3). Some rats ($n = 13/31$, 42%) also showed PFM EMG tonic activity during continence with decreased activity during voiding (Fig. 2). The mean frequency of PFM EMG in these rats during baseline activity was significantly lower than that of the EUS EMG.

In other rats ($n = 18/31$, 58%), there was no PFM EMG tonic activity during either continence or voiding. EUS EMG in contrast, demonstrated tonic activity during continence and bursting activity during voiding (Fig. 3). To verify normal physiological reactivity of the PFM, a small forceps was used to pinch the perineal skin around the urethral meatus (~5 s duration). In all of these rats, PFM EMG was activated and maintained its activity for several seconds beyond the duration of the pinching action. Activity was maintained unless voiding occurred (Fig. 3). This response was abolished by bilateral transection of the levator ani nerve, but not after bilateral pudendal nerve transection.

Different effects of EUS & PFM EMG response during LPP testing

During LPP testing, EUS EMG activity demonstrated a significant increase in both amplitude and frequency, suggesting the presence of a bladder-to-EUS continence response to an increase in bladder pressure (Fig. 2, 3). In contrast, there was no similarly positive response in PFM activity, since no significant change or response was demonstrated in PFM EMG activity during LPP testing either in the rats that showed tonic activity (Fig. 2) or in those that showed no tonic activity (Fig. 3).

Effects of pudendal nerve transection on EUS & PFM EMG and LPP

In six of the rats that showed tonic activity in both EUS and PFM, LPP testing with simultaneous electrophysiological recordings was repeated after bilateral pudendal nerve transection (Fig. 4). EUS EMG demonstrated no activity either before LPP testing or during LPP testing after pudendal nerve transection. In contrast, PFM EMG showed tonic activity before LPP testing and did not change significantly during LPP testing. After bilateral pudendal nerve transection, LPP was significantly decreased (23 ± 5 cmH₂O) compared to the same rats when intact (44 ± 6 cmH₂O).

Effects of levator ani nerve transection on EUS & PFM EMG and LPP

In the other six rats that showed tonic activity in both EUS EMG and PFM EMG, LPP testing was repeated after bilateral levator ani nerve transection (Fig. 5). PFM EMG did not demonstrate activity either before or during LPP testing. EUS EMG maintained tonic activity before LPP testing and had a significant increase in both amplitude and frequency with LPP testing. In contrast to pudendal nerve transection, LPP was not significantly different after bilateral levator ani nerve transection (41 ± 6 cmH₂O) compared to the same animals when intact (47 ± 4 cmH₂O).

Comment

The pelvic and perineal nerve roots are more proximal in rats than in humans. The pudendal nerve arises from L6-S1 roots in rats and S2-S4 roots in humans.^{14,18} Similarly, the levator ani nerve branches from the L6-S1 trunk in rats, enters the pelvic floor along with the pelvic nerve,^{1,20} and innervates the pubococcygeus and iliococcygeus muscles from the superior surface.^{3,17} In humans, the levator ani nerve and its branches arise from sacral nerve roots (S3-S5) and also innervate the PFM from its ventromedial surface.¹ In rats this nerve has had several

different names, including the somato-motor branch of the pelvic nerve, the intrapelvic somatic nerve, and the intrapelvic branch of the pudendal nerve.^{2,20} Nonetheless, dissection of this nerve in female rats demonstrates a general similarity with humans.

The mechanism of urinary continence involves sophisticated structural support and nervous control, including a bladder-to-EUS continence reflex.^{5, 8} Afferent signals are generated from bladder distension or abdominal pressure transmission to the bladder, which communicate with pudendal motoneurons in the sacral spinal cord to activate pudendal efferent fibers, increasing EUS activity. The EUS response during activation of this reflex via the pelvic nerve afferent limb has been validated experimentally in experiments in which the bladder neck was ligated with no urine entering the proximal urethra.¹³

The bladder-to-EUS reflex plays an important role in prevention of urinary leakage, as demonstrated by studies in rats showing that glutamatergic and serotonergic mechanisms modulate this reflex and the response of pudendal motoneurons to increased bladder pressure, contributing to urinary continence.^{6,25} Similarly, in our recordings, we confirmed that the bladder-to-EUS reflex contributes to urethral resistance to leakage in female rats since EUS EMG activity increased during LPP testing and LPP was significantly decreased after bilateral pudendal nerve transection, consistent with animal models of stress urinary incontinence.¹¹

In our simplified experimental setting, we demonstrated that the PFM of female rats does not increase EMG activity in response to an increase in external pressure to the bladder. This is in contrast to the increased EMG activity response of the EUS, indicating that there is not a similar guarding reflex in female rats from the bladder to the PFM when bladder pressure is passively and directly increased via an external pressure. In contrast to our results, other studies have recorded PFM EMG during voiding or electrical stimulation in rats,^{16, 19, 20} suggesting that continence reflexes involving the PFM are complex.

Although application of a passive external increase in bladder pressure is not realistic in women, we expect that the results we have observed in animals could be clarified in women with a clinical study utilizing electrophysiological and urodynamic outcomes. The effectiveness of PFM contraction and training in the treatment of urinary and fecal incontinence in women may have a more complicated physiological mechanism, including support of the bladder, stabilization of the bladder neck and strengthening PFM contraction during reflexes such as cough or Valsalva maneuver.^{4,9}

PFM EMG in some rats showed tonic activity during filling cystometry, while other rats displayed no tonic activity. However, PFM activity in the latter rats could be activated by pinching the perineal skin, suggesting that the innervation of the PFM was not damaged during our surgical procedure. Sensory innervation of the perineal skin in female rats is via either the lumbosacral or L6–S1 trunks with a certain degree of overlapping distribution, including the sensory branch of the pudendal nerve, the viscerocutaneous branch of the pelvic nerve, and the perineal branches of the L6–S1 trunk.⁷ We speculate that the pinching-induced response involves supraspinal neural control because the PFM could be activated by pinching either the contralateral or ipsilateral perineal skin, and had no response after T8–9 spinal cord transection (unpublished observation). In contrast, the bladder-to-EUS continence reflex remained after a spinal cord transection. Therefore, upon pinching in this study, we confirmed that the lack of tonic activity in the PFM in some rats is not due to damage to the innervation during our procedure.

Based on anatomical investigations, it has been proposed that the levator ani muscles have dual innervation from the pudendal nerve on the perineal surface, and from branches of the sacral nerves on the pelvic surface,¹⁰ or have variations of this innervation pattern.²¹ We believe it is nearly impossible to determine PFM innervation solely by gross anatomical observations,

since it can be difficult to differentiate tiny nerve fibers from fascia. Therefore we designed this functional electrophysiological study. We demonstrated that the pudendal nerve does not have a functional branch innervating the PFM in rats since transection of levator ani nerve abolished the pinch response, but transection of the pudendal nerve did not. We therefore confirmed that only the levator ani nerve functionally innervates the levator ani muscles in female rats.

We were unable to demonstrate a bladder-to-PFM reflex contributing to urethral resistance to leakage during an external increase in pressure in female rats. The PFM could nonetheless contribute to prevention of urinary leakage in these animals by supporting the bladder from prolapse, stabilizing the bladder neck, and strengthening PFM contraction during other reflexes such as during a cough or valsalva maneuver.^{4, 9}

Conclusion

In female rats the PFM does not have a reflex contribution to maintenance of continence during an applied external increase in bladder pressure. Even if the PFM is variously innervated, no bladder-to-PFM continence reflex could be demonstrated for prevention of urinary leakage in our experimental setting. The EUS in contrast, demonstrates a strong bladder-to-EUS reflex, increasing activity and urethral resistance in response to an external increase in bladder pressure. Thus, these two muscles are independently controlled and respond differently to a passive increase in bladder pressure in female rats. This result should be confirmed in a clinical study as it may have implications for the mechanism of continence in women.

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Figure 1.

Schematic of the experimental design. An external increase in bladder pressure until urinary leakage was made to determine if there is a bladder-to-pelvic floor muscle continence reflex which would be similar with the bladder-to-external urethral sphincter reflex. PFM, pelvic floor muscles; EUS, external urethral sphincter; EMG, electromyogram; XA, site of bilateral levator ani nerve transection; XB, site of bilateral pudendal nerve transection.

Figure 2.

External urethral sphincter (EUS) electromyogram (EMG) and pelvic floor muscle (PFM) EMG in a rat with tonic PFM activity during filling cystometrograms (CMG) and leak point pressure (LPP) testing (n=13). A: Simultaneous recordings of EUS and PFM EMG during filling cystometry, LPP testing and voiding. Arrows a and b indicate the EUS and PFM response to LPP testing, respectively; arrow c indicates the PFM response to voiding. B: One second samples of EUS and PFM EMG on an expanded time scale showing baseline (solid outline box in panel A) and peak LPP activity (dashed outline box in panel A). C: Frequency of baseline and peak LPP EUS and PFM EMG activity. D: Amplitude of baseline and peak LPP EUS and PFM EMG activity. Each bar shows mean $\pm$ standard error of data from 13 animals. * indicates a significant difference (p < 0.001) compared to EUS EMG baseline activity.

Figure 3.

External urethral sphincter (EUS) electromyogram (EMG) and pelvic floor muscle (PFM) EMG in animals without tonic PFM activity during filling cystometrogram (CMG) and leak point pressure (LPP) testing (n=18). A: Simultaneous recordings of EUS and PFM EMG during filling cystometry, LPP testing, perineal pinching, and voiding. Arrow a indicates EUS response to LPP testing; arrow b indicates PFM response to voiding. B: One second example of EUS and PFM EMG on an expanded time scale showing baseline activity (solid outline box in panel A) and peak LPP activity (dashed outline box in panel A). C: Frequency of baseline and peak LPP EUS EMG activity. D: Amplitude of baseline and peak LPP EUS EMG activity. Each bar shows mean $\pm$ standard error of data from 18 animals. * indicates a significant difference ($p < 0.001$) compared to comparable baseline activity level.

Figure 4.

External urethral sphincter (EUS) electromyogram (EMG) and pelvic floor muscle (PFM) EMG after bilateral pudendal nerve transection or bilateral levator ani nerve transection. A: EUS and PFM EMG at baseline and peak leak point pressure (LPP). B: Frequency and amplitude of PFM EMG at baseline and peak LPP after pudendal nerve transection. C: LPP before and after pudendal nerve transection. Each bar shows mean $\pm$ standard error of data from 6 animals. D: EUS and PFM EMG at baseline and peak LPP. E: Frequency and amplitude of EUS EMG baseline and peak LPP after levator ani nerve transection. F: LPP before and after levator ani nerve transection. Each bar shows mean $\pm$ standard error of data from 6 animals. * indicates a significant difference ($p < 0.001$) compared to LPP testing in the same

animals prior to pudendal nerve transection (intact). † indicates a significant difference ($p < 0.05$) compared to baseline EMG activity.