The role of bladder-to-urethral reflexes in urinary continence mechanisms in rats

Izumi Kamo, Tracy W. Cannon, Deirdre A. Conway, Kazumasa Torimoto, Michael B. Chancellor, William C. de Groat, and Naoki Yoshimura. The role of bladder-to-urethral reflexes in urinary continence mechanisms in rats. Am J Physiol Renal Physiol 287: F434–F441, 2004. First published April 27, 2004; 10.1152/ajprenal.00038.2004.—Urethral closure mechanisms during passive increments in intravesicular pressure (Pves) were investigated using microtip transducer catheters in urethane-anesthetized rats. After a block of reflex bladder contractions by spinal cord transection at T8-T9, abruptly raising Pves to 20, 40, or 60 cmH2O for 2 min induced a bladder pressure-dependent contractile response in a restricted portion of the middle urethra (12.5–15 mm from the urethral orifice) that was abolished by cutting the pelvic nerves bilaterally. In pelvic nerve-intact rats, the bilateral transection of either the pudendal nerves, the nerves to the iliococcygeous/pubococcygeous muscles, or hypogastric nerves significantly reduced (49–74%) the urethral reflex response induced by passive Pves increases, and combined transection of these three sets of nerves totally abolished the urethral-closing responses. In spinal cord-intact rats, similar urethral contractile responses were elicited during Pves elevation (20 or 40 cmH2O) and were also eliminated by bilateral pelvic nerve transection. After spinal cord and pelvic nerve transection, leak point pressures, defined as the pressure inducing fluid leakage from the urethral orifice during passive Pves elevation by either bladder pressure clamping in 2.5-cmH2O steps or direct compression of the bladder, were significantly lowered by 30–35% compared with sham-operated (spinal cord-transected and pelvic nerve-intact) rats. These results indicate that 1) passive elevation of Pves can elicit pelvic afferent nerve-mediated contractile reflexes in the restricted portion of the urethra mediated by activation of sympathetic and somatic nerves and 2) bladder-to-urethral reflexes induced by passive Pves elevation significantly contribute to the prevention of stress urinary incontinence.

STRESS URINARY INCONTINENCE (SUI), defined as the involuntary loss of urine during elevation of abdominal pressure in the absence of bladder contractions, is very common in women over middle age (7, 28). This disorder generally occurs as a result of defects in the various passive and reflex mechanisms that maintain urethral closure in the presence of elevated abdominal pressure. We have recently demonstrated that a nerve-mediated active urethral closure mechanism is crucial for preventing urinary leakage against elevated abdominal pressure during sneezing in rats (11). However, in patients with SUI, urine leakage occurs during abdominal pressure increases not only by sneezing but also by other Valsalva-like stress conditions such as laughing, jogging, or lifting heavy objects (22). Therefore, it is also important to study the role of urethral continence mechanisms that prevent SUI during sudden, passive increases in intravesicular pressure (Pves) that occur during abrupt elevations of abdominal pressure.

Previous studies have demonstrated that the bladder-to-sympathetic (hypogastric) and bladder-to-somatic (pudendal) nerve-mediated urethral reflexes are involved in the urethral closure mechanism during bladder distension in the storage phase (4, 6). However, it is not known whether these neural mechanisms in the urethra also contribute to urethral closure and prevent SUI under stress conditions. Therefore, the present study was performed using microtip transducer catheter techniques and LPP measurements in rats to characterize the neurally mediated active urethral closure mechanisms during sudden, passive increases in Pves, which mimic the condition that occurs during an abrupt elevation in abdominal pressure.

MATERIALS AND METHODS

Animals

Sixty-one adult female Sprague-Dawley rats weighing 209–305 g were studied according to experimental protocols approved by University of Pittsburgh Institutional Animal Care and Use Committee.

Experiment 1: Urethral Responses to Passive Elevation of Pves in Spinal Cord-Transected Rats

In five rats anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ) inhalation, the spinal cord was transected at the T8-T9 spinal cord level after laminectomy. A sterile sponge (Gel-foam, Upjohn, Kalamazoo, MI) was placed between the cut ends of the spinal cord, and the overlying muscle and skin were closed with sutures. Under this condition, it has been reported that reflex voiding that is organized by spinobulbospinal pathways passing through a micturition center in the pons is eliminated, whereas urethral reflexes induced by bladder distension, which are predominantly organized in the lumbosacral spinal cord, are preserved (4, 5). The urinary bladder was exposed through an abdominal incision, and the bladder neck was ligated with a suture to prevent fluid leakage from the bladder into the urethra. A polyethylene catheter with a fire-flared tip (PE-90, Clay Adams, Parsippany, NJ) was inserted into the bladder from the dome and secured with a ligature for bladder filling and pressure recording.

After the surgery, halothane anesthesia was replaced with urethane anesthesia (1.2 g/kg sc, Sigma, St. Louis, MO). A 3.5-Fr-size nylon catheter with a side-mounted microtip transducer located 1 mm from the catheter tip (SPR-524, Millar Instruments, Houston, TX) was inserted into the urethra from the urethral orifice with its side-mounted sensor facing the inner urethral surface in the 3 o’clock position, because measurement in a lateral orientation corresponds most closely to urethral...
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pressure (1). Although transurethral catheters might affect urethral function by dilating the urethra and activating afferent nerves, microtip transducer catheters were used because there are currently no noninvasive methods available for measuring local responses in a restricted portion of the urethra. The length of catheter inserted into the urethra from the urethral oriﬁce and its orientation were monitored to conﬁrm that the position of transducer was not moved throughout the experiments. Pves was kept constant by connecting a bladder catheter to a saline reservoir and a pressure transducer (BLPR, World Precision Instruments, Sarasota, FL) via three-way stopcocks. The reservoir was mounted on a metered vertical pole for controlled height adjustment. Pves was abruptly increased by elevating the reservoir and maintaining it for 2 min at a range of pressures (20, 40, 60 cmH2O). Between each pressure rise, the reservoir was returned to 0 cmH2O for 2 min. A microtip transducer catheter was inserted into the urethra from the urethral oriﬁce and connected to an ampliﬁer (Transbridge 4M, World Precision Instruments), and urethral responses were recorded using data-acquisition software (Chart, ADInstruments, Castle Hill, NSW, Australia) on a computer system equipped with an analog-to-digital converter (PowerLab, ADInstruments). Urethral pressure readings were digitally recorded at a sampling rate of 400 Hz, and all data sampled during an initial 1-min period after each Pves elevation (24,000 data points) were averaged using data-analysis software (Chart, ADInstruments) to calculate the mean urethral response during passive Pves increases. Urethral responses (expressed in cmH2O), which strictly speaking correspond to the force per unit area exerted by the wall of the organ on a catheter-mounted sensor, are considered to be approximately equal to changes in urethral pressure (1, 10).

The urethral length in female rats, which was determined by the length of the microtip transducer catheter inserted into the urethra from the urethral oriﬁce, was ~20 mm. Pves elevation-induced responses in different portions of the urethra were measured in 2.5-mm steps (17.5–15, 15–12.5, 12.5–10, 10–7.5, and 7.5–5 mm from the urethral oriﬁce) by changing the position of the microtip transducer catheter in the same rats. Responses in the proximal (20.0–7.5 mm) and distal urethra (5–0 mm) could not be measured because of the ligation at the bladder neck or the difﬁculty in holding the microtip transducer in position, respectively.

Experiment 2: Effects of Bilateral Transection of Nerves Innervating the Urethra and Pelvic Floor Muscles on Urethral Responses

Twenty-nine spinal cord-transected rats were used to evaluate the contribution of different nerves innervating the urethra or the pelvic ﬂoor to the bladder-to-urethral reﬂex. Under halothane anesthesia, pelvic nerves (n = 5), pudendal nerves (n = 5), nerves to ilioioccocygeous/ pubococcygeous muscles (muscular branch or somatomotor branch of the pelvic nerve as described in a previous report; n = 5) (17, 20), hypogastric nerves (n = 5), or three sets of nerves (pudendal, ilioioccocygeous/pubococcygeous muscles, and hypogastric nerves; n = 4) were transected bilaterally near the internal iliac vessels according to the method of Manzo et al. (17). Five sham-operated rats underwent an exposure of the nerves without transection after the preparations for pressure measurements as described for experiment 1. Urethral responses during passive increases in Pves were recorded as described for experiment 1 before and after the nerve transection.

In other experiments, the effects of intravenous administration of hexamethonium, a ganglionic blocking agent, or α-bungarotoxin, a neuromuscular blocking agent, were investigated. Urethral responses during passive increases in Pves were compared before and after hexamethonium (25 mg/kg iv, Sigma, n = 4) or α-bungarotoxin injection (0.4 mg/kg iv, Sigma, n = 3). After α-bungarotoxin application, animals were artiﬁcially respired via an intratracheal tube.

Experiment 3: Effects of Bilateral Transection of Pelvic Nerves on Urethral Responses in Spinal Cord-Intact Rats

Ten rats were used to examine whether the bladder-to-urethral reﬂex during passive Pves elevation observed in acute spinal cord-transected rats is similarly activated in spinal cord-intact rats. Pves elevation-induced responses in the urethra (12.5–15 mm from the urethral oriﬁce) were compared before and after bilateral transection of pelvic nerves (n = 5) or sham operation (n = 5) as described for experiment 1 without spinal cord transection. Urethral responses during Pves elevation to 20 or 40 cmH2O were assessed during only the period when the increase in urethral activity was stable without the urethral bursting activity that is indicative of the micturition reﬂex.

Experiment 4: Effects of Bilateral Transection of Pelvic Nerves on Leak Point Pressures

Leak point pressure (LPP) measurements were used in 10 rats to evaluate the contribution of bladder-to-urethral reﬂexes to urethral closure and urinary continence. Under halothane anesthesia, spinal cord transection and bladder catheter insertion were performed as described for experiment 1 except for ligation of the bladder neck. After the surgery, halothane anesthesia was switched to urethane anesthesia. Thereafter, LPP measurements were performed during passive Pves elevation induced by either the Pves clamp method or bladder compression from the outside of the bladder before and after bilateral transection of pelvic nerves (n = 5) or sham operation (n = 5).

Pves clamp method. LPP was estimated using the Pves clamp method described for experiment 1. By elevating a reservoir mounted on a metered vertical pole in 2.5-cmH2O steps from 0 cmH2O (90 s at each step), the Pves at which ﬂuid leaked from the urethral oriﬁce was estimated. Pves was returned to 0 cmH2O between the pressure steps for 2 min. The pressure at which ﬂuid leakage occurred was regarded as LPP.

Bladder compression method. The bladder catheter was connected to a pressure transducer, and after the bladder was emptied, 0.3 ml of saline solution was injected into the bladder. Pves, was increased gradually by bladder compression from the outside of the bladder using two cotton swabs until ﬂuid leakage from the urethral oriﬁce was observed. The pressure at ﬂuid leakage was measured as LPP.

Statistical Analysis

Data are expressed as means ± SE. In experiments 1 and 2, urethral responses were measured two times after each increment in Pves, and the mean ± SE in a group of animals was calculated from the averaged value of two trials in each rat. Two-way ANOVA was performed for the analysis of statistical differences in the urethral response before and after nerve transection (experiment 2). For the comparison of statistical differences in the baseline urethral reading before and after nerve transection (experiment 2), % changes after nerve transection were analyzed with a paired t-test. In experiment 3, urethral responses were measured three times at every increment of Pves, and the mean ± SE in a group of animals was calculated from the averaged value of three trials in each rat. For the analysis of statistical differences in the urethral response before and after pelvic nerve transection, two-way ANOVA was performed. In experiment 4, LPP was measured three times by using the Pves clamp method and ﬁve times with the bladder compression method before and after bilateral transection of pelvic nerves. The average of three or ﬁve consecutive LPPs was calculated in each test of each rat (Pves-clamp method or bladder compression method, respectively). The mean ± SE was then obtained in each group of animals. For the analysis of statistical differences, % changes in LPPs after bilateral transection of pelvic nerves were compared in sham-operated and pelvic nerve-transected groups with Student’s t-test. P values <0.05 were considered to be signiﬁcant.

RESULTS

Experiment 1: Urethral Responses to Passive Increases in Pves in Spinal Cord-TRANSECTED Rats

Contractile responses in restricted portions of the urethra (17.5–15, 15–12.5, 12.5–10, 10–7.5, or 7.5–5 mm from the
urethral orifice) during passive elevation of $P_{ves}$ were measured by a microtip transducer catheter ($n = 5$). In the proximal urethra (17.5–15 mm from the urethral orifice) and the distal urethra (10–7.5 and 7.5–5 mm from the orifice), the average baseline pressure readings were 5–20 cmH$_2$O at 0 cmH$_2$O of $P_{ves}$ (Figs. 1A and 2, Table 1), and $P_{ves}$ elevation to 20, 40, or 60 cmH$_2$O did not induce significant urethral responses (Figs. 1A and 2). However, in the middle urethra (15–12.5 and 12.5–10 mm from the orifice), baseline urethral pressures were higher and the urethral responses during passive $P_{ves}$ elevation were more obvious than in the proximal or distal urethra (Fig. 1). In the middle urethra, the baseline pressure reading at 0 cmH$_2$O of $P_{ves}$ was the highest in the urethra at 12.5–10 mm from the urethral orifice, whereas the changes in urethral pressure-induced $P_{ves}$ elevation were the greatest (6.6–11.2 cmH$_2$O) in the urethra at 15–12.5 mm from the orifice (Figs. 1A and 2). The magnitude of urethral responses, which rapidly reached a peak value in 5–10 s after passive $P_{ves}$ elevation and then gradually declined in most experiments over the course of the next 2 min of $P_{ves}$ elevation, was dependent on the magnitude of the $P_{ves}$ increase (Fig. 1). In addition, $P_{ves}$ elevation-induced urethral responses were associated with high-frequency fluctuating activity (Fig. 1). The increased urethral pressures returned to baseline values when $P_{ves}$ was lowered to 0 cmH$_2$O (Fig. 1).

Experiment 2: Effects of Bilateral Transection of Nerves Innervating the Urethra and Pelvic Floor Muscles on Urethral Responses

To investigate which afferent or efferent nerves were responsible for the $P_{ves}$-induced urethral contractile responses, pressures were measured in the urethra at 15–12.5 mm from the urethral orifice before and after bilateral transection of pelvic nerves ($n = 5$), pudendal nerves ($n = 5$), nerves to the iliococcygeous/pubococcygeous muscles ($n = 5$), hypogastric nerves ($n = 5$), or three sets of nerves (pudendal nerves, nerves to the iliococcygeous/pubococcygeous muscles and hypogastric nerves; $n = 4$).

The baseline value in each location of the urethra before transection of the nerve was not statistically different among the groups tested with transection of different nerves (Table 1). Sham operation did not affect baseline pressure readings along the urethra at 0 cmH$_2$O $P_{ves}$ or the responses in the urethra at the 15- to 12.5-mm site during passive $P_{ves}$ elevation (Fig. 3A).
and Table 1). After bilateral pelvic nerve transection, the urethral response to $P_{\text{ves}}$ elevation was totally abolished, whereas the baseline pressure at 0 cmH$_2$O $P_{\text{ves}}$ was not significantly altered (Fig. 3B and Table 1), suggesting that afferent information from the bladder carried through the pelvic nerve triggers the bladder-to-urethral contractile responses during passive $P_{\text{ves}}$ elevation. Bilateral transection of pudendal nerves greatly reduced the $P_{\text{ves}}$ elevation-induced urethral response by

| Nerve Transection | n | Location in Urethra, mm from urethral orifice | Baseline pressure values in different portions of the urethra (2.5-mm steps from the urethral orifice) measured by a microtip transducer catheter at 0 cmH$_2$O $P_{\text{ves}}$ in female rats with acute spinal cord transection |
|-------------------|---|---------------------------------------------|
|                  |   | 17.5–15.0 | 15–12.5 | 12.5–10 | 10–7.5 | 7.5–5 |
| Sham             | 5 | Before | After | Before | After | Before | After | Before | After | Before | After | Before | After |
| Pel X            | 5 | 5.2±3.2 | 4.4±2.3 | 15.2±4.8 | 19.7±4.0 | 23.3±4.7 | 26.1±2.8 | 19.5±4.8 | 18.8±3.4 | 7.7±1.6 | 12.7±2.6 |
| Pud X            | 5 | 4.6±1.8 | 3.7±0.9 | 17.1±3.0 | 17.1±3.6 | 22.9±2.2 | 18.3±2.6 | 18.2±1.6 | 15.9±0.8 | 8.9±3.0 | 13.8±1.9 |
| Ilio/Pubo X      | 5 | 2.5±0.9 | 5.9±1.0 | 13.9±2.2 | 14.7±4.3 | 17.4±5.0 | 16.9±4.7 | 14.3±2.1 | 14.0±2.8 | 10.2±2.8 | 13.3±1.3 |
| Hypo X           | 5 | 8.1±1.9 | 9.1±3.3 | 21.6±1.7 | 14.4±2.0† | 27.8±4.8 | 29.3±3.4 | 18.3±2.9 | 20.0±3.1 | 11.6±1.5 | 14.9±0.4 |
| Pud+Ilio/Pubo+Hypo X | 4 | 5.2±1.9 | 6.5±2.1 | 26.5±6.5 | 8.3±2.0* | 35.2±2.9 | 30.8±4.3 | 20.6±2.7 | 17.7±2.3 | 9.1±2.5 | 13.1±2.5 |

Values are means ± SE. Pressures were measured before and after sham operation and various nerve transections (X). n, No. of animals; Pel, pelvic nerve; Pud, pudendal nerve; Ilio/Pubo, nerves to the iliococcygeous and pubococcygeous muscles; $P_{\text{ves}}$, intravesicular pressure; Hypo, hypogastric nerve. Percent changes in baseline pressure readings after nerve transection were examined by paired $t$-test ($^*P$ < 0.05, †$P$ < 0.01).

Fig. 3. Contractile responses of the urethra at 15–12.5 mm from the urethral orifice measured by a microtip transducer catheter during increments in $P_{\text{ves}}$ to 20, 40, or 60 cmH$_2$O before and after the sham operation (A), bilateral transection of pelvic nerves (B), pudendal nerves (C), nerves to iliococcygeous and pubococcygeous muscles (D), hypogastric nerves (hypogastric X; E), or 3 sets of nerves (pudendal nerves and nerves to iliococcygeous and pubococcygeous muscles and hypogastric nerves; F). Values are means ± SE; n = 5 rats. The changes in responses after the nerve transection were statistically significant in B–F ($P$ < 0.001, 2-way ANOVA).
69% (at 60 cmH₂O Pves) without affecting baseline urethral pressure readings (Fig. 3C and Table 1). The high-frequency fluctuating activity during Pves elevation was reduced but not completely blocked after pudendal nerve transection. Bilateral transection of nerves to iliococcygeus/pubococcygeous muscles also greatly reduced the Pves-induced urethral contractile responses by 74% (at 60 cmH₂O Pves) as well as high-frequency fluctuating activity (Fig. 3D). This treatment also significantly reduced the baseline urethral pressure readings by 34% only at the 15- to 12.5-mm site (Table 1). In addition, bilateral transection of hypogastric nerves partially but significantly reduced the Pves elevation-induced urethral response by 49% (at 60 cmH₂O Pves) without affecting baseline pressure readings (Fig. 3E and Table 1). When three sets of nerves (pudendal nerves, nerves to iliococcygeus/pubococcygeous muscles, and hypogastric nerves) were cut, the urethral response to Pves elevation totally disappeared, and the baseline pressure in the urethra at 15–12.5 mm from the orifice was reduced by 58%, although other parts of the urethra did not show any change in the urethral baseline tone (Fig. 3F and Table 1).

Intravenous injection of hexamethonium (25 mg/kg) also partially but significantly (P = 0.0025, 2-way ANOVA) decreased the Pves-induced urethral response at the 15- to 12.5-mm site from 1.4 ± 0.2, 3.1 ± 1.0, and 5.0 ± 1.2 cmH₂O (at 20, 40, and 60 cmH₂O Pves, respectively; n = 4) to 0.4 ± 0.5, 1.4 ± 0.9, and 3.4 ± 1.3 cmH₂O (at 20, 40, and 60 cmH₂O Pves, respectively; n = 4). Intravenous injection of α-bungarotoxin (0.4 mg/kg) also significantly (P = 0.0055, 2-way ANOVA) decreased the Pves-induced urethral response at the 15- to 12.5-mm site from −0.1 ± 0.4, 1.7 ± 0.2, and 3.2 ± 0.1 cmH₂O (at 20, 40, and 60 cmH₂O Pves, respectively; n = 3) to 0.1 ± 0.2, 0.4 ± 0.8, and 1.1 ± 0.3 cmH₂O (at 20, 40, and 60 cmH₂O Pves, respectively; n = 3). In addition, after α-bungarotoxin treatment, high-frequency fluctuating activity in Pves-induced urethral responses was completely abolished, indicating that fluctuating activity in urethral responses during Pves elevation is induced by striated muscle contractions (data not shown).

Experiment 3: Effects of Bilateral Transection of Pelvic Nerves on Urethral Responses in Spinal Cord-Intact Rats

To examine whether the bladder-to-urethral reflex is also active in spinal cord-intact rats, the contractile responses in the urethra at 15–12.5 mm from the orifice during Pves elevation were evaluated before the occurrence of the oscillatory bursting activity of the urethra that is indicative of the voiding reflex in spinal cord-intact rats (n = 10).

During the elevation of Pves to 20 or 40 cmH₂O, all rats showed an increase in urethral pressure readings measured by a microtip transducer catheter (Fig. 4A). In 3 of 10 rats, at 40 cmH₂O Pves elevation, the initial increment of urethral contractile activity was followed by high-frequency bursting activity of the urethra (Fig. 4B). In this condition, the Pves elevation-induced urethral response was evaluated during the period when urethral activity was stable before urethral bursting activity. At 60 cmH₂O (Pves), in all rats tested the urethra exhibited high-frequency oscillatory bursting activity without a clear initial tonic phase; therefore, the urethral responses at 60 cmH₂O Pves elevation were not evaluated.

When the effects of bilateral pelvic nerve transection on the initial tonic phase of urethral activity were evaluated at 20 or 40 cmH₂O Pves elevations, it was clear that urethral responses were not altered by the sham operation (n = 5) but were abolished by bilateral transection of pelvic nerves (Fig. 5), indicating that the reflex responses were triggered by pelvic nerve afferents as in rats with acute spinal cord transection (experiments 1 and 2).

Experiment 4: Effects of Bilateral Transection of Pelvic Nerves on LPPs

To clarify the contribution of nerve-mediated reflex urethral closure mechanisms during passive Pves elevation to total urethral resistance, LPPs obtained by two different techniques of passive Pves elevation (Pves clamp and bladder compression methods) were compared before and after bilateral transection of pelvic nerves in the same rat. In the Pves clamp method, LPPs were not changed after the sham operation but decreased by 15.7 cmH₂O (32%) after bilateral transection of pelvic nerves (Table 2). In the direct bladder compression method, LPP values were not changed after the sham operation but were significantly decreased by 22.8 cmH₂O (35%) after bilateral transection of pelvic nerves (Table 2).

DISCUSSION

SUI characterized by symptoms of involuntary urine loss due to an increase in abdominal pressure is caused by a dysfunction in urinary continence mechanisms (22). The results in the present study indicate that 1) the bladder-to-urethral
reflex during passive elevation of $P_{ves}$ is prominently observed in the middle urethra; 2) this urethral reflex activity is mediated by activation of afferent pathways in the pelvic nerves and efferent pathways in the hypogastric nerves, pudendal nerves, and somatic nerves innervating pelvic floor muscles; and 3) the bladder-to-urethral reflex contributes to the prevention of SUI by increasing total urethral resistance during passive $P_{ves}$ elevation.

In the first part of this study (experiments 1 and 2), the mechanisms of urethral closure responses in different portions of the urethra during passive $P_{ves}$ increases were studied using microtip transducer catheters in rats with acute spinal cord transection. In this series of experiments, the voiding reflex mediated by spinobulbospinal pathways was eliminated, whereas urethral closure mechanisms during urine storage remained intact because urethral contractile reflexes activated by sympathetic and somatic nerves responding to bladder distension are predominantly organized at the lumbosacral spinal cord level (4–6). In this condition, we found that the baseline urethral activity measured at 0 cmH$_2$O of $P_{ves}$ was the highest in the middle urethra (12.5–10 mm from the urethral orifice), whereas the bladder-to-urethral contractile response during passive $P_{ves}$ elevation was the strongest in the mid- to proximal urethra (15–12.5 mm from the urethral orifice). In addition, when the effect of bilateral transection of efferent nerves innervating the urethra and the pelvic floor was examined, the combined transection of hypogastric nerves, pudendal nerves, and somatic nerves innervating pelvic floor muscles abolished the $P_{ves}$-induced bladder-to-urethral contractile response, whereas the transection of each set of nerves significantly, but only partially, suppressed the responses. Other investigators have reported that 1) a muscular branch of the pelvic nerve (the nerve to iliococcygeus/pubococcygeous muscles in this study) innervates pelvic floor muscles such as iliococcygeus/pubococcygeous muscles in rats (20); 2) the pudendal nerve innervates the external urethral sphincter and other pelvic floor muscles such as coccygeus muscles (20); and 3) the hypogastric nerve, a sympathetic nerve, innervates urethral smooth muscles (6). Previous studies have also demonstrated that 1) electrical stimulation of the coccygeus muscle increases intraurethral pressure (21) and 2) electrical stimulation of the pudendal nerve or the hypogastric nerve induces urethral contractions (15, 16, 29). Based on these findings, it seems likely that $P_{ves}$ elevation-induced bladder-to-urethral contractile responses are induced by reflex activity of three sets of nerves to the urethral smooth and striated muscles as well as pelvic floor muscles.

The resting basal tone of the middle urethra (15–12.5 mm from the urethral orifice), where $P_{ves}$ elevation-induced bladder-to-urethral contractile responses were the greatest, seems to be regulated by somatic nerve-mediated contractions of iliococcygeus/pubococcygeous muscles because baseline pressure in this portion of the urethra was reduced after transection of the nerves to iliococcygeus/pubococcygeous muscles. However, basal urethral pressure values at 0 cmH$_2$O of $P_{ves}$ at all other sites in the urethra including the middle urethra (12.5–10 mm from the urethral orifice), at which the highest resting pressure was recorded, were not affected by any nerve transection. Thus the contribution of nerve-mediated muscle contractions to the resting urethral tone seems to be minimal.

In this study, $P_{ves}$ elevation-induced bladder-to-urethral contractile responses were also eliminated by transection of the pelvic nerves. Although the pelvic nerve contains both afferent and efferent fibers, the contribution of pelvic efferent pathways to the bladder-to-urethral contractile responses seems unlikely because it is known that parasympathetic efferents in the pelvic nerve are quiescent during urine storage (6) and that activation of pelvic nerves induces NO-mediated smooth muscle relaxation rather than contraction in the urethra of female rats (8). Thus the elimination of the $P_{ves}$ elevation-induced bladder-to-urethral reflex after pelvic nerve transection is most reasonably attributed to the ablation of afferent inputs from the bladder to the spinal cord. This conclusion is also supported by our finding that hexamethonium that can block ganglionic transmission in efferent pathways of the pelvic nerve as well as the hypogastric nerve did not produce total suppression of the

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**Table 2. Leak point pressures measured by the $P_{ves}$-clamp method or bladder compression method before and after the sham operation and bilateral of Pel X in female rats with acute spinal cord transection**

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<td>Before</td>
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<tr>
<td>Sham</td>
<td>55.2±2.6</td>
<td>55.0±3.1</td>
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<td>Pel X</td>
<td>48.4±0.8</td>
<td>32.7±4.3*</td>
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Values are means ± S.E (cmH$_2$O) in 5 rats. Sham, sham operation. Percent changes in leak point pressure (LPP) in the sham-operated group and the Pel-X group were compared with a Student’s t-test (*$P < 0.05$ and †$P < 0.01$).
Pves-induced urethral response but rather mimicked the effects of hypogastric nerve transection (i.e., a partial reduction in the urethral response). Overall, it seems reasonable to conclude that the afferent limb of the bladder-to-urethral contractile reflex during passive Pves elevation consists of afferent pathways in the pelvic nerve, which are reportedly connected to tension and volume receptors in the bladder (18), and that the efferent limb of the reflex consists of sympathetic pathways in the hypogastric nerve, innervating urethral smooth muscles, and somatic pathways in the pudendal nerve and other somatic nerves, innervating striated muscles in the urethra and the pelvic floor.

In this study, we further demonstrated that the bladder-to-urethral contractile reflexes during passive Pves elevation also exist in spinal cord-intact rats, although the measurements were only made at lower Pves (20 or 40 cmH2O), because bladder distension at higher pressure induced a voiding reflex, resulting in bursting activity of the urethra that prevented detailed analyses of urethral responses. In this series of experiments, we found that the transection of pelvic nerves totally abolished the Pves elevation-induced urethral contractile response as seen in rats with acute spinal cord transection. Therefore, it is assumed that the bladder-to-urethral reflex induced by passive Pves elevation is organized at the spinal cord level and also functions during the storage phase in a spinal cord-intact condition.

In the last series of the experiments (experiment 4), we examined using LPP measurements whether the Pves-induced bladder-to-urethral reflex contributes to increases in total urethral resistance, which prevent urine leakage. This was necessary because the pressure readings recorded by microtip transducer catheters only reflect local force/unit per area (mechanical stress) exerted by the tissue’s inner surface on a transducer tip but not the entire urethral pressure (10, 23). Because the LPP value during passive Pves elevation measured by the pressure-clamp method was significantly reduced (32%) after pelvic nerve transection that abolished the bladder-to-urethral reflex without affecting baseline tone of urethra (experiment 2), the bladder-to-urethral reflex seems likely to contribute significantly to the continence mechanism, which maintains a high LPP value against a passive elevation of Pves. This assumption is also supported by previous findings that the LPP measured by the Pves clamp method was significantly decreased after bilateral transection of the pudendal nerve and nerves to iliococcygeous/pubococcygeous muscles in rats (3). In this study, additional experiments were also performed to investigate whether the Pves elevation induced by compression from the outside of the bladder produces results similar to those obtained by the pressure-clamp method, which increases Pves from inside the bladder, because passive Pves elevation that leads to urine leakage in patients with SUI is induced by rises in abdominal pressure that, in turn, increase Pves from the outside of the bladder wall. When the bladder was directly compressed from the outside using cotton swabs (bladder compression method in experiment 4), the LPP was significantly reduced by 35% after pelvic nerve transection as found in the pressure-clamp method, suggesting that the bladder-to-urethral reflex would also play an important role in preventing urinary leakage induced by an increase in abdominal pressure.

We have previously demonstrated that the urethral closure mechanism activated by sneezing was mediated by somatic nerves ( pudendal nerves and nerves to iliococcygeous/pubococcygeous muscles) and was crucial for preventing urinary leakage during sneezing in rats (11). Because this urethral closure response during sneezing was not affected by abdominal opening or by bilateral transection of pelvic nerves and hypogastric nerves (11), the sneeze-induced continence mechanism is likely to be activated directly by sneezing but not by activating afferent pathways from the bladder, which triggered Pves-induced bladder-to-urethral continence reflexes, as shown in this study. Therefore, there seem to be at least two different urethral closure mechanisms to prevent SUI. Thus it might be possible to assume that Pves-induced bladder-to-urethral continence reflexes can be activated during abdominal pressure rises induced by Valsalva-like stress conditions such as laughing, jogging, or lifting heavy objects and that another continence reflex can additionally be activated by even stronger, phasic stress conditions such as sneezing or coughing.

In patients with SUI, the incidence of intrinsic sphincter deficiency, characterized by a malfunction of the urethral sphincter mechanism, resulting in a low-pressure urethra, has been reported to be greater than previously thought (13). Previous clinical studies in women with SUI have documented 1) an increased number of pelvic floor muscle fibers that exhibit pathological damage (9, 2) a decrease in fast-twitch type II muscle fibers in the pelvic floor (2), 3) partial denervation of the pelvic floor musculature due to pudendal neuropathy (24, 25), 4) decreased electromyographic activity of the striated urethral sphincter muscle (26), and 5) thinner urethral rhabdosphincter muscles measured by an ultrasound technique in the patients with intrinsic sphincter deficiency (14). Thus it seems reasonable to assume that the reflex urethral closure mechanism against passive Pves increases, induced by the raising of abdominal pressure, is impaired, thereby leading to reduced LPP and urinary incontinence in women with intrinsic sphincter deficiency. Because recent studies have revealed that duloxetine, which is a 5-hydroxytryptamine and norepinephrine reuptake inhibitor, can increase electromyographic activity of the external urethral sphincter in the cat (12, 27) and is effective for treatment of SUI (19), it is also possible that duloxetine might enhance the reflex urethral closure mechanisms during passive Pves elevation; however, further studies are necessary to clarify this point. The method used in the present experiments, which can explore reflex urethral function during passive elevation of Pves, could be useful for studying the pathophysiology of SUI as well as developing new treatment modalities of SUI including the screening of drugs.

In summary, this study demonstrated that passive increases in Pves that activate afferent pathways in the pelvic nerve can elicit reflex contractile responses in a restricted portion of the middle urethra (12.5–15 mm from the urethral orifice) via efferent pathways in three sets of autonomic and somatic nerves (the hypogastric nerve, the pudendal nerve, and the nerves to iliococcygeous/pubococcygeous muscles). This bladder-to-urethral reflex activity also seems to significantly contribute to maintaining the urethral continence mechanism, preventing SUI.
GRANTS
This work was supported by National Institutes of Health Grants DK-067226 and AR-049398.

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