Urethral closure mechanisms under sneeze-induced stress condition in rats - A new animal model for evaluation of stress urinary incontinence

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Running head: Urethral closure under stress condition in rats

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Abstract

The urethral closure mechanism under a stress condition induced by sneezing was investigated in urethane-anesthetized female rats. During sneezing, while the responses measured by micro-tip transducer catheters in the proximal and middle parts of the urethra increased, the response in the proximal urethra was almost negligible when the bladder response was subtracted from the urethral response or when the abdomen was opened. In contrast, the response in the middle urethra during sneezing was still observed after subtracting the bladder response or after opening the abdomen. These responses in the middle urethra during sneezing were significantly reduced approximately 80% by bilateral transection of the pudendal nerves and the nerves to the iliococcygeous and pubococcygeous muscles, but not by transection of the visceral branches of the pelvic nerves and hypogastric nerves. The sneeze leak point pressure was also measured to investigate the role of active urethral closure mechanisms in maintaining total urethral resistance against sneeze-induced urinary incontinence. In sham-operated rats, no urinary leakage was observed during sneeze which produced an increase of intravesical pressure up to $37 \pm 2.2$ cm H$_2$O. However, in nerve-transected rats urinary leakage was observed when the intravesical pressure during sneezing exceeded $16.3 \pm 2.1$ cmH$_2$O.
These results indicate that during sneezing, pressure increases elicited by reflex contractions of external urethral sphincter and pelvic floor muscles occur in the middle portion of the urethra. These reflexes in addition to passive transmission of increased abdominal pressure significantly contribute to urinary continence mechanisms under a sneeze-induced stress condition.

**Keywords**

Stress urinary incontinence, Urethral resistance, Active urethral closure, Sneeze leak point pressure
Introduction

Stress urinary incontinence (SUI) is defined as involuntary loss of urine secondary to an increase in abdominal pressure during events such as sneezing, coughing or laughing, in the absence of bladder contractions. This disorder is very common in women over middle age, occurring in 37.7% of non-institutionalized women older than 60 years of age (7) and in 33.9% of women over 40 years of age (32).

Urinary continence is maintained during elevation of abdominal pressure by multiple mechanisms. The passive closure of the urethra by the transmission of increased abdominal pressure to the urethra has been considered to play an important role in urinary continence; in addition, under stress conditions neurally-mediated urethral closure has also been proposed based on various clinical findings, including: (I) the rise in urethral pressure starts before cough transmission (27, 28, 33), (II) the increase in urethral pressure during coughing exceeds the increase in bladder pressure (5, 6, 27, 28), and (III) urethral closure forces during coughing were significantly reduced by bilateral pudendal nerve blockade (26).

It has recently been reported that in SUI patients the incidence of intrinsic sphincter deficiency, characterized by a malfunction of the urethral sphincter mechanism resulting in
the low-pressure urethra, was greater than previously thought (13). Therefore, it seems important to study the active urinary closure mechanism under stress conditions. In animal studies, the details of urethral closure mechanisms during elevation of abdominal pressure have not been studied except in dogs, in which two components (passive and active) of urethral closure mechanisms were detected (10, 30, 31).

The aim of this study was to clarify urinary continence mechanisms during the elevation of abdominal pressure induced by sneezing in rats. Sneeze, which is a highly coordinated reflex evoked by irritation of nasal mucosa, is designed to remove irritants and clean the airway. Previous studies have used this reflex to induce urinary leakage in a rat model of SUI induced by intravaginal balloon inflation (15, 22). It is hoped that the development of a reproducible animal model of SUI will lead to better understanding of pathophysiology of this condition and to develop new treatments for SUI.

**Materials and Methods**

**Animals**

Forty eight adult female rats of Sprague-Dawley strain weighing 212 – 293 gm were
studied according to experimental protocols approved by University of Pittsburgh Institutional Animal Care and Use Committee.

**Experiment 1  Bladder and urethral responses during sneezing**

Five rats were anesthetized by halothane (Halocarbon Laboratories, River Edge, NJ) inhalation, and a polyethylene catheter (PE-50, Clay Adams, Parsippany, NJ) was inserted into a carotid artery for recording arterial blood pressure. The urinary bladder was exposed through an abdominal incision, and both ureters were cut and their distal ends were ligated. A 3.5 Fr. size nylon catheter with a side-mounted micro-tip transducer located 1mm from the catheter tip (SPR-524, Millar Instruments Inc., Houston, Texas) was inserted into the bladder from the dome. The abdomen was then closed with sutures.

After the surgery, halothane anesthesia was turned off and replaced with urethane anesthesia (0.72 g/kg, s.c., Sigma, St. Louis, MO). Additional doses of anesthetic were then administered intravenously as required during the experiments. Another micro-tip transducer catheter 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 a measurement in a lateral orientation corresponds most closely to the urethral pressure (1).
Although transurethral catheters might affect bladder and urethral functions by dilating the urethra and activating afferent nerves, micro-tip transducer catheters were used because there are currently no non-invasive methods available for measuring local responses in a restricted portion of the urethra. The urethral length in female rats, which was determined by the length of micro-tip transducer catheters inserted into the urethra from the urethral orifice, was approximately 20 mm.

In preliminary experiments, the different characteristics of urethral response during sneezing were observed at proximal, middle, and distal portions of the urethra (15 – 20 mm, 10 – 15 mm and 0 - 10 mm from the urethral orifice, respectively) by changing the position of the micro-tip transducer catheter in the same rats. The length of catheter inserted into the urethra from the urethral orifice was measured before and after inducing the sneeze reflex to confirm that the position of transducer was not moved during the sneeze reflex. The bladder was emptied before starting the experiment so as to measure response under standardized conditions. This procedure was necessary for recording pressures independently using micro-tip transducer catheters in the bladder and urethra and also allowed us to focus on the sneeze-induced urethral reflex without influences of possible bladder-to-urethral reflexes induced by bladder distention. A rat whisker was cut and
inserted into the nostrils to induce the sneeze reflex. Micro-tip transducer catheters were connected to an amplifier (Transbridge 4M, World Precision Instruments, Sarasota, FL) and bladder (Ves) and urethral (Ura) responses, and their difference (Ura-Ves difference) during sneezing were recorded using data acquisition software (Chart, ADInstruments, Castle Hill, NSW, Australia) on a computer system equipped with an analog-to-digital converter (Power lab, ADInstruments). Sneeze-induced bladder and urethral responses measured by micro-tip transducer catheters, which strictly speaking correspond to the force per unit area exerted by the wall of the organ on the catheter-mounted sensor, are approximately equal to changes in the bladder and urethral pressures and were expressed in cmH₂O (1, 9). As the magnitude of the sneeze reflex varied in each episode, at least 10 sneeze reflexes were induced in each test. In 3 out of 5 rats, the sneeze reflex was also induced after opening the abdomen. In addition, simultaneous recordings of rectal responses using micro-tip transducer catheters were performed in 3 other rats to examine whether sneeze-induced pressure increases in the abdomen produce similar responses in the bladder and rectum. We also confirmed in preliminary experiments (n=3) that sneeze-induced rectal responses were similar before and after opening and subsequent closing of the abdomen with sutures. Thus it seems that when the abdomen was closed with the method used in this study it was
reliably airtight.

**Experiment 2  Effects of bilateral transection of nerves innervating urethra on the urethral responses during sneezing**

Twenty eight rats were used to evaluate the contribution to urethral closure of each nerve innervating the urethra. Under halothane anesthesia, pudendal nerves (n=6), nerves to iliococcygeous and pubococcygeous muscles (somatomotor branch of pelvic nerve as described in a previous report (16); n=7), or both pudendal nerves and nerves to iliococcygeous and pubococcygeous muscles (n=5) were transected bilaterally near internal iliac vessels according to the method of Manzo et al (16). In 3 rats, hypogastric nerves and visceral branches of the pelvic nerves were transected bilaterally near the major pelvic ganglia. In 7 rats, a sham operation was performed. The bladder and middle urethral responses during sneezing were recorded as described in experiment 1.

**Experiment 3  Effects of bilateral transection of pudendal nerves on electromyogram (EMG) recordings of external urethral sphincter (EUS)**

In another series of experiments, the change in EUS-EMG activity during sneezing was
evaluated (n=7). In 3 out of 7 rats, pudendal nerves were transected bilaterally under halothane-anesthesia. A pair of wire electrodes (1512F, Life-Tech, Inc., Stafford, TX) was inserted into the EUS under urethane-anesthesia and connected to an amplifier (Grass P511, Astro-Med. Inc., West Warwick, RI). The sneeze reflex was induced as described above, and changes in EMG recordings of EUS were recorded on a computer system equipped with an analog-to-digital converter using the data acquisition software. Recorded EMG activity was integrated for each 1 second before and after starting the sneeze reflex, and the increase in integrated EMG value during sneezing was calculated. In addition, EMG activity in anal sphincter muscles was also evaluated in 3 other rats to examine whether similar reflex activities during sneezing occur in the anal sphincter.

**Experiment 4  Effects on sneeze leak point pressure (SLPP) of bilateral transection of the pudendal nerves and nerves to iliococcygeous and pubococcygeous muscles**

Eight rats were anesthetized by halothane inhalation and the urinary bladder was exposed through an abdominal incision. A catheter (PE-50) connected to a pressure transducer (BLPR, World Precision Instruments) was inserted into the bladder from the dome for recording the intravesical pressure. The visceral branches of pelvic nerves were
cut bilaterally near internal iliac vessels so that reflex bladder contractions as well as the bladder-to-urethral reflex induced by bladder distention were prevented. Then, the 8 rats were divided into 2 groups (each 4 rats) with or without bilateral transection of both the pudendal nerves and the nerves to the iliococcygeus and pubococcygeus muscles. The abdomen was then closed with sutures.

After the surgery, the anesthesia was switched to urethane anesthesia. After emptying the bladder, 0.4 ml of saline solution containing Evans blue (100 $\mu$g/ml) (Sigma) was injected into the bladder. The sneeze reflex was induced as described above at least 50 times while recording changes in intravesical pressure to examine whether urinary leakage from the urethral orifice was induced. The maximal intravesical pressure was measured during each sneeze event and the lowest pressure value that induced fluid leakage from the urethral orifice was defined as the sneeze leak point pressure.

Statistical analysis

Data are expressed as the mean ± SEM. In experiments 1 to 3, at least 10 sneeze-evoked responses were recorded in one series under the same experimental conditions and the values were averaged in each rat. The mean ± SEM in a group of
animals was then calculated from the averaged value in each rat except for Fig. 5, where the method of analysis was different as described in the Results section below. To analyze the statistical difference in responses of proximal, middle, and distal urethra during sneezing (Experiment 1, Table-1), Dunnett’s test was used. For the analysis of statistical differences in the mean values among nerve-transected groups (Experiment 2, Table-2), Dunnett’s test or Student’s t-test was used. The statistical difference of the mean change in EMG activity during sneezing (Experiment 3) was analyzed between the sham-operated and the pudendal nerve-transected groups by a Student’s t-test. Probability values (P) smaller than 0.05 were considered to be significant. In experiments 4, at least 50 sneeze reflexes were induced and the following parameters (SLPP, maximal intravesical pressure without fluid leakage among all sneeze events, average of maximal intravesical pressure in each sneeze event, and incidence of fluid leakage) were calculated in each rat. The mean ± SEM was then obtained in each group of animals.

Results

Experiment 1  Changes in the bladder and urethral responses during sneezing
When the sneeze reflex was induced by stimulation of nostril inner surface with a rat whisker, blood pressure consistently decreased following induction of a sneeze (Figs 1 and 2). Bladder and urethral responses in the proximal, middle and distal parts during sneezing were measured using micro-tip transducer catheters under an abdomen-closed condition (n=5), and then, in 3 out of 5 rats, the abdomen was opened to eliminate the increase in abdominal pressure during sneezing, and responses induced by sneezing were then recorded.

**Bladder**

The readings measured by a micro-tip transducer catheter in the bladder increased during sneezing and returned to the baseline within 0.15 sec under an abdomen-closed condition (Figs 1 and 3). When rectal responses were measured using a micro-tip transducer catheter, a similar shape of short-lasting pressure rises was observed simultaneously in the bladder and rectum during sneezing, indicating that these short-lasting sneeze-induced pressure rises in the bladder are not movement artifacts. In addition, long-lasting (>5s) small bladder responses were sometimes observed following short-lasting large responses during sneezing (Fig. 1a). Since similar pressure traces were
also observed after opening the abdomen (Fig. 2a), these responses might be artifacts due to the movement of animals during sneezing. Thus we did not evaluate these long-lasting, small bladder responses for further data analyses. The response during individual sneeze episodes varied from 2.6 cmH₂O to 128.9 cmH₂O depending on the strength of the sneeze (173 events in 5 rats). The average sneeze-induced bladder response was 36.6±6.7 cm H₂O (n=5), and this reduced to 11.4±0.7 cm H₂O (n=3) after opening the abdomen (Figs 2 and 3).

**Proximal urethra**

The baseline reading of the micro-tip transducer in the proximal urethra was not significantly different from the baseline in bladder (Fig. 3a and Table 1). The response in the urethra increased during sneezing and returned to the baseline within 0.15 second under an abdomen-closed condition (Figs 1a and 3a). The time course of the urethral response was similar to the time course of the bladder response (Fig. 3a), and the responses in the proximal urethra were lower compared with those in the bladder (approximately 60% of the bladder response; Table 1). The Ura-Ves difference, which is the difference of urethral and bladder responses induced by sneezing, showed mostly negative changes during sneezing,
confirming that the urethral response was generally lower than the bladder response (i.e., no obvious positive change of urethral responses exceeding bladder responses) (Fig. 3a and Table 1). After the abdomen was opened, the urethral response during sneezing was significantly reduced to 3.0±0.6 cmH₂O (n=3) (Figs 2a and 3d).

**Middle urethra**

The baseline reading of the micro-tip transducer in the middle urethra was significantly higher than the baseline in the bladder (Figs 1b and 3b and Table 1). During sneezing under an abdomen-closed condition, the urethral response started 18.9±5.5 ms before the bladder response (n=5) (Fig. 3b) and reached a peak value 14.7±1.5 ms after the peak of bladder response (n=5) (Fig. 3b). Urethral responses measured by micro-tip transducer catheters during sneezing were much longer in duration (10 times) than the bladder responses (Fig. 3b) lasting approximately 1.5 second. In addition, the urethral response during sneezing (44.5±4.9 cmH₂O, Table-1) was approximately 30% greater than the response in bladder. The average Ura-Ves difference was positive during sneezing, showing that the sneeze-induced urethral response was always higher than the bladder response (Figs 1b, 3b and Table 1). After opening the abdomen, the response measured by
micro-tip transducer catheters in the urethra still increased by 48.8±8.1 cmH2O during sneezing (n=3; Figs 2b and 3e), indicating that the urethral response during sneezing was caused not only by passive transmission of increased abdominal pressure but also by active reflex contractions of the middle part of the urethra.

**Distal urethra**

The baseline reading of micro-tip transducer catheters in the distal urethra was higher than the baseline in bladder (Fig. 3c and Table 1). The reading in the urethra increased during sneezing and returned to the baseline in 0.15 second under an abdomen-closed condition, but the increase was significantly lower than the changes in the proximal or middle urethra (Figs 1c and 3c and Table 1). The Ura-Ves difference was mostly negative during sneezing and the maximum positive change was 3.0 cmH2O (Figs 1c and 3c and Table 1). After the abdomen was opened, the urethral response measured by micro-tip transducer catheters during sneezing was 6.8±1.1 cmH2O (n=3; Figs 1f and 3f).

**Relationship between bladder and urethral responses**

In order to examine which part of the urethra is the most important for urinary
continence, the maximal readings of the micro-tip transducer catheters in the bladder and urethra during sneezing under an abdomen-closed condition are plotted in Fig. 4 (these data were from the same experiments shown in table 1). In the proximal and distal urethra, the maximal reading was lower (approximately 60% and 50%, respectively) than the maximal reading in the bladder during sneezing. In contrast, in the middle urethra the maximum reading was higher (approximately 180%) than that in the bladder.

We also examined whether active urethral closure responses in the middle urethra can be affected by the strength of sneezing reflex (Fig 5). At least 10 sneeze events were induced in each rat, and 64 sneeze events were measured in total from 5 rats in the experiment shown in table 1. The increase in Ura-Ves differences during sneezing were divided into 8 groups according to the size of bladder response from 0 to 80 cm H₂O with each bin of 10 cm H₂O. As shown in Fig. 5, no significant difference in the increase in Ura-Ves difference was observed among groups. Even when the bladder response was less than 10 cmH₂O, the increase in the Ura-Ves difference reached around 40 cmH₂O, indicating that the active urethral closure response during sneezing was not affected by the strength of sneeze reflex.
Experiment 2  Effects of bilateral transection of nerves innervating urethra on the urethral responses during sneezing

In order to investigate whether contractions of striated muscles such as the EUS and pelvic floor muscles are responsible for the active closure responses in the middle portion of the urethra during sneezing, the sneeze reflex was induced under an abdomen-closed condition in rats whose pudendal nerves (n=6), nerves to the iliococcygeous and pubococcygeous muscles (n=7), or both the pudendal nerves and the nerves to the iliococcygeous and pubococcygeous muscles (n=5) were cut bilaterally. The bladder response during sneezing was not different among groups with or without nerve transection (Table 2), indicating that the degree of sneeze reflex was not different among groups. However, the active component of the urethral closure response (i.e., increase in Ura-Ves difference) during sneezing were significantly reduced by 67% after bilateral transection of pudendal nerves (Table 2). While the transection of the nerves to the iliococcygeous and pubococcygeous muscles alone did not produce a significant change (34% reduction) in the urethral active response, transection of both groups of nerves (pudendal nerves and nerves to the iliococcygeous and pubococcygeous muscles) produced a further significant reduction in the mid-urethral active response (additional 25% reduction) compared with the
pudendal nerves-transected group (Table 2). After the two groups of nerves (pudendal nerves and nerves to the iliococcygeous and pubococcygeous muscles) were transected bilaterally, sneeze-induced responses in the middle urethra were approximately 35% of those in the bladder, with the time course of response during sneezing being similar in the middle urethra and the bladder (Fig. 6).

The effects of bilateral transection of hypogastric nerves and visceral branches of pelvic nerves were also investigated (n=3). The transection of these nerves did not affect the middle urethral response and Ura-Ves difference during sneezing (Table-2), indicating that these nerves were not involved in active urethral closure responses in the middle urethra during sneezing.

**Experiment 3  Effects of bilateral transection of pudendal nerves on EMG activity of EUS during sneezing**

In order to confirm the participation of the EUS in the sneeze reflex, EMG recordings of the EUS were compared before and after bilateral transection of the pudendal nerves under an abdomen-opened condition. EUS-EMG activity was increased during sneezing (Fig. 7) and not altered in sham-operated animals (116±23% of controls, n=4). However,
EUS-EMG activity was markedly decreased to 18 ± 9% of control values after bilateral transection of pudendal nerves (n=3; P<0.05, Student’s t-test) when compared with the sham-operated group. In addition, anal sphincter EMG activity was increased during sneezing (n=3) (data not shown), indicating that the sneeze-induced reflex activity is present not only in the EUS but also in the anal sphincter.

Experiment 4   Effects on sneeze leak point pressure of bilateral transection of the pudendal nerves and nerves to iliococcygeous and pubococcygeous muscles

In order to evaluate total urethral resistance generated against urinary leakage, leak point pressure during sneezing was measured using pressure transducers which were connected to bladder catheters inserted into the bladder from the dome. To clarify the contribution of nerve-mediated active urethral closure mechanisms to the total urethral resistance under a stress condition, sneeze leak point pressure was compared in the sham-operated group and the nerve-transected group (i.e., both pudendal nerves and nerves to the iliococcygeous and pubococcygeous muscles were transected).

Maximal intravesical pressure during each sneeze event varied from 11.3 cmH₂O to 40.8 cmH₂O (Sham-operated group; 220 events in 4 rats) and 8.2 cmH₂O to 40.0 cmH₂O
(Nerves-transected group; 217 events in 4 rats), and the average value did not differ between the sham-operated and nerve-transected groups (20.5±1.2 cmH2O and 20.6±1.0 cmH2O, respectively, n=4 each). In all 4 sham-operated rats tested, no fluid leakage from the urethral orifice was observed during sneezing which produced an increase in intravesical pressure reaching to as high as 37.0±2.2 cmH2O. However, fluid leakage from the urethral orifice was observed in all 4 nerve-transected rats, with the incidence rate of leakage during sneezing being 53.7±6.8%. Sneeze leak point pressures in nerve-transected rats averaged 16.3±2.1 cmH2O.

Discussion

SUI which is characterized by symptoms of involuntary urine loss due to an increase in abdominal pressure is considered to be caused by dysfunction of urinary continence mechanisms under stress conditions (20). The results in this study clearly indicated that the urethral response induced by reflex contractions of striated urethral sphincter and pelvic floor muscles greatly contributes to urinary continence mechanisms during stress conditions such as sneezing.

The present study revealed that the urethral closure response during the sneeze reflex
differed in proximal, middle and distal portions of the urethra. In the proximal or distal urethra, the time course of the urethral response during sneezing was similar to the time course observed in the bladder, and responses in the proximal urethra did not exceed those in the bladder (i.e., no obvious positive increase in Ura-Ves difference) during sneezing. In addition, sneeze-induced urethral and bladder responses were markedly reduced when the abdomen was opened to eliminate the rise of abdominal pressure during sneezing. Taken together, these results suggest that a part of increased abdominal pressure is passively transmitted to the proximal or distal urethra without reflex urethral contractions.

However, the behavior of the middle urethra during sneezing was markedly different from the proximal or distal urethra. The present study revealed: (I) the duration of sneeze-induced response in the middle urethra was significantly longer than the duration of bladder response, (II) the Ura-Ves difference was high during sneezing, and (III) the sneeze-induced urethral response was still obvious after opening the abdomen. These results indicate that the middle urethral response is mediated at least in part by active urethral closure mechanisms induced by sneezing. This is in line with the previous findings in dog that sneezing induced a pressure increase in the urethra that was higher than pressures in the rectum or bladder and did not disappear after opening the abdomen (30, 31).
In the dog, pressure increases in the urethra during sneezing disappeared when the urethra was shielded from the reflex contraction of pelvic floor muscles, indicating that active contractions of pelvic floor muscles during sneezing caused pressure increases in the urethra and must be important for maintaining urinary continence in dogs (10).

The present study also provided insights into the mechanisms underlying active urethral contraction during sneezing by examining the effects of somatic nerve transection on sneeze-induced urethral responses. It has been reported that the somatomotor branch of pelvic nerve (i.e., nerves to the iliococcygeous and pubococcygeous muscles) innervates pelvic floor muscles such as iliococcygeus muscles and pubococcygeous muscles in rats and that the pudendal nerve innervates the EUS and other pelvic floor muscles such as coccygeous muscles (16, 18). It has also been demonstrated that electrical stimulation of the pelvic nerve, which contains the nerves to the iliococcygeous and pubococcygeous muscles, increases EMG activity of iliococcygeus muscles and pubococcygeous muscle (18), that electrical stimulation of pubococcygeous muscles increases intraurethral pressure (19), and that electrical stimulation of the pudendal nerve induces urethral contraction (14). Based on these findings, we examined the effects of bilateral transection of the pudendal nerves, the nerves to the iliococcygeous and pubococcygeous muscles, or both sets of
nerves (pudendal nerves and nerves to the iliococcygeous and pubococcygeous muscles) and found that transection of pudendal nerves significantly reduced the sneeze-induced urethral responses measured by micro-tip transducer catheters as well as the Ura-Ves difference in the middle urethra during sneezing under an abdomen-closed condition. Moreover, the effects of combined transection of pudendal nerves and nerves to iliococcygeous and pubococcygeous muscles further reduced significantly the mid urethral responses during sneezing compared with pudendal nerve-transected rats, while transection of iliococcygeous and pubococcygeous muscle nerves alone did not significantly reduce the sneeze-induced mid-urethral response. Therefore, these results suggest that somatic pathways in the pudendal nerves and the nerves to the iliococcygeous and pubococcygeous muscles are both significantly involved in the sneeze-induced active closure response in the middle urethra and that the contribution of pudendal nerve-mediated striated muscle activity to the continence mechanisms during stress may be greater than that of iliococcygeous and pubococcygeous muscle activity. This conclusion is further supported by the reduction in EMG activity of EUS muscles during sneezing after transection of bilateral pudendal nerves.

We also observed that after transection of both groups of somatic nerves the
sneeze-induced response in the middle urethra was 65% lower than the bladder response, indicating that passive transmission of the sneeze-induced increased abdominal pressure to the middle urethra was less effective than the transmission to the bladder. This difference was also noted in the proximal or distal urethra.

As shown in Fig. 4, during a stress condition induced by sneezing the urethral response exceeded the bladder responses only at the middle portion of the urethra, which exhibited both active and passive components of urethral closure mechanisms, but not in the other parts (proximal and distal) of the urethra, which showed only a passive pressure transmission mechanism. Thus it seems reasonable to assume that the middle urethra is most important for increasing urethral resistance under sneeze-induced stress condition in the female rat.

However, since micro-tip transducers only measure the local force/unit area (mechanical stress) exerted by the tissue inner surface on the transducer tip, recorded values do not necessarily reflect the true urethral pressure (9, 21). Therefore, in order to examine the role of the active urethral closure mechanism mediated by activation of somatic nerves innervating the EUS and pelvic floor muscles in urinary continence, we measured sneeze leak point pressure, which is defined as the minimal intravesical pressure during sneezing.
that can open up the urethra and induce fluid leakage from the urethral orifice without reflex bladder contractions. We found that in normal rats, no urinary leakage occurred during sneezing which increased intravesical pressure as high as 37 cmH₂O. However, in all rats, in which the active closure mechanism was abolished by transection of both the pudendal nerves and the nerves to the iliococcygeous and pubococcygeous muscles, fluid leakage from the urethral orifice during sneezing was observed at a considerably lower leak point pressure of 16.3 cmH₂O. These results indicate that the sneeze-induced active urethral closure mechanism in the middle urethra detected by micro-tip transducer methods is indeed critical for preventing stress urinary incontinence in rats. In addition, micro-tip transducer measurements of bladder and urethral responses seem to be useful to examine the detailed mechanisms of active urethral closure mechanisms even though micro-tip transducers may not measure the urethral pressure as strictly defined. The major contribution of striated muscle contraction to sphincteric function in the middle urethra has also been reported in a previous in vitro muscle strip study in female rats (2). It was demonstrated that: (1) the circular muscle strip preparation of rat female urethra responds to electrical field stimulation in a succinylcholine-sensitive manner, which is indicative of striated muscle contractions, and (2) the most prominent muscular component in the middle
urethra is circularly oriented striated muscle cells, while smooth muscle fibers dominate near the bladder orifice. In addition, it should be noted that the present study was not designed to investigate the bladder-to-urethral reflex, another important continence mechanism, since the experiments were performed in the emptied bladder condition (Experiments #1 and 2) or in the pelvic nerve transected condition (Experiment #4). Thus, further studies are needed to investigate the bladder-to-urethral reflex that contributes urinary continence.

Previous clinical findings also suggested the existence of active urethral closure mechanisms during stress conditions in humans. It has been demonstrated in healthy women that urethral closure forces during coughing were significantly reduced by bilateral pudendal nerve blockade (26). It has also been reported in humans that urethral pressure started rising prior to the pressure rise in the bladder during coughing and that this preceding pressure increment disappeared in women with SUI (27, 28, 33). The present study in rats also showed that during the sneeze reflex the response in the middle urethra preceded the bladder response. Similar arrangements of smooth and striated muscles has been described in female rat and human urethra (11); i.e, an inner longitudinal smooth muscle layer as a continuation of the bladder musculature, proximal circular smooth muscle
layer, and circular or obliquely oriented striated muscle fibers starting from the mid-urethra distally. Thus it is assumed that the active urethral closure mechanism under stress conditions such as coughing and sneezing is important for preventing stress urinary incontinence in humans as well as animals.

Furthermore, it has been documented in women with SUI that (I) the number of pelvic floor muscle fibers which exhibit pathologic damage increased (8), (II) fast-twitch type II muscle fibers in the pelvic floor decreased (4), (III) pelvic floor musculature showed evidence of partial denervation due to pudendal neuropathy (23, 24), (IV) EMG activity of the striated urethral sphincter muscle decreased (25), and (V) the ability to voluntarily increase urethral pressure decreased (3). Thus it is possible that the active urethral closure mechanisms under stress conditions might be impaired in these SUI women, thereby leading to stress urinary incontinence. Recent studies have revealed that duloxetine, which is a 5-hydroxytryptamine and norepinephrine reuptake inhibitor, also increases EMG activity of EUS in the cat (12, 29) and is effective for treatment of SUI (17). Therefore, it is also possible that duloxetine might act by enhancing active urethral closure mechanisms, although further studies are necessary to clarify this point. Thus, as demonstrated in the present experiments, animal models which can explore the active
urethral closure mechanism during stress conditions such as sneezing can be useful for studying the pathophysiology of SUI as well as developing new treatment modalities of SUI.

In summary, this study demonstrated that under stress conditions such as sneezing the rat urethra has active urethral closure mechanisms mediated by somatic nerve-induced reflex contractions of EUS and pelvic floor muscles, in addition to passive transmission of increased abdominal pressure, and that this active urethral pressure response during sneeze serves as an important mechanism for preventing stress urinary incontinence. Evaluation of these active urethral closure mechanisms using micro-tip transducer catheters and sneeze leak point pressure measurements could be useful techniques for developing new treatments of SUI.
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Fig. 1 Typical recordings by micro-tip transducers in the bladder and urethra during sneezing under an abdomen-closed condition in rats. Urethral responses were measured at proximal (a), middle (b) and distal (c) parts of the urethra. Ura-Ves represents the difference of sneeze-induced urethral and bladder responses. The arrow indicates the events of the sneeze reflex.
Fig. 2  Typical recordings by micro-tip transducers in the bladder and urethra during sneezing under an abdomen-opened condition in rats. Urethral responses were measured at proximal (a), middle (b) and distal (c) part in a row. The arrow indicates the start of the sneeze reflex.
Fig. 3  Expanded recordings by micro-tip transducers showing responses in the bladder and urethra during sneezing under abdomen-closed (a to c) or abdomen-opened (d to f) conditions in rats. Urethral responses were measured at proximal (a, d), middle (b, e) and distal (c, f) parts of the urethra. Ura-Ves is the difference of sneeze-induced urethral and bladder responses.
Fig. 4 The comparison of maximal readings of urethral and bladder responses during sneezing in the proximal, middle and distal urethra in rats. Data are expressed as mean±SEM in 5 rats under an abdomen-closed condition.
Fig. 5  The lack of correlation between active urethral responses during sneezing and bladder response. The increase in Ura-Ves difference (the difference of sneeze-induced urethral and bladder responses) during sneezing was divided into 8 groups according to the size of the bladder response from 0 to 80 cm H$_2$O with each bin of 10 cm H$_2$O. Abscissa shows the possible maximal value of each bin in the respective group (e.g., 10=0-10 cmH$_2$O, 20=10-20 cmH$_2$O). Data are expressed as mean±SEM from 3 to 11 sneeze events in 5 rats.
Fig. 6  Expanded recordings by micro-tip transducers showing the effect of nerve transection on the responses measured in bladder and the middle urethra during sneezing under abdomen-closed conditions. Sham-operation (a) or bilateral transection of the pudendal nerves and the somatomotor branches of pelvic nerves (b) was conducted. Ura-Ves is the difference of sneeze-induced urethral and bladder responses.
Fig. 7  Typical EMG recordings of EUS during sneezing before (a) and after (b) pudendal nerve transection in a rat.
Table 1. Baseline values and responses measured in the urethra with micro-tip transducers during sneezing under an abdomen-closed condition in rats. Ura-Ves represents the difference of sneeze-induced urethral and bladder responses. Data are expressed as mean±SEM in 5 rats.

<table>
<thead>
<tr>
<th>Urethral Position</th>
<th>Bladder (cmH₂O)</th>
<th>Urethra (cmH₂O)</th>
<th>Ura-Ves (cmH₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Increase</td>
<td>Baseline</td>
</tr>
<tr>
<td>Proximal</td>
<td>5.9±0.7</td>
<td>34.0±6.2</td>
<td>5.8±0.6</td>
</tr>
<tr>
<td>Middle</td>
<td>4.7±0.4</td>
<td>34.6±4.5</td>
<td>26.5±2.2</td>
</tr>
<tr>
<td>Distal</td>
<td>5.1±0.4</td>
<td>41.5±10.6</td>
<td>15.0±2.1</td>
</tr>
</tbody>
</table>

**P<0.01 compared with the value in proximal or distal urethra (Dunnett’s test).
Table 2  Effects of nerve transection on active urethral responses during sneezing in rats.

Ura-Ves is the difference of sneeze-induced urethral and bladder responses. Ilio/Pubo: nerves to the iliococcygeous and pubococcygeous muscles, Pudend: pudendal nerve, Hypo: hypogastric nerve, VP: visceral branch of pelvic nerve. N expresses the number of animals.

Data are expressed as mean±SEM.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Ura</th>
<th>Ves</th>
<th>Ura-Ves</th>
<th>VP</th>
<th>VP+Hypo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>7</td>
<td>5.5±1.5</td>
<td>24.5±4.6</td>
<td>21.2±3.1</td>
<td>48.3±8.5</td>
<td>43.6±8.1</td>
</tr>
<tr>
<td>Ilio/Pubo</td>
<td>7</td>
<td>5.7±1.0</td>
<td>23.0±3.9</td>
<td>16.7±3.4</td>
<td>33.7±6.9</td>
<td>28.6±7.6</td>
</tr>
<tr>
<td>Pudend</td>
<td>6</td>
<td>5.1±0.8</td>
<td>26.6±4.4</td>
<td>17.1±2.3</td>
<td>22.1±4.0</td>
<td>14.6±3.3</td>
</tr>
<tr>
<td>Pudend+Ilio/Pubo</td>
<td>5</td>
<td>5.6±0.6</td>
<td>25.8±6.9</td>
<td>26.4±2.5</td>
<td>8.8±1.8</td>
<td>3.7±2.4</td>
</tr>
<tr>
<td>VP+Hypo</td>
<td>3</td>
<td>10.0±1.7</td>
<td>18.2±5.7</td>
<td>16.1±3.2</td>
<td>54.6±10.0</td>
<td>45.2±8.3</td>
</tr>
</tbody>
</table>

*** P<0.001 compared with the value for the sham-operated group (Dunnett’s test).

# P<0.05 compared with the value for the pudendal nerve-transected group (Student’s t-test).