Urethral closure mechanisms during sneezing-induced stress in anesthetized female cats

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1Pelvipharm, Centre National de la Recherche Scientifique, Gif sur Yvette; 2Assistance Publique-Hôpitaux de Paris (AP-HP), Neuro-Urology-Andrology, Department of Physical Medicine and Rehabilitation, Raymond Poincaré Hospital, Garches, University of Versailles Saint Quentin, Unité de Formation et de Recherche Paris Ile de France Ouest; and 3AP-HP, Department of Urology, La Pitié-Salpêtrière Hospital, Paris, France

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Julia-Guilloteau V, Denys P, Bernabé J, Mevel K, Chartier-Kastler E, Alexandre L, Giuliano F. Urethral closure mechanisms during sneezing-induced stress in anesthetized female cats. Am J Physiol Regul Integr Comp Physiol 293: R1357–R1367, 2007. —During stress-induced increase in abdominal pressure, urinary continence is maintained by urethral closure mechanisms. Active urethral response has been studied in dogs and rats. Such an active urethral response is also believed to occur in humans during stress events. We aimed to investigate urethral closure mechanisms during sneezing in cats. Urethral pressures along the urethra (UP1–UP4), with microtip transducer catheters with UP4 positioned in the distal urethra where the external urethral sphincter (EUS) is located, and intravesical pressure were measured, and abdominal wall, anal sphincter (AS), levator ani (LA), and EUS electromyograms (EMGs) were recorded during sneezing under closed-abdomen and open-abdomen conditions in eight anesthetized adult female cats. Proximal and middle urethral response induced by sneezing was not different from bladder response. Distal urethral response was greater compared with proximal and middle urethral and bladder response. In the open-abdomen bladder, proximal and middle urethral responses were similarly decreased and distal urethral response was unchanged compared with the closed-abdomen bladder. Bladder and urethral responses were positively correlated to sneeze strength. EUS, LA, and AS EMGs increased during sneezing. No urine leakage was observed, regardless of the strength of sneeze. In cats urethral closure mechanisms are partly passive in the proximal and middle urethra and involve an active component in the distal urethra that is believed to result from EUS and possibly LA contractions. Because central serotonin exerts similar effects on the lower urinary tract in cats and humans, the cat may represent a relevant model for pharmacological studies on continence mechanisms.

external urethral sphincter; urethra; urinary continence; stress urinary incontinence

STRESS URINARY INCONTINENCE (SUI) is defined as an involuntary loss of urine during effort or during sneezing or coughing (1). Approximately one-half of women with urinary incontinence are experiencing stress incontinence (16, 20). SUI is the most common type of urinary incontinence in women and is highly prevalent over the age of 40 (18). During urodynamic testing, SUI can be confirmed by identifying urinary leakage from the urethra during elevation of abdominal pressure in the absence of bladder contractions (1).

Urinary continence depends on a complex coordination between bladder/detrusor, urethral, and pelvic striated muscles. During stress events such as coughing or during physical activity, the urethra maintains sufficient pressure to prevent urine passage. The urethra is composed of striated muscle fibers constituting the external urethral sphincter (EUS) and of smooth muscle fibers organized in longitudinal and circular layers.

The urethral closure mechanisms during elevation of abdominal pressure have been studied experimentally in rats (9, 21, 23) and dogs (19, 32, 33). Earlier studies of anesthetized male dogs reported that bladder and urethral pressures increased during sneeze reflex, with increases in pressure in the distal urethra (at the level of EUS) being greater than intravesical pressure increases (19, 33). In anesthetized female rats, urethral closure response during sneeze reflex was different in proximal, middle, and distal parts of the urethra. Increases in abdominal pressure were partly passively transmitted to the proximal and distal urethra. During the sneeze reflex, the medial urethral response at the EUS level was partly mediated by an active urethral closure mechanism, since the duration and magnitude of middle urethral response have been reported to be superior to the duration and magnitude of concomitant bladder response. Moreover, the sneeze-induced middle urethral response was still present after the abdomen was opened. Bilateral transection of pudendal nerves and/or nerves destined for iliococcygeus and pubococcygeus muscles reduced the middle urethral response to the sneeze reflex, thus indicating that the sneeze-induced active urethral closure mechanism at the level of EUS is controlled by somatic innervation (23).

There is a paucity of pharmacological research targeting SUI. The modulatory effects of central serotonin (5-HT) on lower urinary tract (LUT) function in rats are different from those in cats (10). Micturition in rats is sensitive to both excitatory and inhibitory serotonergic mechanisms, whereas in cats central 5-HT appears to act primarily to promote urine storage by enhancing EUS activity and suppressing bladder activity. Duloxetine, a combined 5-HT and norepinephrine reuptake inhibitor (30) was authorized for the treatment of moderate to severe stress urinary incontinence in women in 2004 by the European Agency for the Evaluation of Medicinal Products (EMEA). Because of the similarities between cats and humans, supported by the role of central 5-HT in the control of urinary continence (29, 31), it has been
proposed that micturition in the cat may be a useful model for studying centrally acting serotonergic agents for the treatment of LUT dysfunction (11). The central neurophysiology of micturition and urine storage has been studied extensively in cats (12); nevertheless, the urethral closure mechanisms in stress condition have yet to be described in cats. Accordingly, we investigated and described urethral closure mechanisms in female cats during the elevation of abdominal pressure induced by sneeze reflex in an attempt to determine the role of the EUS, the smooth urethral musculature, and the levator ani (LA) and anal sphincter (AS) in urinary continence.

METHODS

Preliminary anatomic study. Three urethras from adult female cat cadavers were carefully dissected. The urethras ended in a vestibule measured ~4 cm in length. The urethras were subsequently frozen and transversally sectioned on a freezing rotary microtome. Sections (5 μm) were collected and stained with hematoxylin-eosin-safran. Urethral sections were studied with a light microscope. Striated muscle fibers constituting the EUS were found to be located in the distal part of the urethra. Proximal and middle urethra contained circular and longitudinal smooth muscle fiber layers (Fig. 1). This information was used to identify the location of the EUS in the in vivo experiments.
Urethral closure mechanisms in female cats during stress.

Animals. The in vivo experiments were performed on eight European female cats (Isoquimen, Spain), weighing 2.5–3 kg, in accordance with European Community Council directives (86/609/EEC). All possible measures were taken to minimize animal suffering. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, revised 1996) and Animal Care Regulations in force in France since 1988 (with authorization from French Ministry of Agriculture Agreement No. 91-86, 7/20/2001).

Surgical preparation. Cats were anesthetized with 3% isoflurane (Centravet) inhalation. Polyethylene catheters (PE-50, Phymep, Paris, France) filled with heparinized saline (25 IU/ml) were inserted into the femoral artery and vein for recording arterial blood pressure and drug delivery, respectively. The urinary bladder was exposed through a midline suprapubic abdominal incision, both ureters were cut in the vicinity of the bladder, and their distal ends were ligated. A polyethylene catheter (1.5-mm external diameter, Phymep) was then inserted into the bladder from the dome, to record intravesical pressure (IVP) and to fill or empty the bladder. Intravesical and arterial catheters were connected to pressure transducers (Elcomatic EM 750, Glasgow, UK), with the signal being amplified (Bionic Instruments, Nozay, France), digitized (DAS1000, Measurement Computing), and converted to millimeters of mercury after calibration (Elphy software, Centre National de la Recherche Scientifique (CNRS), Gif sur Yvette, France).

A pair of stainless wire electrodes (Cooner Wire) was implanted into the anterior abdominal wall striated musculature. The abdomen was closed with sutures once the experiments in the open-abdomen condition were completed. Pairs of stainless wire electrodes were also implanted into the EUS, whose exact location was identified based on the preliminary anatomic study results, LA, and AS. Electromyographic (EMG) signals from these striated muscles were amplified (DP-301, Warner Instrument, Phymep; gain 1,000, low pass 3 Hz, high pass 3 KHz). For each muscle, the EMG was rectified and analyzed with Elphy software (CNRS) to assess contractile activity.

Urethral pressure recording. A catheter (size 5 F) with four pressure microtip transducers separated by a distance of 8 mm (K5468-E2-0451, Unisensor, Attikon, Switzerland) was used (Fig. 2). Urethral pressure transducers were connected, and their signals were amplified by an eight-channel Multiplexor (DAS16 board, Measurement Computing). Urethral pressure signals provided by the readings of the microtip transducers were converted to millimeters of mercury after calibration (Elphy software, CNRS). The bladder was emptied before the urethral pressure catheter was inserted. The pressure catheter was then retrogradely inserted into the urethra from the urethral meatus with the side-mounted transducers facing the urethral inner surface in the 12 o’clock position. Before sneeze reflex induction, urethral profilometry (pressure recording along the entire length of the urethra by slowly moving the catheter backward) was performed in each animal to check the precise location of the EUS. The correct positioning of the distal transducer [urethral pressure (UP4) at the EUS level was determined based on the preliminary anatomic study according to which the EUS is located in the distal part of the urethra and the greater basal urethral pressure at the EUS level. The three other transducers were positioned proximally in the middle and proximal urethra (Fig. 2).

After surgery, isoflurane inhalation was turned off and replaced by an intravenous infusion (4.76 ml/h) of ketamine (6 mg/kg iv, Centravet) and acepromazine (0.5 mg/kg iv, Centravet). Sneeze reflexes were induced by repetitive air puffs into the nasal cavity of the cat generated by a mechanical air puff stimulator (35, 36).

Experimental protocol. To discriminate between active and passive components of the urethral closure mechanisms, the experiment was divided into three phases (A, B, and C).

In phase A, the abdomen was left open before, during, and after multiple sneeze reflexes. Bladder (IVP) and urethral (UP1, UP2, UP3, and UP4) pressures were simultaneously recorded, and anterior abdominal wall, AS, LA, and EUS EMG recordings were performed.

In phase B, the same procedure was repeated after closure of the abdominal wall. During phase C, the bladder was filled with Evans blue dye at ~50% capacity, and then the urethral catheter was removed and IVP was again recorded during the sneeze reflex. A possible urine leakage was visually checked. The maximal IVP induced by sneeze reflex did not provoke urine leakage.

Sneeze-evoked urethral reflexes were determined with empty bladder (phases A and B) to study urethral continence. Before leak point pressure (LPP) test (phase C), bladder capacity was determined: the empty bladder was perfused, and ketamine-acepromazine anesthesia allowed micturition. Bladder capacity was then calculated for each animal.

Data collection and analysis. The following parameters were calculated for each sneeze reflex episode in both open-abdomen and closed-abdomen conditions: 1) magnitude of bladder and urethral responses during sneezing estimated at each location (in the bladder and proximal, middle, and distal parts of the urethra) from the difference between the maximal pressure during sneezing and the resting pressure before sneezing; 2) duration of the bladder and urethral responses, i.e., of the increase in IVP, UP1, UP2, UP3, and UP4 during a sneeze reflex; 3) magnitude of the increase in abdominal wall, AS, LA, and EUS EMGs during the sneeze reflex, corresponding to the difference between maximal electrical signal during sneezing and baseline signal measured after sneezing; and 4) area under the curve (AUC) of abdominal wall, AS, LA, and EUS EMGs before and during sneeze. For each parameter the mean was calculated for all sneezing episodes for each cat in both conditions, i.e., abdomen opened and closed, and then averaged for the eight investigated cats. Data are expressed as means ± SE.

To compare responses between closed-abdomen and open-abdomen conditions, it was necessary to compare the following parameters during comparable sneeze reflexes: 1) magnitude of bladder and urethral responses during sneezing, 2) duration of the bladder and urethral responses, and 3) magnitude of the increase in abdominal wall, AS, LA, and EUS EMGs during the sneeze reflex. Comparable...
sneeze reflexes were defined as sneeze reflexes eliciting an increase in abdominal wall EMG activity of comparable magnitude. Comparable sneezes during closed-abdomen and open-abdomen conditions were selected for each cat, between one and four per cat, with the following criterion: the variation of the magnitude of anterior abdominal wall rectified EMG must be <10% between all selected sneeze episodes. An average value for each parameter characterizing the response to comparable sneezing was calculated per condition and per cat and then averaged for the eight cats.

The maximal bladder pressure without fluid leakage for the strongest sneeze was determined for each animal and averaged and expressed as mean ± SE.

**Statistical analysis.** Increases in UP1, UP2, UP3, UP4, and IVP and the duration of urethral and bladder responses during sneeze reflex were compared with a one-way ANOVA with repeated measures whenever \( P < 0.05 \) by post hoc test (Newman-Keuls test).

Comparison between rectified AUCs of AS, LA, and EUS EMGs before and during the sneeze was performed with paired Student’s \( t \)-test.

A regression curve was calculated between strength of sneeze reflex (evaluated by the magnitude in anterior abdominal wall rectified EMG) and the magnitudes of the four urethral and bladder responses under the closed-abdomen condition. For each animal, 1) Pearson coefficients \( r \) were calculated to assess the correlation between strength of sneezing and magnitudes of bladder or urethral responses and 2) \( r \) were calculated to assess the correlation between magnitudes of bladder response and urethral responses.

For comparable sneeze reflexes, UP1, UP2, UP3, UP4, IVP, and AS, LA, and EUS EMG magnitudes were compared between closed-abdomen and open-abdomen conditions with paired Student’s \( t \)-test.

**RESULTS**

**Bladder and urethral responses during sneeze reflex under closed-abdomen condition.** During each sneeze reflex, increases in bladder and urethral pressures were noted. These increases started simultaneously with the beginning of the increase in EMG activity of the striated muscles of the anterior abdominal wall induced by sneeze reflex (Fig. 3A). The number of analyzed sneeze reflex episodes varied from 15 to 37 in the closed-abdomen condition depending on each cat’s ability to respond to air puffs into the nasal cavity. Considering the mean of all sneeze-induced responses per cat, averaged for eight cats, ANOVA revealed a difference between magnitudes of urethral and bladder responses, i.e., UP1, UP2, UP3, UP4, and IVP \( (P = 0.0123, 1\text{-way ANOVA with repeated measures; } n = 8) \). The magnitude of the distal urethral response during sneezing (UP4), 59.8 ± 20.04 mmHg, was significantly higher compared with IVP \( (17.16 ± 4.37 \text{ mmHg}), \) UP1 \( (19.56 ± 3.15 \text{ mmHg}), \) UP2 \( (18.67 ± 2.88 \text{ mmHg}), \) and UP3 \( (19.72 ± 2.54 \text{ mmHg}) \) \( (P < 0.05, \text{Newman-Keuls test; Fig. 3B}) \). It is noteworthy that during a single sneeze reflex the maximal UP4 magnitude was highly variable, from 4.46 to 346.68 mmHg, because of interanimal variability, the variable strength of the sneeze reflex, and the location of the UP4 microtip transducer.

During sneezing, the bladder (IVP) and the proximal and middle urethral (UP1, UP2, UP3) responses lasted 0.32 ± 0.03, 0.30 ± 0.03, 0.31 ± 0.03, and 0.33 ± 0.03 s, respectively, whereas the distal urethral response lasted significantly longer \((0.43 ± 0.03 \text{ s}; \text{ANOVA with repeated measures } P < 0.001, \text{followed by post hoc Newman-Keuls test } P < 0.05; n = 8)\) (Fig. 3C).

**Bladder and urethral responses during sneeze reflex under open-abdomen condition.** When the abdomen was left open, the sneeze reflex elicited a rise in both bladder and urethral pressure, as measured by the four microtip transducers positioned along the urethra. The mean magnitude of the response in the distal urethra (UP4) reached 45.65 ± 25.89 mmHg (Fig. 4A). The mean magnitudes of bladder (IVP) and proximal and middle urethral responses (UP1, UP2, and UP3) were 9.61 ± 2.78, 6.99 ± 1.17, 7.58 ± 2.10, and 11.36 ± 4.97 mmHg,
respectively (Fig. 4A). Despite the fact that UP4 was numerically greater than IVP, UP1, UP2, and UP3, ANOVA \((P = 0.134, 1\text{-way ANOVA with repeated measures})\) did not yield any statistical difference when these various responses were compared.

Under open-abdomen conditions, the duration of the response in the distal urethra (UP4, 0.41 \(\pm\) 0.04 s) was significantly longer than in the bladder (0.29 \(\pm\) 0.02 s) and in the proximal and middle urethra (UP1 0.34 \(\pm\) 0.02 s, UP2 0.34 \(\pm\) 0.02 s, and UP3 0.35 \(\pm\) 0.02 s) \((\text{ANOVA repeated-measures} P < 0.001)\) followed by post hoc Newman-Keuls test \(P < 0.05\); \(n = 8\); Fig. 4B).

Relationship between sneeze reflex strength and bladder/urethral responses and relationship between bladder and urethral responses under closed-abdomen condition. For each cat, in the closed-abdomen condition an increase in sneeze strength reflex was found to elicit a proportional increase in bladder and urethral responses (Fig. 5A). The parameters of the regression curves between sneeze strength and the magnitude of bladder and urethral responses are shown in Fig. 5B and averaged for eight cats in Table 1. The slopes and offsets of the regression curves for the relationship between sneeze strength and bladder or urethral pressures were similar for IVP and UP1, UP2, and UP3 (Fig. 5B, 2 and 4), whereas those focused on the distal part of the urethra (UP4) (Fig. 5B3) were higher (Table 1). The Pearson coefficient \(r\) for the correlations between urethral and bladder responses and sneeze strength was 0.73 \(\pm\) 0.03, 0.73 \(\pm\) 0.04, 0.66 \(\pm\) 0.04, and 0.59 \(\pm\) 0.06 for UP1, UP2, UP3, and UP4, respectively, and 0.57 \(\pm\) 0.11 for the bladder response (data not shown). In one cat, no correlation between sneeze reflex strength and bladder response was found.

In six of the eight cats, there was a strong correlation between bladder and urethral responses (Pearson coefficients varying from 0.48 to 0.99; data not shown). In these six cats the Pearson coefficient for the correlation between bladder and urethral responses was superior to the Pearson coefficient for the correlation between sneeze reflex strength and urethral responses (data not shown). In the other two cats investigated \((\text{cats} 2 \text{ and} 7),\) there was no correlation between bladder and urethral responses. Because of the existing correlations between sneeze strength and bladder and urethral responses, parameters of the regression curves have been calculated for six cats.

Comparison of bladder and urethral responses between open-abdomen and closed-abdomen conditions during comparable sneeze reflex. The strength of the sneeze reflex strongly influenced both the urethral and bladder responses. To compare the responses in open-abdomen and closed-abdomen conditions, we compared urethral and bladder responses for comparable sneeze reflex events \((i.e., \text{sneeze reflexes of equivalent strength elicited in both conditions, based on comparable magnitude of abdominal EMGs})\).

In the open-abdomen condition, there was a significant decrease in the mean magnitude of the proximal and middle urethral responses \((\text{UP1, UP2, and UP3 during sneezing compared with the closed-abdomen condition} [\text{UP1} 6.64 \pm 0.98 \text{ vs.} 17.43 \pm 4.29 \text{ mmHg} (P = 0.0239), \text{UP2} 5.07 \pm 1.36 \text{ vs.} 16.21 \pm 3.68 \text{ mmHg} (P = 0.0268), \text{UP3} 6.11 \pm 1.60 \text{ vs.} 20.83 \pm 4.29 \text{ mmHg} (P = 0.0122), \text{paired Student’s} t\text{-test}; n = 8])\) (Fig. 6). The response in the distal urethra \((\text{UP4})\) was not statistically different between open-abdomen and closed-abdomen conditions \((54.55 \pm 31.21 \text{ vs.} 42.71 \pm 15.01 \text{ mmHg}; P = 0.5591, \text{paired Student’s} t\text{-test}; n = 8)\). The mean magnitude of the bladder response was decreased in the open-abdomen condition compared with the closed-abdomen condition \((9.79 \pm 2.46 \text{ vs.} 15.27 \pm 5.34 \text{ mmHg}),\) although no statistical difference could be observed \((P = 0.5339, \text{paired Student’s} t\text{-test}; n = 8)\). This result was likely caused by interanimal variability.

Comparison between open-abdomen and closed-abdomen conditions for AS, LA, and EUS EMG responses during sneezing. Anterior abdominal wall, LA, AS, and EUS EMG activities increased during sneeze-induced reflex \((\text{Fig. 7})\). During sneezing, AUC of rectified LA, EUS, and AS EMGs increased compared with AUC before sneeze reflex in both open-abdomen and closed-abdomen conditions \((P < 0.05, \text{paired} t\text{-test})\) \((\text{Table 2})\). The EMG responses of LA, AS, and EUS appeared to be dependent on the strength of sneezing \((\text{Fig. 7})\). Accordingly, a comparison of LA, EUS, and AS EMG activities during sneeze reflex between open-abdomen and closed-abdomen conditions was performed for comparable sneezes.

Comparison of AS, LA, and EUS EMG responses between open-abdomen and closed-abdomen conditions during comparable sneezing. For comparable sneezing, no difference between open-abdomen and closed-abdomen conditions in the mean magnitude of AS, LA, and EUS rectified EMG activities was found \((\text{Fig. 8})\). For AS, the mean magnitude of rectified EMG activity was 5.19 \(\pm\) 0.86 \(\mu\text{V}\) in the open-abdomen condition and 6.94 \(\pm\) 1.98 \(\mu\text{V}\) in the closed-abdomen condi-
Fig. 5. Correlation between strength of sneeze reflex and bladder and urethral responses. A: original recording of anterior abdominal wall electromyogram (EMG) and urethral response at the EUS level. Each arrow represents a sneeze reflex elicited by air puffs into the nasal cavity. B: slope and offset of regression curves between strength of sneeze reflex evaluated by magnitude of rectified abdominal wall EMG and magnitude of bladder and urethral pressure responses for a single female cat. B1, theoretical regression curve. B2, 3 regression curves between proximal and middle urethral responses (UP1, UP2, UP3) and strength of sneeze. B3, regression curve between distal urethral responses (UP4) at the EUS level and strength of sneeze. B4, regression curve between bladder (Ves) and strength of sneeze.

Table 1. Mean slope and offset of regression curves between sneeze strength and magnitude of bladder and urethral responses under open- and closed-abdomen conditions

<table>
<thead>
<tr>
<th></th>
<th>UP1</th>
<th>UP2</th>
<th>UP3</th>
<th>UP4</th>
<th>IVP</th>
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<tbody>
<tr>
<td><strong>Open abdomen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.21±0.11</td>
<td>0.26±0.11</td>
<td>0.15±0.05</td>
<td>0.68±0.37</td>
<td>0.24±0.12</td>
</tr>
<tr>
<td>Offset</td>
<td>2.77±0.90</td>
<td>-0.07±2.29</td>
<td>3.12±1.81</td>
<td>8.61±2.23</td>
<td>1.64±3.71</td>
</tr>
<tr>
<td><strong>Closed abdomen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.59±0.17</td>
<td>0.58±0.18</td>
<td>0.64±0.16</td>
<td>2.56±1.25</td>
<td>0.46±0.16</td>
</tr>
<tr>
<td>Offset</td>
<td>4.05±1.76</td>
<td>3.47±2.06</td>
<td>3.95±3.30</td>
<td>24.14±12.93</td>
<td>2.91±1.93</td>
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</tbody>
</table>

Values are means ± SE of parameters (slope and offset) of the regression curves between sneeze strength and the magnitude of bladder (intravesical pressure, IVP) and urethral responses [urethral pressure (UP1, UP2, UP3, and UP4)] under open- and closed-abdomen conditions. UP4 was located at the distal part of the urethra (external urethral sphincter, EUS) and UP1, UP2, and UP3 at the middle and proximal parts of the urethra.
Urine leakage test. In the present experimental conditions, no urine leakage was observed regardless of sneeze strength. The strongest sneeze recorded (135.1 ± 39.9 μV) elicited a bladder response of 35.4 ± 11.02 mmHg.

DISCUSSION

The present results support a partly passive component of the closure mechanism in the proximal and middle urethra, and, more importantly, an active component of urethral closure mechanisms in the distal urethra was involved in the response to sneezing. The increase in distal urethral pressure was likely due to EUS contractions, with a possible additional participation of LA. It is also noteworthy that no urine leakage was observed regardless of the intensity of sneeze.

The existence of active urethral closure mechanisms induced by sneezing was previously reported in rats (9, 21, 23) and dogs (19, 32, 33). These mechanisms are likely important for maintaining urinary continence in these animal species.

In anesthetized female cats, the sneeze-induced response in the distal urethra, at the level of the EUS, lasted longer than the bladder and middle and proximal urethral responses. In rats (23) and dogs (32, 33) the active component of the urethral closure mechanisms precedes the sneeze-induced passive transmission to the bladder and urethra of the increase in abdominal pressure. In contrast, distal urethral response in cats started simultaneously with proximal urethral and bladder responses. In female cats, the simultaneous recording of urethral responses with perineal striated muscle EMG activity provided additional information on the urethral closure mechanisms in stress-induced conditions like sneezing. The lack of change in the distal urethral response when the abdominal wall was opened compared with the closed condition must be related to the unmodified contractions of EUS, LA, and AS in both conditions. This provides direct physiological evidence of the role in cats of EUS and possibly LA contractions in the nonpassively transmitted distal urethral response. Moreover, in cats, sacral sphincter motoneurons have a voltage-sensitive, nonlinear membrane response to depolarization that could contribute to the sustained activity of these motoneurons during sneeze-induced stress (25).
In humans, to prevent urine leakage during cough a simultaneous elevation in bladder and sphincteric pressure are required \((5, 14, 15)\). Nevertheless, the physiology of urinary continence during stress is complex, and the respective roles of passive and active mechanisms remain unclear in humans. In healthy volunteers, it has been demonstrated that during voluntary cough increases in intraurethral pressure precede bladder pressure increases \((7, 28)\). This latency was interpreted as an active pressure generation by the peri- and/or intraurethral structures to preserve a positive urethral closure pressure \((34)\). Additional clinical findings have suggested the presence of active urethral closure mechanisms in women \((27)\). In female cats, we have demonstrated the existence of both an active and a passive component of the urethral closure mechanisms during

Fig. 7. Typical simultaneous EMG recordings of anterior abdominal wall, levator ani (LA), EUS, and anal sphincter (AS) during sneeze reflexes of different strengths and EMGs in an anesthetized female cat. Arrows indicate sneeze reflex events.

Table 2. Comparison between area under the curve of levator ani, anal sphincter, and EUS rectified EMG before and during sneezing under open- and closed-abdomen conditions

<table>
<thead>
<tr>
<th></th>
<th>LA EMG</th>
<th></th>
<th>AS EMG</th>
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<th>EUS EMG</th>
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<tbody>
<tr>
<td></td>
<td>Before Sneezing</td>
<td>During Sneezing</td>
<td>Before Sneezing</td>
<td>During Sneezing</td>
<td>Before Sneezing</td>
</tr>
<tr>
<td>Open abdomen</td>
<td>0.76±0.15</td>
<td>1.35±0.23*</td>
<td>0.88±0.19</td>
<td>1.41±0.25*</td>
<td>0.59±0.09</td>
</tr>
<tr>
<td>Closed abdomen</td>
<td>0.72±0.18</td>
<td>2.43±0.88</td>
<td>0.71±0.13</td>
<td>1.88±0.33*</td>
<td>0.85±0.13</td>
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Values (in \(\mu V\cdot s\)) are mean ± SE area under the curve of levator ani (LA), anal sphincter (AS), and EUS rectified electromyogram (EMG) before and during sneezing under open- and closed-abdomen conditions. \(*P < 0.05\), paired Student’s t-test; \(n = 8\).
stress-induced sneeze. In humans, pelvic floor muscle contractions increase with the level of intra-abdominal pressure generated during cough (2). Similarly, in cats, bladder and urethral responses during sneeze were correlated to the magnitude of abdominal EMG, which determines the magnitude of stress. The smaller correlation between bladder response and sneeze strength compared with the correlation between the urethral responses and sneeze strength could be explained by the different recording techniques used to record the responses in the urethra with microtip transducers and in the bladder with a catheter filled with saline. A positive correlation between the elevation of abdominal pressure and pelvic floor muscle contraction exists in humans (2). The similarity between cats and humans in the correlation between the strength of sneeze and the urethral and bladder responses is one of the arguments for developing a cat model to investigate urethral closure mechanisms. Women affected with SUI exhibit urine leakage after repeated efforts, which could be explained by an increased fatigue of the urethral sphincter and periurethral muscles (13). Further investigations are necessary to determine whether urethral muscle fatigue also exists in cats and could lead to urine leakage.

To examine the role of active urethral closure mechanisms, sneeze LPP was measured. Sneeze LPP is defined as the minimal IVP during sneeze reflex that can open the urethra and induce urine leakage without bladder contractions. We found that in cats no urine leakage occurred during sneeze reflex regardless of the strength of sneeze. This result is comparable to the results obtained in anesthetized female rats, in which sneeze did not induce urine leakage (23). In intact adult rats, urine leakage was present when the elevation in abdominal pressure was elicited by manual external pressure to mimic Crede or vertical tilt table methods (8). In the same species, urine leakage could occur during sneezing after transection of both pudendal nerves and nerves to the iliococcygeus and pubococcygeus muscles (23). Similarly, in rats, it was proposed that during sneezing the middle urethral response induced by contractions of EUS and pelvic floor muscles greatly contributes to urinary continence mechanisms (8). In the present experiments, the maximal IVP magnitude recorded without urine leakage was 35.4 mmHg. These results are consistent with those reported in rats, in which during sneezing the maximal IVP magnitude without leakage was 45.5 mmHg (8).

There are neuroanatomic differences between cats and rats regarding the innervation of the striated pelvic musculature. In both species, the EUS is innervated by the pudendal nerve. In cats, cellular bodies of the pudendal motoneurons are located in the ventral part of the Onuf nucleus in the lateral ventral horn of the S1–S2 spinal cord (3), with the dorsomedial part of the Onuf nucleus containing motoneurons innervating the external anal sphincter. This organization is similar in humans (4). Conversely, in rats the motoneurons to the anal and urethral sphincters originate from two separate nuclei (dorsolateral and dorsomedial nuclei) located in the ventral horn of the spinal cord at the L6–S1 level (4). Furthermore, in rats, the modulatory effects of 5-HT on LUT function are different than those in cats (10). While in rats 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a 5-HT1A receptor agonist, facilitates the micturition reflex (6, 24), in cats 8-OH-DPAT acts in the spinal cord to inhibit the micturition reflex (31). In cats administration of the 5-HT precursor 5-hydroxy-
triptamine enhanced the pudendal efferent reflex output, thereby facilitating EUS tonic activity (17). Accordingly, the suitability of the rat for study of the effect of pharmacological intervention in the serotonergic system targeting active urethral closure mechanisms during stress conditions is questionable, while the cat might represent a useful model for the investigation of the effects of serotonergic agents for the treatment of SUI. Based on the various models developed to mimic SUI in rats (8, 21–23, 26), further investigations are necessary to investigate experimental procedures that could elicit urine leakage in female cats caused by the impairment of the active urethral closure mechanisms evidenced in the present experiments.

In conclusion, this study showed that under stress conditions (sneeze reflex) in cats the distal urethra develops active closure mechanisms that are believed to be mediated by contractions of EUS and possibly LA. According to differences between the various mammalian species studied so far in terms of physiology of their continence mechanism and neuroanatomy and the neurochemistry of the control of the LUT, the female cat appears to be a potential animal model for human urinary continence. The female cat model may allow investigation of the physiological events occurring during stress to prevent urine leakage as well as pharmacological studies targeting the continence mechanisms. Further experiments in female cats are needed to determine the role of pudendal and pelvic nerves and nerves to the LA in urethral closure mechanisms to design a relevant cat SUI model.

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REFERENCES


