Effect of duloxetine, a norepinephrine and serotonin reuptake inhibitor, on sneeze-induced urethral continence reflex in rats

Minoru Miyazato,1,4 Yasuhiro Kaiho,4 Izumi Kamo,1 Michael B. Chancellor,3 Kimio Sugaya,4 William C. de Groat,2 and Naoki Yoshimura1,2

1Department of Urology and 2Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; 3Department of Urology, William Beaumont Hospital, Royal Oak, Michigan; and 4Division of Urology, Department of Organ-Oriented Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan

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Miyazato M, Kaiho Y, Kamo I, Chancellor MB, Sugaya K, de Groat WC, Yoshimura N. Effect of duloxetine, a norepinephrine and serotonin reuptake inhibitor, on sneeze-induced urethral continence reflex in rats. Am J Physiol Renal Physiol 295: F264–F271, 2008. First published May 14, 2008; doi:10.1152/ajprenal.90241.2008.—We investigated the effect of duloxetine, a norepinephrine (NE) and serotonin (5-HT) reuptake inhibitor, on the neurally evoked urethral continence reflex induced by sneezing in rats. To clarify the role of noradrenergic and serotonergic mechanisms in preventing stress urinary incontinence (SUI) during sneezing, we examined the effect of duloxetine followed by intrathecal (it) methiothepin maleate (5-HT receptor and α1-adrenoceptor antagonist) or terazosin or idazoxan (selective α1- and α2-adrenoceptor antagonists, respectively). Amplitude of urethral pressure responses during sneezing (A-URS), urethral baseline pressure (UBP) at the midurethra, and sneeze-induced leak point pressure (S-LPP) were measured in normal adult female rats and rats with SUI induced by vaginal distension (VD). In normal and VD rats, intravenous application of duloxetine (1 mg/kg) increased A-URS by 35% and 34% and UBP by 21% and 34%, respectively. Sneezing-induced fluid leakage from the urethral orifice was observed in VD rats but not in normal rats. S-LPP was increased from 39.1 to 92.2 cmH2O by intravenous duloxetine in incontinent VD rats. Duloxetine-mediated enhancement of A-URS was inhibited by terazosin but not methiothepin maleate (it). In addition, simultaneous intrathecal application of methiothepin and terazosin induced a reduction in A-URS during sneezing, which was not increased by intravenous duloxetine. However, the reduced A-URS after intrathecal application of methiothepin and terazosin returned to the control level when duloxetine (iv) was applied after intrathecal idazoxan administration. These results indicate that duloxetine can prevent SUI by facilitating noradrenergic and serotonergic systems in the spinal cord to enhance the sneeze-induced active urethral closure mechanism.

urinary incontinence; neural pathway; adrenergic and serotonergic reuptake inhibitors; birth trauma

STRESS URINARY INCONTINENCE (SUI) is the most common type of urinary incontinence in women (10). This type of incontinence is often seen in women after middle age, and it can be caused by impaired closure mechanisms of the urethra due to a weak pelvic floor or poorly supported urethral sphincter (urethral hypermobility) and/or a damaged urethral sphincter system (intrinsic sphincter deficiency). Duloxetine, a norepinephrine (NE) and serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitor, has demonstrated clinical efficacy in the treatment of SUI (9). The action of this drug is considered to be associated with reuptake inhibition of 5-HT and NE at presynaptic terminals in Onuf’s nucleus of the sacral spinal cord (21, 22). However, the precise mechanisms by which duloxetine improves SUI during stress conditions such as sneezing have not been fully clarified.

We previously established (13) a rat model that can be used to examine sneeze-induced active urethral closure mechanisms that are mediated by somatic nerve-induced reflex contractions of external urethral sphincter and pelvic floor striated muscles. Our recent study (12) has also shown that these active urethral closure mechanisms during sneezing are impaired in a rat model of SUI induced by simulated birth trauma. We have also reported (11) that activation of the noradrenergic system by nisoxetine, a NE reuptake inhibitor, can prevent SUI via activation of α1-adrenoceptors by enhancing the sneezed-induced active urethral closure mechanisms at the spinal level and augmenting urethral baseline pressure at the periphery. However, the role of serotonergic and noradrenergic mechanisms in sneeze-induced urethral continence reflex and the relationship between the two monoaminergic pathways have not been fully examined.

Thus, in the present study, we investigated the effect of duloxetine on the sneeze-induced continence reflex by using urethral microtransducer-tipped catheter methods and leak point pressure measurements in normal rats and in rats with simulated birth trauma induced by vaginal distension (VD). To clarify the role of noradrenergic and serotonergic mechanisms in the active urethral pressure responses that prevent SUI during sneeze, we also investigated the effect of duloxetine after pretreatment with intrathecal methiothepin maleate, a 5-HT receptor and α1-adrenoceptor antagonist, terazosin, an α1-adrenoceptor antagonist, or idazoxan, an α2-adrenoceptor antagonist.

MATERIALS AND METHODS

Animals. Sixty-seven adult female Sprague-Dawley rats weighing 236–265 g were studied with experimental protocols approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Experiments were performed in normal rats and rats with simulated birth trauma induced by VD (3, 15).

Vaginal distension. VD rats were used as a rat model of SUI for experiments 1 and 3. Under pentobarbital (30 mg/kg ip; Ovation, Deerfield, IL), a modified 10-Fr Foley balloon catheter (5 ml;
RUSCH, Salt Lake, UT) with the tip cut off was inserted into the vagina and the vaginal orifice was closed with suture. The balloon catheter was inflated with 4 ml of water to distend the vagina for 3 h (12). The experiments were conducted 4 days after the VD.

Surgical procedures for experiments. Under isoflurane anesthesia, a polyethylene catheter (PE-10, Clay-Adams, Parsippany, NJ) was inserted into a jugular vein for drug injection. The urinary bladder was exposed through an abdominal incision, ureters were cut bilaterally, and their distal ends were ligated. The visceral branches of pelvic nerves were transected bilaterally near internal iliac vessels to prevent reflex bladder contractions (16).

A polyethylene catheter (PE-90, Clay-Adams) connected to a pressure transducer (Transbridge 4M, World Precision Instruments, Sarasota, FL) was then inserted into the bladder through the dome for recording intravesical pressure to detect leak point pressure during sneezing in experiment 1. Feces were removed from the distal colon through a small incision of the colon wall in experiments 1 and 2. A handmade small balloon catheter with a 1-cm-diameter balloon connected to a pressure transducer was then inserted through the rectum into the abdominal cavity to record abdominal pressure (Pabd) during sneezing. The abdomen was then closed with sutures.

After the surgery, isoflurane anesthesia was turned off and replaced with urethane anesthesia (0.72 g/kg ip; Sigma Chemical, St. Louis, MO), and additional doses of anesthetic (0.1 g/kg per injection) were administered as required before sneeze reflex testing started to obtain the sufficient level of anesthesia, which was confirmed by negative reflex responses to toe pinch. The final dose of urethane ranged from 1.0 to 1.2 g/kg in 67 animals tested. Rats were placed in a supine position for the experiments.

Sneeze reflex and sneeze-induced urethral continence reflex. The sneeze reflex, which is a highly coordinated reflex evoked by irritation of nasal mucosa, is designed to remove irritations and clean the airway. In this study, the sneeze reflex was induced by a rat’s whisker cut and inserted gently into the nostril under urethane anesthesia. Sneeze induces a urethral pressure increase, which is elicited by reflex contractions of external urethral sphincter and pelvic floor muscles (13). We previously examined this active urethral closure mechanism during sneezing in rats and reported that the reflex is mediated by somatic nerves and occurs only at the middle portion of the urethra (13).

Experiment 1: Effect of duloxetine on midurethral pressure responses and baseline pressure. Normal and VD rats (n = 12 each) were examined after the bladder was emptied, and a 3.5-Fr nylon catheter with a side-mounted microtransducer located 1 mm from the catheter tip (SPR-524, Millar Instruments, Houston, TX) was inserted into the urethra from the urethral orifice. The side-mounted sensor was positioned to face the inner urethral surface in the 3 o’clock position, because measurement in a lateral orientation corresponds most closely to urethral pressure in human studies (1). The microtransducer-tipped catheter was connected to a pressure transducer (Transbridge 4M, World Precision Instruments), and urethral pressures were recorded with a data-acquisition software (sampling rate 400 Hz; Chart, AD Instruments, Castle Hill, NSW, Australia) on a computer system equipped with an analog-to-digital converter (Power Lab, AD Instruments, Colorado Springs, CO). By monitoring changes in urethral pressure on the computer screen during the insertion of the microtransducer-tipped catheter into the urethra, the tip of a microtransducer-tipped catheter was fixed at the middle urethra (inserted catheter length; 10–15 mm from the urethral orifice), where the highest basal urethral pressure was observed. This location coincided with the urethral portion where neurally evoked active urethral pressure responses during sneeze were observed in our previous study (13). The catheter position was monitored throughout the experiments to confirm that the location of the transducer had not changed.

Both in normal and VD rats, the sneeze reflex was induced, and amplitude of urethral pressure responses during sneezing (A-URS) and urethral baseline pressure (UBP) were measured. A-URS was determined as the maximal pressure change (cmH2O) from the baseline. The averaged UBP was obtained from a plateau section of pressure recordings just before the sneeze response according to our previous reports (11). Baseline sneeze-induced responses were measured before and after intravenous injection of duloxetine (1 mg/kg; Kemprotec, Middlesbrough, UK). Sneeze reflexes were evoked repeatedly to obtain at least 10 measurable responses before and after duloxetine treatment.

To evaluate the intensity of the induced sneeze, which varied with each sneeze event, pressure increases in Pabd during sneezing were also measured during each sneeze event via an intra-abdominal balloon catheter inserted through the rectum because it was not possible to measure intravesical pressure in the emptied bladder. Sneeze-induced increases in Pabd were determined as pressure values (cmH2O) measured from the baseline to the peak of the pressure responses according to our previous reports (11).

Experiment 2: Effects of 5-HT receptor and α1-adrenoceptor antagonist methiothepin maleate, α1-adrenoceptor antagonist terazosin, or α2-adrenoceptor antagonist idazoxan on duloxetine-induced changes in urethral pressure responses. Twenty-nine normal rats were implanted with an intrathecal catheter 1–2 days before the experiment under isoflurane anesthesia. Laminectomy was performed at the level of the third lumbar vertebra, and a polyethylene catheter (PE-10, Clay-Adams) was implanted at the level of the L3–S1 spinal cord through a small hole in the dura. The catheter was secured to the vertebral with sutures and placed subcutaneously underneath the skin on the back. One or two days after the catheter insertion, the intrathecal catheter was exteriorized through the skin incision for intrathecal drug administration (it) during the experiments. At the end of each experiment, a laminectomy was performed to verify the location of the catheter tip and the distribution of injected dye (2 μl Evans blue, Sigma) flushed with 10 μl of saline.

Initially, sneeze reflexes were induced, and A-URS and UBP were measured before drug application as baseline values by using microtransducer-tipped catheters as described in experiment 1. In 8 of 29 rats, after methiothepin maleate, a 5-HT receptor and α1-adrenoceptor antagonist (6 nmol; Tocris Cookson, Ellville, MO), was administered via the intrathecal catheter and flushed with 10 μl of saline, sneeze reflexes were induced again and the effects of the drug on both A-URS and UBP were evaluated. Duloxetine (1 mg/kg) was then injected intravenously, and its effects on A-URS as well as UBP in the presence of methiothepin maleate were evaluated. Sneeze reflexes were evoked repeatedly to obtain at least 10 measurable responses before and after the drug administration.

In another eight rats, the effects of intrathecal administered terazosin (2 nmol; Tocris Cookson), an α1-adrenoceptor antagonist, were examined on A-URS and UBP in the same manner. Duloxetine (1 mg/kg iv)-induced changes in A-URS and UBP were then evaluated in the presence of terazosin (2 nmol).

In eight other rats, the effects of simultaneous intrathecal administration of methiothepin maleate (6 nmol) and terazosin (2 nmol) were also examined on A-URS and UBP in the same manner as described above. Duloxetine (1 mg/kg iv)-induced changes in A-URS and UBP were then evaluated in the presence of the two drugs.

In five additional rats, the role of spinal α2-adrenoceptor activation was examined. First, simultaneous intrathecal administration of methiothepin maleate (6 nmol) and terazosin (2 nmol) on A-URS and UBP was examined. Idazoxan (4 nmol, Sigma Chemical), a selective α2-adrenoceptor antagonist, followed by duloxetine (1 mg/kg iv) was then administered intrathecally.

To measure the averaged intensity of induced sneezes, pressure changes in Pabd during sneezing were also measured with the intra-abdominal catheter as described in experiment 1.

Experiment 3: Effects of duloxetine on sneeze leak point pressure. In normal and VD rats (n = 6 and 8, respectively) after the bladder was emptied, 0.4 ml of saline solution containing Evans blue (100 μg/ml; Sigma Chemical) was injected into the bladder. The sneeze...
reflex was induced to examine whether fluid leakage from the urethral orifice was induced by sneezing. Intravesical pressure changes were recorded to monitor the increase in abdominal pressure during sneezing with data acquisition software (sampling rate 400 Hz; Chart, AD Instruments) on a computer system equipped with an analog-to-digital converter (Power Lab, AD Instruments). The sneeze reflex was induced at least 50 times to obtain large sneezes with high intravesical pressures sufficient to induce fluid leakage from the urethral orifice. The maximal intravesical pressure was measured in each sneeze event, and the lowest pressure value that induced fluid leakage from the urethral orifice was defined as the sneeze leak point pressure (S-LPP). After control S-LPPs were obtained, duloxetine (1 mg/kg iv) was injected intravenously and sneeze reflexes were induced again to evaluate the effect of the drug on sneeze-induced fluid leakage and S-LPP.

**Drugs.** Duloxetine, methiothepin maleate, terazosin, and idazoxan were dissolved in distilled water and administered in doses based on the results of a previous study (14) and our own preliminary experiments. Intravenous duloxetine was administered in a volume of 0.1 ml/100 g body wt. For intrathecal application, 1 µl of drug solution was given via the implanted intrathecal catheter and flushed by 10 µl of saline. In our preliminary study, intrathecal saline injection had no effect on urethral activity during sneezing.

**Statistical analysis.** Data are expressed as means ± SE. Excessively large sneezes that induced increases in $P_{abd}$ greater than +2 SD above the average and very small responses inducing increases in $P_{abd} < 3$ cmH$_2$O were excluded from data analyses. The values of the A-URS and UBP as well as increases in $P_{abd}$ during sneezing were averaged in each rat. The mean ± SE in a group of animals was then calculated from the averaged value in each rat.

A paired t-test was used to compare A-URS, UBP, and increases in $P_{abd}$ during sneezing before and after duloxetine in the absence or presence of methiothepin maleate, terazosin, and idazoxan. Student’s t-test for unpaired data was used to compare A-URS and UBP as well as increases in $P_{abd}$ during sneezing before and after duloxetine in the absence or presence of methiothepin maleate, terazosin, and idazoxan. Student’s t-test for paired data was also used to compare the duloxetine-induced increase in S-LPP. P values <0.05 were considered to be significant.

**RESULTS**

**Experiment 1: Effects of duloxetine on midurethral pressure responses and baseline pressure.** Representative traces of urethral pressure responses measured by a microtransducer-tipped catheter are shown in Fig. 1. Both A-URS and UBP were significantly lower in incontinent VD rats (13.1 ± 3.1 and 27.4 ± 6.1 cmH$_2$O, respectively) than in normal rats (36.4 ± 3.1 and 27.4 ± 6.1 cmH$_2$O, respectively) ($P < 0.05$). In normal and VD rats, duloxetine (1 mg/kg iv) significantly increased A-URS (48.0 ± 3.9 and 17.5 ± 1.8 cmH$_2$O, respectively; $P < 0.05$) (Fig. 1). Duloxetine treatment also significantly increased UBP in normal and VD rats (33.3 ± 2.4 and 26.2 ± 2.0 cmH$_2$O, respectively; $P < 0.05$) (Fig. 1).

The average value (range 8.2–8.5 cmH$_2$O) of sneeze-induced increases in $P_{abd}$ measured by intra-abdominal catheters was not significantly different in normal and VD rats and was not significantly changed by duloxetine in these rats (Table 1).

**Experiment 2: Effects of 5-HT receptor and α$_1$-adrenoceptor antagonist methiothepin maleate and/or α$_1$-adrenoceptor antagonist terazosin or α$_2$-adrenoceptor antagonist idazoxan on duloxetine-induced changes in urethral pressure responses.** Intrathecal application of methiothepin maleate (6 nmol) alone did not significantly change A-URS and UBP or the duloxetine-induced increase in A-URS (44.3 ± 4.4 and 58.4 ± 5.4 cmH$_2$O before and after 1 mg/kg duloxetine iv, respectively).

The duloxetine-induced increase in UBP (6.0 ± 1.2 cmH$_2$O increment) also still occurred in the presence of methiothepin maleate (6 nmol it) (Table 2, Fig. 2A).

Intrathecal application of terazosin (2 nmol), which alone did not significantly change A-URS and UBP, suppressed the duloxetine-induced increases in A-URS (44.3 ± 5.8 and 43.9 ± 4.5 cmH$_2$O before and after duloxetine, respectively) but did not block the duloxetine-induced increase in UBP (7.0 ± 1.2 cmH$_2$O increment) (Table 2, Fig. 2B).

Intrathecal coapplication of methiothepin maleate (6 nmol) and terazosin (2 nmol) significantly reduced ($P < 0.05$) A-URS during sneezing from 44.9 ± 4.4 to 32.5 ± 5.5 cmH$_2$O without altering UBP. Duloxetine (1 mg/kg iv) applied in the absence of methiothepin and terazosin elicited a further decrease in A-URS to 25.1 ± 3.8 cmH$_2$O ($P < 0.01$ vs. predrug control value of 44.9 ± 4.4 cmH$_2$O; Table 2, Fig. 2C). In addition, duloxetine-induced increases in UBP were significantly decreased in the presence of methiothepin and terazosin.

In the next series of experiments, the effects of an addition of idazoxan (4 nmol) were examined. Simultaneous intrathecal application of methiothepin maleate (6 nmol) and terazosin (2 nmol) reduced A-URS significantly ($P < 0.05$) from 49.5 ± 5.7 to 36.1 ± 4.4 cmH$_2$O but did not alter UBP. Therefore,
when duloxetine (1 mg/kg iv) was applied after intrathecal application of idazoxan (4 nmol), the A-URS value (39.8 ± 8.9 cmH2O) was not significantly different from the predrug control value (49.5 ± 5.7 cmH2O), indicating that α2-adrenoceptor inhibition by idazoxan restored the A-URS value after duloxetine to the control level in the presence of methiothepin and terazosin. Duloxetine tended to increase UBP in the presence of the three drugs, but UBP values before and after duloxetine were not significantly different (P = 0.11; Table 2, Fig. 2D).

The averaged values of sneeze-induced increases in Pabd measured by intra-abdominal catheters were not significantly different among the different experimental groups before and after duloxetine treatment with and without intrathecal methiothepin maleate, terazosin, and/or idazoxan application, indicating that there was no significant difference in the intensity of sneezing among the groups (Table 2).

Experiment 3: Effect of duloxetine on sneeze leak point pressure. Intravesical pressures during sneezing were higher than the averaged value of Pabd during sneezing (8 cmH2O) examined in experiment 1 because it was necessary to produce large sneezes to induce fluid leakage from the urethral orifice in order to obtain S-LPP.

In six normal rats, no fluid leakage was observed during sneezing before and after duloxetine injection (1 mg/kg iv). Sneeze-induced intravesical pressure as high as 80.4 ± 9.1 cmH2O without fluid leakage from the urethral orifice. Duloxetine (1 mg/kg iv) did not affect the peak intravesical pressure during sneezing.

In all eight VD rats, leakage was observed during sneezing before duloxetine injection (1 mg/kg iv). S-LPP of these VD rats averaged 39.1 ± 3.8 cmH2O. After the duloxetine treatment (1 mg/kg iv), fluid leakage during sneezing disappeared in two of eight incontinent VD rats, and in the remaining six incontinent VD rats S-LPP was significantly increased from 39.1 ± 3.8 to 92.3 ± 15.8 cmH2O (136% increase, P < 0.01). In the two VD rats in which leakage was not seen after duloxetine (1 mg/kg iv), sneeze-induced leakage was observed at S-LPP of 46.5 and 30.2 cmH2O, respectively, before duloxetine; however, leakage did not occur after duloxetine (1 mg/kg iv) even when the maximum intravesical pressure reached 119.2 and 92.9 cmH2O, respectively, during sneezing.

### DISCUSSION

Effects of duloxetine. Sneeze-induced active urethral closure is elicited by reflex contractions of external urethral sphincter and pelvic floor muscles (13). We previously reported (13) that the active urethral closure mechanism during sneezing is mediated by activation of somatic nerves innervating urethral and pelvic floor striated muscles. This sneeze-induced continence reflex is considered to be different from the bladder-to-urethra reflex that is activated by afferent firing in the pelvic nerve in response to an increase in intravesical pressure (11, 13).

In the present study, in which microtransducer-tipped catheters were used to measure urethral reflexes, intravenous application of duloxetine in normal and VD rats increased A-URS by 35% and 34% and UBP by 21% and 34%, respectively. Because microtransducer-tipped catheters can only measure the local force/unit area exerted by the tissue on the catheter, recorded values do not necessarily reflect the true urethral pressure. Thus, to assess overall urethral resistance during sneezing, we also measured the changes in S-LPP, which is defined as the minimal intravesical pressure during sneezing that induces fluid leakage from the urethral orifice. We found that after the duloxetine treatment (1 mg/kg iv), fluid leakage during sneezing disappeared in two of eight incontinent VD rats and S-LPP was significantly increased from 39.1 to 92.3 cmH2O (136% increase) in the remaining six incontinent VD rats. These results

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**Table 1. Effects of duloxetine on sneeze-induced pressure changes in normal and vaginally distended rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A-URS (cmH2O)</th>
<th>UBP (cmH2O)</th>
<th>Increase in Pabd (cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (n = 12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>36.4 ± 3.1</td>
<td>27.4 ± 1.6</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>After</td>
<td>48.0 ± 3.9*</td>
<td>33.3 ± 2.4*</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>VD rats (n = 12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>13.1 ± 1.2†</td>
<td>20.4 ± 1.6†</td>
<td>8.4 ± 1.2</td>
</tr>
<tr>
<td>After</td>
<td>7.5 ± 1.8*</td>
<td>26.2 ± 2.0*</td>
<td>8.5 ± 0.7</td>
</tr>
</tbody>
</table>

Values (in cmH2O) are means ± SE. Significant difference vs. predrug values: *P < 0.05, †P < 0.01.

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**Table 2. Effects of intrathecal application of methiothepin maleate and/or terazosin or idazoxan and intravenous duloxetine on sneeze-induced pressure changes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A-URS (cmH2O)</th>
<th>UBP (cmH2O)</th>
<th>Increase in Pabd (cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methiothepin maleate (n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predrug control</td>
<td>44.3 ± 4.4</td>
<td>28 ± 2.4</td>
<td>7.5 ± 0.6</td>
</tr>
<tr>
<td>After methiothepin</td>
<td>48.3 ± 6.0</td>
<td>28.2 ± 1.9</td>
<td>8.6 ± 1.8</td>
</tr>
<tr>
<td>After duloxetine in presence of methiothepin</td>
<td>58.4 ± 5.4*</td>
<td>34.1 ± 2.3*</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>Predrug control</td>
<td>44.3 ± 5.8</td>
<td>26.3 ± 2.3</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td>After terazosin</td>
<td>44.1 ± 5.3</td>
<td>27.2 ± 2.0</td>
<td>8.5 ± 1.3</td>
</tr>
<tr>
<td>After duloxetine in presence of terazosin</td>
<td>43.9 ± 4.5</td>
<td>33.2 ± 2.3*</td>
<td>8.0 ± 1.2</td>
</tr>
<tr>
<td>Predrug control</td>
<td>44.9 ± 4.4</td>
<td>23.2 ± 1.8</td>
<td>8.1 ± 0.6</td>
</tr>
<tr>
<td>After methiothepin + terazosin</td>
<td>32.5 ± 5.5*</td>
<td>22.9 ± 1.8</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>After duloxetine in presence of methiothepin + terazosin</td>
<td>25.1 ± 3.8†</td>
<td>25.1 ± 1.9</td>
<td>7.8 ± 0.8</td>
</tr>
</tbody>
</table>

Terazosin (n = 8)  
| Predrug control    | 49.5 ± 5.7    | 21.7 ± 1.7  | 8.0 ± 0.6               |
| After methiothepin + terazosin | 36.1 ± 4.4*   | 21.1 ± 1.7  | 7.8 ± 0.8               |
| After duloxetine in presence of methiothepin + terazosin + idazoxan | 39.8 ± 8.9   | 25.2 ± 2.1  | 8.6 ± 0.5               |

Methiothepin maleate + terazosin and idazoxan (n = 5)  

Values (in cmH2O) are means ± SE. Significant difference from predrug values: *P < 0.05, †P < 0.01.
indicate that complete remission of SUI after duloxetine was achieved in only two VD rats (25%), but the urethral continence reflex during sneezing was also improved in other VD rats. We showed previously that the VD rat, which is a model of SUI that reproduces the injuries associated with human vaginal parity (3, 15), exhibits a damaged active urethral closure mechanism at the middle urethra, resulting in SUI (12). In the present study, we also found that the sneeze-induced continence reflex (A-URS) and UBP measured with microtransducer-tipped catheters were lower in VD rats compared with normal rats. Thus duloxetine appears to enhance baseline pressure at the midurethra and the active urethral closure reflex during sneezing, thereby preventing sneeze-induced urine leakage in a rat SUI model induced by VD.

In addition, in our previous study (11), intravenous application of nisoxetine, a NE reuptake inhibitor, increased A-URS by 17% and 18% in normal and VD rats, respectively, and increased S-LPP by 30% in incontinent VD rats. These values are lower than those after duloxetine (34–35% and 136% increases in A-URS and S-LPP, respectively) in the present study. Therefore, duloxetine, a NE and 5-HT reuptake inhibitor, seems to be superior to nisoxetine to enhance the active urethral closure mechanisms, possibly because of additional activation of the serotonergic system by duloxetine.

Adrenergic and serotonergic mechanisms controlling sneeze-induced continence reflex. In this study, we found that intrathecal application of either methiothepin maleate or terazosin alone did not affect sneeze-induced urethral responses (A-
URT), whereas the responses were reduced when methiothepin and terazosin were applied intrathecally together. Thus it is assumed that endogenous noradrenergic and serotonergic mechanisms in the spinal cord are complementing each other to maintain the sneeze-induced urethral continence reflex and that the removal of these two systems in the spinal cord results in the reduction of the reflex.

We also found that intrathecal terazosin, but not methiothepin maleate, suppressed duloxetine-induced increases in A-URS, indicating that the enhancing effect of duloxetine on the urethral continence reflex during sneezing was mainly mediated by activation of $\alpha_1$-adrenoceptors, but not 5-HT receptors, in the spinal cord. It has been reported that methiothepin has high affinity not only to 5-HT receptors, but also to $\alpha_1$-adrenoceptors (4, 18). Katofiasc et al. (14) reported that duloxetine (iv) produces marked increases in bladder capacity and external sphincter activity that are reversed by methiothepin (iv) in cats. However, in the present study intrathecal application of methiothepin maleate alone did not affect the sneeze-induced urethral responses or suppress the duloxetine (iv)-induced increases in A-URS at the midurethra, while terazosin, a selective $\alpha_1$-adrenoceptor antagonist, suppressed duloxetine-induced increases in A-URS during sneezing and also reduced sneeze-induced A-URS when applied in combination with methiothepin. Therefore, it seems that the antagonistic effects of methiothepin at the dose (6 nmol) used in this study on $\alpha_1$-adrenoceptors in the somatic nerve-induced active urethral closure mechanisms might be weak compared with the effects on the guarding reflex (i.e., bladder-to-urethra reflex) in cats. This discrepancy could be explained by species difference; however, further studies are needed to clarify this point.

Furthermore, in the presence of both terazosin and methiothepin, which reduced A-URS during sneezing, duloxetine induced a greater reduction in sneeze-induced A-URS compared with the predrug control value. However, the reduction in A-URS after duloxetine in the presence of terazosin and methiothepin was abolished by intrathecal application of idazoxan, an $\alpha_2$-adrenoceptor antagonist. These results suggest that suppression by duloxetine of the sneeze-induced urethral continence reflex (i.e., reduced A-URS) seen after inhibition of spinal $\alpha_1$-adrenergic and 5-HT receptors is due to activation of inhibitory $\alpha_2$-adrenoceptors in the spinal cord. Danuser et al. (6) previously demonstrated that modulation of somatic reflex pathways to the lower urinary tract in cats by NE reuptake inhibition caused activation of not only facilitatory $\alpha_1$-receptors, but also inhibitory $\alpha_2$-receptors in the spinal cord. Thus the present results further confirm that NE reuptake inhibition by duloxetine exerts opposite effects (excitatory and inhibitory) on the sneeze-induced continence reflex via spinal $\alpha_1$- and $\alpha_2$-adrenoceptors, respectively. However, because an $\alpha_2$-receptor-mediated reduction in A-URS after duloxetine treatment was not observed when only spinal $\alpha_1$-adrenoceptors or 5-HT receptors were blocked by terazosin or methiothepin, respectively, duloxetine-induced inhibition of the sneeze-induced continence reflex via $\alpha_2$-adrenoceptors seems to be masked when $\alpha_1$-adrenoceptors or 5-HT receptors are simultaneously activated. Overall, urethral sphincter continence reflexes during sneezing are likely to be regulated by a complex balance among facilitatory 5-HT receptors, $\alpha_1$-adrenoceptors and inhibitory $\alpha_2$-adrenoceptors.

In this study, we used a nonspecific 5-HT antagonist (methiothepin) to examine the overall effect of 5-HT receptor activation by duloxetine on the sneeze-induced continence reflexes. However, 5-HT receptors are divided into seven families (5-HT$_{1-7}$) (20). Among these, it is well known that 5-HT$_2$ receptor stimulation activates the pudendal nerve, causing an increase in external sphincter activity in cats, guinea pigs, and rats (7, 8, 20). In addition, Thor et al. (23) showed

![Hypothetical schema](image-url)
that 5-HT_{1A} receptor activation can also enhance external sphincter activity in cats. Chang et al. (5) also have shown that WAY-100635, a 5-HT_{1A} antagonist, reversed the effect of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} agonist that increased external sphincter activity during stimulation of the pelvic nerve in rats. It is also possible that activation of other 5-HT receptor subtypes after duloxetine treatment might have affected the results in the present study. However, which 5-HT receptor subtypes are involved in the regulation of the somatic nerve-induced urethral continence reflex during sneezing has not been fully examined. Further studies using specific 5-HT receptor agonists and antagonists are necessary to clarify these points.

**Adrenergic and serotonergic mechanisms controlling baseline urethral pressure.** In this study, UBP was not affected by intrathecal application of methiothepin and terazosin (either alone or combined), indicating that spinal noradrenergic (via α_{1}-receptors) and serotonergic systems do not tonically contribute to the maintenance of baseline pressure of the urethra. However, duloxetine that elevates NE and 5-HT concentrations at nerve terminals increased UBP in the study. We have previously shown (11) that nisoxetine (a NE reuptake inhibitor)-induced increases in UBP were inhibited by bilateral transection of hypogastric nerves or intravenous application of phentolamine or prazosin in rats. Thus it is assumed that duloxetine or nisoxetine can increase UBP due to activation of sympathetic pathways carried through the hypogastric nerves, which innervate urethral smooth muscles (2).

We observed in this study that duloxetine-mediated increases in UBP were not affected by intrathecal application of methiothepin or terazosin alone but were suppressed when the two drugs were coapplied. These results suggest that sympathetic preganglionic neurons in the spinal cord receive facilitatory inputs from both spinal noradrenergic (via α_{1}-receptors) and serotonergic systems to increase UBP, and that activation of either α_{1}-adrenoceptors or 5-HT receptors is enough to saturate the duloxetine-mediated enhancing effects on UBP.

**Adrenergic and serotonergic controls of the urethral continence reflex.** According to our present and previous studies (11), there seem to be at least two separate urethral continence mechanisms involving noradrenergic and serotonergic pathways that prevent SUI during sneezing in rats (Fig. 3). One mechanism enhances the urethral continence reflex during sneezing by activation of α_{1}-adrenoceptors and 5-HT receptors at the spinal cord level. The site of the action of NE/5-HT reuptake inhibitors in the spinal cord is probably in the Onuf’s nucleus, where dense NE- and 5-HT-containing terminals and urethral rhabdosphincter motoneurons are located (19). Descending signals in the bulbospongial noradrenergic and serotonergic pathways might enhance excitatory interneurons, such as glutamatergic neurons (17), or directly act on the Onuf’s nucleus, thereby maintaining and enhancing the active urethral closure mechanisms, while α_{2}-adrenoceptor stimulation by noradrenergic pathways can inhibit activity of the Onuf’s nucleus.

Then urethral baseline pressure can be modulated at the urethral smooth muscle level, where sympathetic postganglionic nerves release NE to directly stimulate α_{1}-adrenoceptors on smooth muscles.

**Perspectives and Significance**

In the present study, we investigated the role of the noradrenergic and serotonergic systems in prevention of urinary incontinence by using a rat model that allows the study of the neurally evoked continence reflex during sneezing. Duloxetine-mediated enhancement of sneeze-induced urethral responses is predominantly dependent on activation of spinal α_{1}-adrenoceptors. However, activation of both noradrenergic and serotonergic mechanisms via α_{1}-adrenoceptors and 5-HT receptors, respectively, must be important to maintain the active urethral closure during sneezing because combined treatment with methiothepin and terazosin suppressed A-URS. These results provide further insights into the mechanisms underlying the clinical efficacy of NE/5-HT reuptake inhibitors such as duloxetine in the treatment of SUI in humans, and also demonstrate that the sneeze-induced incontinence model in rats is useful for testing new drugs that are being developed to treat SUI.

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