Role of noradrenergic pathways in sneeze-induced urethral continence reflex in rats

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Kaiho Y, Kamo I, Chancellor MB, Arai Y, de Groat WC, Yoshimura N. Role of noradrenergic pathways in sneeze-induced urethral continence reflex in rats. Am J Physiol Renal Physiol 292: F639–F646, 2007. First published October 17, 2006; doi:10.1152/ajprenal.00226.2006.—To clarify the role of noradrenergic pathways in preventing stress urinary incontinence (SUI) during sneezing, we investigated the effect of the norepinephrine reuptake inhibitor nisoxetine and α1-adrenoceptor antagonists phentolamine (nonspecific blocker) and prazosin (α1-receptor-selective blocker) on the neurally evoked urethral continence reflex induced by sneezing in rats. The 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 female adult rats and rats with SUI induced by vaginal distention (VD). In normal rats, intrathecal (it) phentolamine (0.02 nmol) and prazosin (0.02 nmol) decreased A-URS by 11.9 and 15.7%, respectively, without affecting UBP. In both normal and VD rats, intravenous (iv) application of nisoxetine (1 mg/kg) increased A-URS by 17.2 and 18.3% and UBP by 23.7 and 32.7%, respectively. Phentolamine or prazosin (both it) eliminated nisoxetine-induced increases in A-URS, but not the increases in UBP, which were, however, suppressed by iv phentolamine (5 mg/kg) or prazosin (1 mg/kg). Sneeze-induced fluid leakage from the urethral orifice in VD rats, but not in normal rats. In VD rats, S-LPP was increased by 30.2% by iv nisoxetine. Application of phentolamine and prazosin (both it) decreased S-LPP by 15.7 and 20.6%, respectively, and nisoxetine induced increases in S-LPP to 13.2 and 12.3%, respectively. These results indicate that activation of the noradrenergic system by a norepinephrine reuptake inhibitor can prevent SUI via α1-adrenoceptors by enhancing the sneeze-induced active urethral closure mechanism at the spinal level and augmenting UBP at the periphery.

STRESS URINARY INCONTINENCE (SUI) is a very common condition affecting mainly women and is growing as a significant health problem with a rapid increase in elderly population worldwide (18). A search for effective and well-tolerated drugs for treatment of SUI has demonstrated clinical efficiency of norepinephrine (NE) and/or serotonin reuptake inhibitors, such as duloxetine, in patients with SUI (2, 5). The action of these drugs is considered to be associated with reuptake inhibition of serotonin and NE at the presynaptic terminal in Onuf’s nucleus of the sacral spinal cord (17). However, the mechanisms for improving continence during stress conditions such as sneezing have not been studied in detail. Therefore, in the present study we examined the effects of activation of noradrenergic pathways on the continence reflex preventing SUI during sneezing in rats.

We have previously established a rat model that can examine the neurally evoked continence reflex during sneezing (12). Under stress conditions such as sneezing, the rat urethra has an active urethral closure mechanism that is mediated by somatic nerve-induced reflex contractions of external urethral sphincter and pelvic floor striated muscles. The active urethral pressure responses during sneezing in addition to passive transmission of increased abdominal pressure play an important role in preventing SUI (12). We have also reported that these active urethral closure mechanisms during sneezing are impaired in a rat model of SUI induced by simulated birth trauma (11).

Thus to clarify the role of noradrenergic pathways in the active pressure responses preventing SUI during sneeze in rats, we investigated the effect of a NE reuptake inhibitor, nisoxetine, on the sneeze-induced continence reflex by using leak point pressure measurements and urethral microtip transducer catheter methods in normal rats and rats with simulated birth trauma induced by vaginal distension (VD). The site of action of nisoxetine, spinal vs. peripheral, for the enhancement of sneeze-induced continence reflexes was also examined by intrathecal and intravenous administration of α1-adrenoceptor antagonists phentolamine or prazosin.

MATERIALS AND METHODS

Animals. Eighty-three adult female Sprague-Dawley rats, weighing 214–292 g, were studied, and the experimental protocols were evaluated and 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 (4, 13).

VD. VD rats were used as a rat model of SUI for experiments 1, 2, and 4. Under halothane anesthesia (Halocarbon Laboratories, River Edge, NJ), a modified 10-Fr Foley balloon catheter (5 ml, RUSCH) 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 water to distend the vagina for 3 h (11). The experiments were conducted 4 days after VD.

Surgical procedures for experiments. Under halothane 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, and ureters were cut bilaterally and their distal ends were ligated. Feces were removed from the distal colon through a small incision of the colon wall. The visceral branches of pelvic nerves were transected bilaterally near internal iliac vessels to prevent reflex bladder contractions.

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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 from the dome for recording intravesical pressure to detect leak point pressure during sneezing in the experiments 1 and 4. A handmade small balloon catheter with a 1-cm-diameter balloon connected to a pressure transducer was also inserted through the rectum into the abdominal cavity before the start of sneeze induction. The final dose of urethane ranged from 1.02 to 1.22 g/kg in 83 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. A sneeze induces a urethral pressure increase that is elicited by reflex contractions of external urethral sphincter and pelvic floor muscles (12). We have previously examined this active urethral closure mechanism during sneezing in rats and reported that the reflex is mediated by somatic nerves and occurred only at the middle portion of the urethra (12).

**Experiment 1: effects of nisoxetine on sneeze leak point pressure.** In five normal and normal VD rats, after the bladder was emptied 0.4 ml of saline solution containing Evans blue (100 µg/ml, Sigma) was injected into the bladder. The sneeze reflex was induced to examine whether fluid leakage from the urethral orifice was induced by sneezing, and intravesical pressure changes were recorded to monitor an increase in abdominal pressure during sneezing using 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). 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 S-LPP without any drug application was determined, nisoxetine (1 mg/kg, Tocris Cookson, Ellisville, MO) was injected intravenously. Nisoxetine (1 mg/kg) was then injected intravenously. Sneeze reflexes were induced again, and the effect of the nisoxetine treatment on both A-URS and UBP was evaluated. Sneeze reflexes were evoked repeatedly to obtain at least 10 measurable responses in each condition.

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. We confirmed in preliminarily experiments that pressures measured by an intra-abdominal catheter were nearly equal to those measured by an intravesical catheter in the fluid-filled bladder during sneezing (data not shown). Sneeze-induced increases in Pabd were determined as pressure values (H2O) measured from the baseline to the peak of the pressure responses (Fig. 1).

**Experiment 3: effects of a-adrenoceptor antagonists phentolamine and prazosin on nisoxetine-induced changes in urethral pressure responses.** Twenty normal rats were implanted with an intrathecal catheter 1–2 days before the experiment. Under halothane anesthesia, a laminectomy was performed at the Th11 vertebra through a skin incision on the back, and a polyethylene catheter (PE-10, Clay Adams) was inserted intrathecially and the end of the catheter was positioned at the level of the L6-S1 spinal cord. The catheter was secured to the vertebra with sutures and placed subcutaneously underneath the skin on the back. One or 2 days after the catheter insertion, the intrathecal catheter was exteriorized through the skin incision for intrathecal phentolamine or prazosin injection during the following experiments. At the end of every experiment, a laminectomy was performed to verify the location of the catheter tip and the distribution of injected dye (2 µl Evans blue flushed with 10 µl saline).

Initially, sneeze reflexes were induced, and A-URS and UBP were measured without drug application as baseline values by using microtip transducer catheters as described in experiment 2. In 12 of 20 rats, after a nonselective a-adrenoceptor antagonist, phentolamine
(0.02 nmol, Sigma), was administered via the intrathecal catheter and flushed with 10 µl saline, sneeze reflexes were induced again and the effects of phenotolamine on both A-URS and UBP were evaluated. Then, nisoxetine (1 mg/kg) was injected intravenously and its effects on A-URS as well as UBP in the presence of phenotolamine were evaluated. Sneeze reflexes were evoked repeatedly to obtain at least 10 measurable responses.

In 8 of 20 rats, the effects of an intrathecally administered α1-adrenoceptor antagonist, prazosin (0.02 nmol, Tocris Cookson), were examined in the same manner as described above for phenotolamine injection. The effect of intrathecal prazosin on A-URS and UBP was examined, and then nisoxetine-induced changes in A-URS and UBP in the presence of prazosin were evaluated.

In an additional six rats, to estimate the involvement of peripheral α-adrenoceptor activation, the effects of intravenous application of phenotolamine (5.0 mg/kg, n = 3) or prazosin (1 mg/kg, n = 3) on nisoxetine-induced changes in UBP were examined under urethane anesthesia (1.0 g/kg sc).

In another five rats, the hypogastric nerves were transected bilaterally at the level of the major pelvic ganglia to disrupt sympathetic outflow to the urethra under halothane, in which the effects of nisoxetine on UBP were examined to clarify the contribution of peripheral sympathetic pathways to nisoxetine-induced changes in urethral activity.

To measure the averaged intensity of induced sneezes, pressure changes in Pabd during sneezing were also measured using the intraperitoneal catheter as described in experiment 2.

Experiment 4: effect of intrathecal phenotolamine and prazosin on S-LPP. In 16 VD rats, in which sneezing induced urinary leakage, S-LPP was examined in the presence of intrathecal α1-adrenoceptor antagonists phenotolamine (n = 7) or prazosin (n = 9). In seven rats, after control S-LPP values were measured in the absence of drugs as described in experiment 1, phenotolamine (0.02 nmol) was administered via the implanted intrathecal catheter, and the effect of phenotolamine on S-LPP was evaluated. Then, nisoxetine (1 mg/kg) was injected intravenously and the effect of nisoxetine on the S-LPP in the presence of phenotolamine was also evaluated. The nisoxetine-induced increase in S-LPP in the presence of phenotolamine was compared with the increase in S-LPP without phenotolamine, which was evaluated in experiment 1. In another nine rats, the effects of intrathecally applied prazosin were examined in the same way as performed for phenotolamine injection. Prazosin (0.02 nmol) was administered intrathecally, and the effect of prazosin on S-LPP and nisoxetine-induced increases in S-LPP after prazosin treatment were examined.

Drug dosage. The doses of nisoxetine, phenotolamine, and prazosin were chosen on the basis of published results (10) and our own preliminary experiments. Phenotolamine or prazosin was administered intrathecally in saline at concentrations of 10 µmol/l and in a volume of 2 µl flushed by 10 µl saline. Intravaginal nisoxetine, phenotolamine, and prazosin were administered in a volume of 0.1 ml/100 g body wt.

Statistical analysis. Data are expressed as means ± SE. The excessively large sneezes that induced increases in Pabd greater than +2 SD above the average and very small responses inducing increases in Pabd <3 cmH2O were excluded from data analyses. The values of the A-URS and UBP as well as increases in Pabd 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 the A-URS and UBP and increase in Pabd during sneezing before and after nisoxetine in the absence or presence of phenotolamine or prazosin. Student’s t-test was used to compare the A-URS and UBP as well as increases in Pabd during sneezing between normal and VD rats. In experiment 4, Student’s t-test was also used to compare the nisoxetine-induced increase in S-LPP with or without phenotolamine or prazosin treatment.

RESULTS

Experiment 1: effect of nisoxetine on S-LPP. In five normal rats, no fluid leakage was observed during sneezing before and after nisoxetine injection (1 mg/kg iv). Sneezing increased intravesical pressure as high as 78.2 ± 6.9 cmH2O without fluid leakage from the urethral orifice. Nisoxetine (1 mg/kg iv) did not affect the peak intravesical pressure during sneezing.

In seven of eight VD rats, leakage was observed during sneezing before nisoxetine injection (1 mg/kg iv). S-LPP of seven incontinent VD rats averaged 45.9 ± 5.8 cmH2O. After the nisoxetine treatment (1 mg/kg iv), fluid leakage during sneezing disappeared in two out of seven incontinent VD rats, and S-LPP was significantly increased from 46.4 ± 6.4 to 61.5 ± 10.2 cmH2O (P < 0.05) in the remaining five incontinent VD rats (Fig. 2A). In the two VD rats, in which leakage was eliminated by nisoxetine (1 mg/kg iv), sneeze-induced leakage was observed at S-LPPs of 62.1 and 27.3 cmH2O before nisoxetine, respectively; however, leakage did not occur after nisoxetine (1 mg/kg iv) even when the maximum intravesical pressure reached 100.8 and 63.3 cmH2O, respectively.

![Graph showing drug-induced changes in sneeze leak point pressure (S-LPP) in vaginal distended rats. A: nisoxetine (1 mg/kg iv) increased S-LPP. B: intrathecal application of phenotolamine (0.02 nmol) significantly decreased S-LPP and reduced the increase in S-LPP induced by nisoxetine. *P < 0.05.](http://ajprenal.physiology.org/DownloadedFrom/article/2007/02/561/figure/F641)
Experiment 2: effects of nisoxetine on midurethral pressure responses and baseline pressure. Both A-URS and UBP were significantly lower in incontinent VD rats (11.4 ± 1.6 and 16.2 ± 2.0 cmH2O, respectively) than in normal rats (37.6 ± 4.5 and 30.4 ± 2.1 cmH2O, respectively) (P < 0.05). Nisoxetine (1 mg/kg iv) increased both A-URS and UBP (Fig. 3). In normal and VD rats, nisoxetine treatment (1 mg/kg iv) significantly increased A-URS by 17.2 ± 3.6 and 20.2 ± 6.5%, respectively (P < 0.05). Nisoxetine treatment also increased UBP significantly by 23.7 ± 2.9 and 32.7 ± 3.9% in normal and VD rats, respectively (P < 0.05) (see Fig. 5A, Table 1).

The average value of sneeze-induced increases in Pabd measured by intra-abdominal catheters was not significantly changed in normal and VD rats, indicating that there was no significant difference in the intensity of sneezing between normal and VD rats before and after nisoxetine treatment (Table 1). The averaged value of Pabd during sneezing (8–9 cmH2O) was smaller than intravesical pressures during sneezing (in experiment 1) because it was necessary to produce large sneezes to induce fluid leakage from the urethral orifice to obtain S-LPP.

Experiment 3: effects of α-antagonists phentolamine and prazosin on nisoxetine-induced changes in urethral pressure responses. Representative traces of urethral pressure responses measured by a microtip transducer catheter are shown in Fig. 4. Intrathecal application of phentolamine (0.02 nmol) significantly decreased A-URS (P < 0.05) from 40.2 ± 5.2 to 35.8 ± 4.8 cmH2O (Table 2). On the other hand, intrathecal phentolamine did not significantly change UBP.

After intrathecal phentolamine, the nisoxetine-induced increase in A-URS was blocked (35.8 ± 4.8 vs. 34.4 ± 4.1 cmH2O after nisoxetine). On the other hand, the nisoxetine-induced increase in UBP (6.0 ± 0.9 cmH2O) still occurred after phentolamine administration (0.02 nmol iv) (Fig. 5, B and C, Table 2).

Intrathecal application of prazosin (0.02 nmol) produced effects similar to those elicited by phentolamine. Prazosin (0.02 nmol it) significantly reduced A-URS from 39.5 ± 4.7 to 33.5 ± 4.2 cmH2O (P < 0.05) but did not alter the UBP. Prazosin (0.02 nmol it) also eliminated the nisoxetine-induced enhancement of the continence reflex during sneezing (A-URS before and after nisoxetine was 33.5 ± 4.2 and 32.5 ± 4.1 cmH2O, respectively) without affecting the nisoxetine-induced increase (7.8 ± 1.8 cmH2O) in the UBP (Fig. 5, B and C, Table 2).

On the other hand, in another set of experiments, when phentolamine or prazosin was injected intravenously (5 and 1 mg/kg, respectively; n = 3 for each drug), the subsequent nisoxetine injection (1 mg/kg iv) did not increase UBP, indicating that systemically administered α-adrenoceptor antagonists inhibited nisoxetine-induced increase in UBP (Fig. 5, B and C). Moreover, in another five rats with bilateral hypogastric nerves transection, nisoxetine did not increase UBP significantly (UBP before and after nisoxetine: 23.5 ± 3.9 and 24.8 ± 3.5 cmH2O, respectively).

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 nisoxetine treatment with and without intrathecal phentolamine/prazosin application, indicating that there was no significant difference in the intensity of sneezing among the groups (Table 2).

Experiment 4: effects of intrathecal phentolamine and prazosin on S-LPP. In 16 incontinent VD rats, intrathecal application of phentolamine (0.02 nmol) or prazosin (0.02 nmol) significantly decreased S-LPP and the effect of the subsequent administration of nisoxetine (Table 3, Fig. 2B).
S-LPP induced by nisoxetine (1 mg/kg iv) in the presence of intrathecal phentolamine or prazosin was significantly smaller (13.2 and 12.3% increase, respectively) than that induced by nisoxetine alone (1 mg/kg iv) in experiment 1 (30.2% increase) (Table 3). This significantly smaller nisoxetine-induced increase in S-LPP following phentolamine or prazosin treatment was consistent with the results obtained with microtip transducer catheter methods in experiment 3, in which the nisoxetine-induced increase in A-URS was blocked by intrathecal phentolamine or prazosin application.

DISCUSSION

Sneezing can induce an active urethral closure that is elicited by reflex contractions of external urethral sphincter and pelvic floor muscles. We have previously reported that the active urethral closure mechanism during sneezing is mediated by activation of somatic nerves innervating urethral and pelvic floor striated muscles (12). This sneeze-induced continence reflex is considered to be different from the bladder-to-urethra reflex which is activated by afferent firing in the pelvic nerve (10). This sneeze-induced continence reflex is considered to be different from the bladder-to-urethra reflex which is activated by afferent firing in the pelvic nerve (10). This sneeze-induced continence reflex and increased UBP in both normal and VD rats; 4) intrathecally administered phentolamine/prazosin decreased the sneeze-induced urethral responses (A-URS) without affecting UBP; 5) intrathecal phentolamine or prazosin decreased the sneeze-induced urethral responses (A-URS) without affecting UBP. In the presence of phentolamine, the nisoxetine-induced increase in A-URS was blocked; however, the nisoxetine-induced increase in UBP was still observed.

Table 2. Effects of intrathecal application of phentolamine/prazosin and following intravenous nisoxetine 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>Phenolamine (n = 12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before phentolamine</td>
<td>40.2±5.2</td>
<td>28.4±3.5</td>
<td>8.0±0.8</td>
</tr>
<tr>
<td>After phentolamine</td>
<td>35.2±4.8*</td>
<td>28.1±3.7</td>
<td>8.6±1.0</td>
</tr>
<tr>
<td>After nisoxetine</td>
<td>34.4±4.1</td>
<td>34.2±4.0*</td>
<td>8.5±0.9</td>
</tr>
<tr>
<td>After nisoxetine (n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before prazosin</td>
<td>39.5±4.7</td>
<td>27.5±1.9</td>
<td>8.4±0.9</td>
</tr>
<tr>
<td>After prazosin</td>
<td>33.5±4.2*</td>
<td>27.2±1.9</td>
<td>8.7±1.1</td>
</tr>
<tr>
<td>After nisoxetine</td>
<td>32.5±4.1</td>
<td>35.0±2.3*</td>
<td>9.0±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05.
tolamine/prazosin only blocked the nisoxetine-induced enhancement of the continence reflex during sneezing, without blocking nisoxetine-induced increases in UBP; and 6) the nisoxetine-induced increase in UBP was inhibited by systemically applied phentolamine and prazosin as well as bilateral transection of the hypogastric nerves.

These results suggest that there are at least two separate urethral continence mechanisms involving noradrenergic pathways that prevent SUI during sneezing in rats. One pathway enhances the A-URS by activation of $\alpha_1$-adrenoceptors at the spinal cord level and the other augments the UBP via peripheral $\alpha_1$-adrenoceptors. Thus it seems likely that at the spinal cord level, descending signals in the bulbospinal noradrenergic pathways were enhanced by nisoxetine, the NE reuptake inhibitor, to strengthen the sneeze-induced continence reflex. This mechanism was suppressed by intrathecal nonspecific and $\alpha_1$-selective adrenoceptor antagonists. The site of the action of NE reuptake inhibitors in the spinal cord is probably at Onuf’s nucleus, where dense NE-containing terminals and urethral rhabdosphincter motor neurons are located (14). NE is considered to facilitate pudendal nerve efferent activity via interaction with glutamic acid, which is a primary excitatory neurotransmitter for rhabdosphincter motor neurons (16). Therefore, it is assumed that the descending signals strengthening the sneeze-induced continence reflex would be facilitated by intravenous nisoxetine or suppressed by intrathecal phentolamine/prazosin in Onuf’s nucleus, resulting in the enhanced or decreased continence reflex in the rhabdosphincter during sneezing, respectively.

Although the effects of $\alpha$-adrenoceptor antagonists on urethral activity have been extensively studied, most of previous studies have focused on the peripheral effects of these drugs and there have not been many reports about the central $\alpha$-adrenergic mechanisms controlling urethral activity. Gajewski et al. (9) and Danuser et al. (7) observed that intravenous administration of prazosin or phentolamine inhibited action potentials of the pudendal nerve evoked by stimulation of the contralateral pudendal or pelvic nerve in cats. Chen et al. (6) reported that intrathecal administration of prazosin depressed tonic external urethral sphincter electromyography activity induced by acetic acid infused into the urethra in cats with acute spinal cord injury. These results of intravenous and intrathecal administration of $\alpha$-adrenoceptor antagonists indicate that $\alpha$-adrenergic antagonists depressed the somatic control of the external sphincter through an action in the spinal cord. In the present study, $\alpha$-adrenoceptor antagonists were administered intrathecally and found to be effective in suppressing the

Table 3. Sneez leak point pressure increased by nisoxetine with or without intrathecal phentolamine/prazosin in VD rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>S-LPP, cmH2O</th>
<th>%Change in S-LPP</th>
</tr>
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<tbody>
<tr>
<td>Nisoxetine ($n = 5$)</td>
<td>Before nisoxetine</td>
<td>46.4±6.4</td>
</tr>
<tr>
<td></td>
<td>After nisoxetine</td>
<td>61.5±10.2</td>
</tr>
<tr>
<td>Nisoxetine in the presence of phentolamine ($n = 7$)</td>
<td>Before phentolamine</td>
<td>49.7±4.3</td>
</tr>
<tr>
<td></td>
<td>After phentolamine</td>
<td>41.4±4.1*</td>
</tr>
<tr>
<td></td>
<td>After nisoxetine in the presence of phentolamine</td>
<td>46.6±5.1*</td>
</tr>
<tr>
<td>Nisoxetine in the presence of prazosin ($n = 9$)</td>
<td>Before prazosin</td>
<td>47.3±7.0</td>
</tr>
<tr>
<td></td>
<td>After prazosin</td>
<td>37.0±6.4*</td>
</tr>
<tr>
<td></td>
<td>After nisoxetine in the presence of prazosin</td>
<td>41.0±8.6*</td>
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Values are means ± SE. S-LPP, sneeze-induced leak point pressure. *$P < 0.05$.

Fig. 5. Effects of nisoxetine on A-URS and UBP with and without phentolamine or prazosin. A: nisoxetine increased A-URS and UBP in both normal and vaginal distention (VD) rats. Intrathecal application of phentolamine (B) or prazosin (C) eliminated nisoxetine-induced increases in A-URS, but not the increases in UBP after nisoxetine, which, however, were suppressed by intravenous phentolamine (0.02 nmol) and prazosin (0.02 nmol). *Significant increase by nisoxetine treatment in A-URS or UBP ($P < 0.05$).
neurally evoked continence reflex during sneezing, indicating that the spinal noradrenergic system tonically enhances the sneeze-induced urethral continence mechanism. To the best of our knowledge, this is the first report that examines the contribution of spinal noradrenergic descending pathways in urinary continence during sneezing.

Danuser et al. (7) also described that intravenous administration of prazosin inhibited action potentials of the hypogastric nerve evoked by stimulation of the pelvic nerve in cats. However, our results showed intrathecal administration of neither prazosin nor phentolamine decreased UBP. This discrepancy could be due to species difference, or, alternatively, it is possible that intrathecal administration of prazosin or phentolamine did not reach the spinal levels that originate sympathetic outflow because the drugs were applied at the L6-S1 spinal cord levels in this study.

This study also revealed a peripheral noradrenergic mechanism that prevents SUI by increasing UBP. Pretreatment with intrathecal phentolamine or prazosin, which blocked the effect of nisoxetine at the spinal level on sneeze-induced urethral pressure increases, did not change the nisoxetine-induced increase in the UBP. However, the UBP increases after nisoxetine were inhibited by intravenously injected phentolamine and prazosin, and they also significantly diminished after transection of the hypogastric nerves that provide major sympathetic inputs to urethral smooth muscles. Since it is well known that sympathetic pathways innervating the urethra can directly stimulate adrenoceptors in the urethral smooth muscles to increase urethral pressure, it seems reasonable to assume that the second site of the action of the NE uptake inhibitor is probably noradrenergic mechanisms in the urethral smooth muscle, where the drug enhances the extracellular concentration of NE, leading to activation of $\alpha_1$-adrenoceptors in the urethral smooth muscles to increase the UBP (Fig. 6).

In the present study, a nonselective $\alpha$-adrenoceptor antagonist (phentolamine) and an $\alpha_1$-adrenoceptor antagonist (prazosin) were examined although the precise contribution of different subtypes of adrenoceptors in the spinal cord or in the periphery was not examined. Our results showed the effects of these two drugs on the mechanisms to prevent SUI in rats were almost the same at the dosage in the experiments. Prazosin ($\alpha_1$-receptor-selective blocker) as well as phentolamine (non-specific blocker) suppressed the sneeze-induced continence reflex at the spinal level and the nisoxetine-induced enhancement of UBP in the periphery. It is well known that $\alpha_1$-adrenoceptors play a major role in controlling urethral smooth muscles (3). In experiments examining the effects of duloxetine, a NE and serotonin reuptake inhibitor, on the striated urethral sphincter in cats, the effects of duloxetine were blocked by an $\alpha_1$-adrenoceptor antagonist, but not by an $\alpha_2$- or $\beta$-adrenoceptor antagonist (17). We assume that $\alpha_1$-adrenoceptors are also important in the prevention of SUI during sneezing in rats. However, other adrenoceptor subtypes such as $\alpha_2$-receptors or $\beta$-receptors should be examined to elucidate the contributions of these adrenoceptors in the sneeze-induced continence reflex because previous studies have shown activation of $\alpha_2$- and $\beta$-adrenoceptor subtypes has prominent effects on urethral functions (7, 8, 15). Springer et al. (15) reported that NE reuptake inhibition produced a greater increase in activation of $\beta$-adrenoceptors compared with $\alpha$-receptors in the peripheral urethral smooth muscle. Danucer et al. (7) also demonstrated that NE reuptake inhibition caused activation of not only facilitatory $\alpha_1$-receptors but also inhibitory $\alpha_2$-receptors in the spinal cord to modulate somatic reflex pathways to the lower urinary tract in cats. Although the present study showed that prazosin and phentolamine had similar effects on nisoxetine-induced changes in urethral activity, it is possible that antagonistic activity of phentolamine for $\alpha_2$-adrenoceptors may be insufficient at the dose tested due to the difference in the number of $\alpha$-receptor subtypes and/or unequal tissue distribution of the drug despite that phentolamine has similar affinities for $\alpha_1$- and $\alpha_2$-adrenoceptors. Further studies are needed to clarify these points.

Because microtip transducers can only measure the local force/unit area exerted by the tissue on the inner surface of the transducer tip, recorded values do not necessarily reflect the true urethral pressure. Therefore, in experiment 4 we examined the changes in S-LPP, which is defined as the minimal intra-vesical pressure during sneezing that induces fluid leakage from the urethral orifice without reflex bladder contractions. The results showed that phentolamine or prazosin significantly decreased S-LPP, and the following application of nisoxetine significantly increased S-LPP even in the presence of intrathecal phentolamine or prazosin. However, the increase in S-LPP induced by the nisoxetine treatment after $\alpha$-adrenoceptor...
blockade in the spinal cord was significantly smaller than that without the phenolamine/prazosin treatment. Thus the small nisoxetine-induced increase in S-LPP after phenolamine/prazosin treatment is likely to correspond to the results obtained using microtip transducer catheters, showing that intrathecal phenolamine blocks the nisoxetine-mediated effects only at the spinal level (i.e., increases in sneeze-induced urethral responses) but not the effects at the peripheral level, where nisoxetine can still increase UBP in the presence of intrathecal α1-adrenoceptor antagonists.

In conclusion, in the present study we investigated the role of the noradrenergic system in prevention of urinary incontinence by using a rat model that can examine the neurally evoked continence reflex during sneezing. The noradrenergic system can prevent SUI via α1-adrenoceptors by promoting the descending signals in bulbospinal noradrenergic pathways to strengthen the sneeze-induced continence reflex at the spinal cord level while increasing UBP at the peripheral level. These results provide further insights into the mechanisms underlying the clinical efficacy of NE/serotonin reuptake inhibitors such as duloxetine in the treatment of SUI in humans and also demonstrate that the sneeze-induced incontinence model in rats might be useful for testing new drugs that are being developed to treat SUI.

GRANTS

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