Sexually dimorphic urethral activity in response to pharmacological activation of 5-HT$_{1A}$ receptors in the rat

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Fan WJ, Li YT, Chen JJJ, Chen SC, LinYS, Kou YR, Peng CW. Sexually dimorphic urethral activity in response to pharmacological activation of 5-HT$_{1A}$ receptors in the rat. Am J Physiol Renal Physiol 305: F1332–F1342, 2013. First published September 18, 2013; doi:10.1152/ajprenal.00261.2013.—In this study, we examined the possibility that 5-HT$_{1A}$ receptors may underlie sexually dimorphic mechanisms affecting the regulation of urethral functions in anesthetized rats. Simultaneous recordings of intravesical pressure under isovolumetric conditions, external urethral sphincter-electromyography, and urethral perfusion pressure were used to examine the effects of a 5-HT$_{1A}$ receptor agonist [8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)] and antagonist (WAY-100635) on bladder and urethral functions. This research also evaluated the effects of 8-OH-DPAT and α-bungarotoxin (a neuromuscular blockade agent) on the urethral continence using leak point pressure testing, and the distribution of 5-HT$_{1A}$ receptors in the lower urinary tract was assessed by immunohistochemistry. The serotoninergic mechanism that controls the urinary bladder and external urethral sphincter-electromyography activity showed no significant sexual differences, but urethral activity in urethral perfusion pressure and leak point pressure values exhibited some sexual differences. 8-OH-DPAT enhanced urethral pressure during continence in rats of both sexes, but the drug elevated the pressure during voiding in male rats and reduced it in female rats. The distribution of 5-HT$_{1A}$ receptors in the spinal cord also showed some sexual differences. The present study contributes to our understanding of the role of 5-HT$_{1A}$ receptors in physiological and immunohistochemical properties of urethral smooth muscle in rats of different sexes. These findings may be a basis for the future development of pharmacotherapies for stress urinary incontinence in men.

intravesical pressure; external urethral sphincter-electromyography; urethral perfusion pressure; 8-hydroxy-2-(di-n-propylamino)tetralin; WAY-100635; serotonin

SEROTONERGIC AGENTS, such as duloxetine, are clinically used to treat stress urinary incontinence (SUI) in women (14). Despite satisfactory results in most female patients, there are few reports regarding the effect of duloxetine on SUI in men. Because the incidence of SUI in men is dramatically lower than in women, SUI is generally considered to be a female issue (27, 42). However, epidemiological studies have suggested that men are sometimes severely afflicted with SUI (27) and suffer greater emotional impacts than women (44). Currently, duloxetine has not been approved for treating SUI in men, but the drug has been used off label in some pilot studies; the studies (17, 40, 48) showed that the drug may produce a marked improvement in men with urinary incontinence.

The rat model has now gained great popularity as the main species for developing pharmacologically based treatments of SUI. Effects of a 5-HT receptor agonist on regulatory bladder functions have been extensively investigated in female animals (5, 6, 15, 19, 23, 28, 43). To the best of our knowledge, few studies have explored the role of 5-HT$_{1A}$ receptors in regulating urethral functions in male rats. In addition, many cases of sexually anatomic and functional dimorphism in the lower urinary tract (LUT) have been reported in humans and rats. Anatomically, the distribution of the external urethral sphincter (EUS) was considered to be a sexual difference in both humans and rats (4, 13, 31, 37, 39, 47). In rats, the bulbospongiosus, ischiocavernosus, and cremaster are well developed in adult male rats but are vestigial or absent in adult female rats (20, 34). Some sexual dimorphisms in the functions of urethral striated smooth muscles have been reported (12, 25, 26). For example, Kontani and Shiraoya (25, 26) indicated that urethral activity in rats in response to electrical stimulation of the hypogastric or pudendal nerve differed between the sexes, which might result from the different neurotransmitters that control urethral smooth muscle activity.

Because of the existence of anatomic and functional differences in the LUT between the sexes, the primary goal of this study was to examine whether the 5-HT$_{1A}$ receptors that control urethral activity in rats exhibit sexual dimorphism. Serotonergic receptors were activated or blocked by systemic administration of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a receptor agonist, or WAY-100635, a 5-HT$_{1A}$ receptor antagonist. Simultaneous recordings of intravesical pressure (IVP) under isovolumetric conditions (isovolumetric IVP), EUS-electromyography (EUS-EMG), and urethral perfusion pressure (UPP) were used to evaluate contributions of striated and smooth muscles to urethral responses during isovolumetric IVP. In addition, an immunohistochemical (IHC) examination was conducted on rats to localize 5-HT$_{1A}$ receptors in the tissues of the lumbosacral spinal cord. This examination may provide an anatomic basis to correlate with urethral responses induced by 5-HT agents.

* Y. R. Kou and C.-W. Peng contributed equally to this work.
MATERIALS AND METHODS

General preparations. The Institutional Animal Care and Use Committee of Taipei Medical University and Hospital approved the experiment protocols involving the use of animals in this study. In total, 22 male (276–300 g) and 22 virgin female (201–225 g) Sprague-Dawley rats were used in this study. All rats were anesthetized with urethane (1.2 g/kg SC). The trachea was cannulated to facilitate respiration. The femoral vein was catheterized for fluid and drug administration, and body temperature was maintained at 36–38°C with a recirculating water blanket. Most animals breathed spontaneously. However, a few animals were artificially ventilated after an intravenous injection of α-bungarotoxin (Tocris Bioscience, Bristol, UK), a neuromuscular blocking agent, which was used to block urethral striated muscle activity during the experiment.

Simultaneous recordings of isovolumetric IVP, UPP, and EUS-EMG. Several rats (n = 10 rats of each sex) were used to conduct simultaneous recordings of isovolumetric IVP, UPP, and EUS-EMG. The procedure for simultaneous recordings was performed as previously reported (2, 7). The experimental drug was administered during the recordings. For each trial, simultaneous recordings were turned off every 35–45-min recording trial and the bladder was then emptied. This was followed by a 10-min equilibration period before the next trial.

The analyst who examined isovolumetric IVP, UPP, and EMG activity was blinded to the status of the rats. Seven isovolumetric IVP and UPP parameters were measured: 1) the maximum amplitude of bladder contraction, 2) the duration of bladder contraction, 3) the area of reflex bladder contraction, 4) the frequency of bladder contraction, 5) the UPP baseline of the continence phase (i.e., the average UPP during bladder relaxation), 6) the UPP baseline of the voiding phase (i.e., the average UPP during the period of high-frequency oscillations (HFOs)), and 7) the difference between the two UPP baselines (i.e., the UPP baseline of the continence phase minus the UPP baseline of the voiding phase) (Fig. 1) (2, 7, 45).

For the analysis of EUS-EMG activity (9, 35), five parameters were measured: 1) the burst period (Fig. 3), 2) the silent period, 3) the active period, 4) the amplitude of the EMG during the continence phase (Fig. 1), and 5) the amplitude of the EMG during the voiding phase.

LPP testing. Six rats of each sex were used to measure LPP via the vertical-tilt table method (11, 29). The vertical-tilt table method was used in this study since it is more sensitive and reliable for measuring resting state LPP than is manual compression of the Crede method (11). Before the tests, the spinal cord was acutely transected at the T9–T10 level to eliminate reflex bladder activity in response to increasing IVP, but this did not interfere with the spinal continence reflexes of the bladder neck or urethra (11, 22, 24). Rats then underwent surgical preparations for LPP measurements. The surgical and measurement procedures for LPPs were performed as previously reported (11, 29).

Drug administration. 8-OH-DAPT and WAY-100635 (both from Sigma, St. Louis, MO) were dissolved in saline. To assess the role of 5-HT1A receptors in bladder and urethral activity, 8-OH-DAPT (0.3 mg/kg iv) and WAY-100635 (0.1 mg/kg iv) were administrated to rats at intervals of no less than 1 h. The drug dose was based on dosages used in previous studies (8, 10, 28). The first posttreatment urodynamics and EUS-EMG examinations were completed within 20–45 min after drug administration because of the short half-life of 8-OH-DAPT (15, 28). To further determine the independent effect of 8-OH-DPAT on urethral smooth muscle activity, some rats received an intravenous injection of α-bungarotoxin (0.1 mg/kg dissolved in saline), a neuromuscular blocking agent, at 30 min before the 8-OH-DPAT treatment. The effects of α-bungarotoxin generally last for 3–5 h (36).

IHC examinations. Six male and six female rats were used for 5-HT1A receptor IHC. Under terminal anesthesia, animals were transcardially perfused with 50 ml of a heparin solution (20 U/ml heparin in normal saline) followed by 300 ml of fixative (4% paraformaldehyde in 10 mM PBS at pH 7.4). The L1–L2 and L6–S1 segments of the spinal cord were carefully harvested and then immersed in 4% paraformaldehyde overnight. Subsequently, all spinal cord tissues were soaked in a 20% sucrose solution at 4°C for cryoprotection. The solution was changed every 24 h until samples sank to the bottom of the container. Spinal cord tissues were embedded in OCT compound, stored at ~80°C, cut into 5-μm-thick sections, and then placed onto gelatinized slides. A rabbit anti-5-HT1A receptor antibody (1:300, Abcam, Cambridge, UK) was used as the primary antibody. A NovolinkTM polymer detection system (Novocastra, Yerevan, Armenia) was used to localize the expression of 5-HT1A receptors on samples. Negative controls were samples without the primary antibody in rats of both sexes. To identify the tissue morphology and
location of immunoreactivity in detail, hematoxylin counterstaining was also conducted in some immunostained specimens.

Digital images of the samples were captured with a whole slide scanner (ScanScope CS, Aperio Technologies, Vista, CA) at ×40 magnification. To quantify the density of 5-HT<sub>1A</sub> receptors in the spinal cord, MetaMorph 7.7 software (Universal Imaging, Downingtown, PA) was used to digitally measure the average optical density (OD) in immunostained specimens with no counterstaining (33). The average OD was the ratio of the total ODs of all pixels to the total pixel area. For OD measurements, areas of gray matter related to neural control of the urethra were measured, including the dorsal horn (DH), sympathetic preganglionic neurons [lying at both intermediolateral neuron (IML) and dorsal gray commissure (DGC) areas in L<sub>1</sub>–L<sub>2</sub> segments], parasympathetic preganglionic neurons (lying only at the IML area in L<sub>6</sub>–S<sub>1</sub> segments), and pudendal motoneurons [at the dorsolateral nucleus (DLN) area in L<sub>6</sub>–S<sub>1</sub> segments] (Fig. 5, E and F) (32, 38).

**Statistical analysis.** Data are presented as means ± SD. Two-way ANOVA was used to compare parameters obtained from isovolumetric IVP, EUS-EMG, UPP, LPP, and IHC. ANOVA was followed by Tukey highly significant difference post hoc paired comparisons (SigmaStat, SPSS, Chicago, IL). P values of <0.05 were considered statistically significant for all analyses.

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**Fig. 2.** Typical patterns of IVP (**top**), EUS-EMG (**middle**), and UPP (**bottom**) recorded in anesthetized male rats. As with female rats, HFOs of urethral pressure appeared during the isovolumetric contraction of the bladder, but three types of UPP baseline values during the voiding phase were observed in male rats: type 1 (**A**), in which the UPP baseline of the voiding phase dramatically increased compared with the baseline before voiding; type 2 (**B**), in which the UPP baseline of the voiding phase did not significantly change compared with the baseline before voiding; and type 3 (**C**), in which the UPP baseline of the voiding phase dramatically decreased compared with the baseline before voiding.
RESULTS

Typical pattern of isovolumetric IVP, EUS-EMG, and UPP. Figure 1 shows typical patterns of isovolumetric IVP, EUS-EMG, and UPP in female rats. Isovolumetric contractions of the bladder were associated with relaxation of the urethra and HFOs of urethral pressure appearing during urethral relaxation. In addition, EUS-EMG exhibited a marked increase in amplitude during bladder contractions compared with the low-amplitude tonic activity between bladder contractions. The pattern of isovolumetric IVP, EUS-EMG, and UPP activities in female rats occurred rhythmically, and its detailed features were consistent among all cycles (n = 384 of 384 cycles from 10 female rats).

In male rats, although the general patterns of rhythmic isovolumetric IVP and EUS-EMG activities were consistent and similar to those of female rats, three different patterns of UPP activity during reflex bladder contractions were detected during the simultaneous recordings, as shown in Fig. 2. In type 1, the UPP baseline of the voiding phase dramatically increased compared with the UPP baseline of the continence phase (Fig. 2A). In type 2, the UPP baseline of the voiding phase showed no significant changes compared with the baseline before voiding occurred (Fig. 2B). In type 3, the UPP baseline of the voiding phase dramatically decreased during voiding (Fig. 2C).

UPP types 1 and 2 were usually observed in male animals during the first 90 min after the simultaneous recordings began (or within ~3.5 h after induction with urethane). After the first 90 min of simultaneous recording trials, the UPP pattern was dominated by the type 3 pattern, and ~90% of the UPP cycles were type 3 (n = 312 of 398 cycles from 10 male rats; Fig. 2C). Types 1 and 2 appeared to result from detrusor sphincter dyssynergia because the animal’s metabolism had not reached equilibration subsequent to anesthesia or surgery. Thus, in this study, the analysis of IVP, UPP, and EUS recordings in male rats only included the type 3 pattern of UPP and its accompanying IVP and EUS-EMG for statistical comparison with female rats.

In male rats, the basic pattern of EUS-EMG activity during UPP recordings among the three types was characterized by low-amplitude tonic activity between bladder contractions and a marked increase in amplitude during bladder contractions (Fig. 2). Detailed features of EUS-EMG activity during a single bladder contraction clearly presented a long burst period in both male and female rats, as shown in Fig. 3, A and C, respectively. Burst discharges in the burst period showed clusters of high-frequency spikes (corresponding to an active period) separated by periods of quiescence (corresponding to a silent period), as shown in Fig. 3, B and D. In addition, the EUS burst period was accompanied by the UPP superimposed with a series of HFOs, but it did not cause any oscillation waves in IVP (Fig. 3, B and D). The appearance of active periods in male rats was usually of short duration, beginning from the bottom and ending at the initial rising segment of UPP (Fig. 3B). In female rats, the active period lasted from the bottom to approximately the peak of UPP (Fig. 3D).

Effects of 8-OH-DPAT and WAY-100635 on isovolumetric IVP. Table 1 shows all isovolumetric IVP parameters obtained from male and female rats both before and after 5-HT drug treatment. In male rats, the amplitude of bladder contractions...
significantly increased and decreased with 8-OH-DPAT and WAY-100635 treatment, respectively, whereas this IVP parameter was unaffected by the drugs in female rats. In contrast, the duration of bladder contractions in male rats significantly decreased and increased with 8-OH-DPAT and WAY-100635, respectively, but in female rats, the drugs produced opposite effects to those observed in male rats. Although there were no consistent responses in the amplitude or duration of bladder contractions between the sexes, the effects of the drugs on the area of bladder contraction exhibited similar results in both sexes. In rats of either sex, 8-OH-DPAT treatment significantly increased the area of bladder contractions, and exactly opposite effects were detected in rats with WAY-100635 treatment. Because the area of bladder contraction is proportional to the product of the amplitude multiplied by the duration of bladder contractions, the results indicated that in rats of both sexes, the 5-HT1A receptor agonist produced excitatory effects on bladder activity, and opposite effects were generated by administration of the 5-HT1A receptor antagonist. These findings were also supported by results of a statistical analysis of the frequency of bladder contractions in rats of both sexes (Table 1).

**Effects of 8-OH-DPAT and WAY-100635 on UPP.** UPP measurements of male and female rats before and after drug treatment are shown in Table 2. Experimental results showed that in both males and female rats, administration of 8-OH-DPAT significantly increased the UPP baseline of the continence phase. Conversely, WAY-100635 treatment reduced the UPP baseline of the continence phase in both sexes. Interestingly, the UPP baseline of the voiding phase exhibited a different drug response in the two sexes. In male rats, 8-OH-DPAT significantly increased the UPP baseline of the voiding phase, whereas the drug significantly decreased this baseline in female rats. These results imply that the 5-HT1A receptor agonist enhanced urethral resistance during continence in both sexes, but urethral resistance during voiding was elevated and reduced by the drug in male and female rats, respectively.

The pressure difference in UPP between the average baselines of the continence and voiding phases was further analyzed in this study (Table 2). In male rats, the average baselines of the continence and voiding phases both increased with 8-OH-DPAT, and the pressure difference after 8-OH-DPAT treatment slightly increased compared with the control value (5.70 ± 1.17 vs. 4.37 ± 1.15 cmH2O, P = 0.76). However, in female rats, the pressure difference between the two UPP baselines significantly increased from 4.66 ± 1.25 to 9.50 ± 1.11 cmH2O with 8-OH-DPAT treatment (P < 0.05). This significant increase could have resulted from 8-OH-DPAT treatment, which, in turn, elevated the baseline of the continence phase and reduced the baseline of the voiding phase, causing the drug-enlarged UPP difference in female rats. Conversely, the pressure difference between the two UPP baselines was significantly reduced by WAY-100635 treatment in rats of both sexes.

**Effects of 8-OH-DPAT and WAY-100635 on EUS-EMG.** Table 3 shows EUS-EMG measurements in male and female rats before and after 8-OH-DPAT or WAY-100635 treatment. All EUS-EMG parameters in rats before any drug treatment exhibited significant sexual differences. Differences detected in male rats compared with female rats included long burst periods and silent periods, large amplitudes of EUS-EMG during the continence and voiding phases, and a short active period.

Subsequently, experiments showed that EUS-EMG activities of male and female rats treated with 8-OH-DPAT exhibited similar drug effects. The influence of 8-OH-DPAT treatment in both sexes included significant increases in the burst period, silent period, and amplitudes of EUS-EMG during the continence and voiding phases. In contrast, rats of both sexes treated with WAY-100635 showed effects opposite to those produced by 8-OH-DPAT. Note that the active period was not significantly affected by 8-OH-DPAT or WAY-100635 treatment in rats of either sex.

**Effects of α-bungarotoxin and 8-OH-DPAT on UPP.** Our results demonstrated that 8-OH-DPAT induced a differential UPP response in the sexes during the voiding phase (Table 2). To further determine the independent effect of 8-OH-DPAT on urethral smooth muscle, α-bungarotoxin was administrated in some rats during the UPP experiments (n = 3 rats of each sex). The drug was administrated to rats 30-40 min before 8-OH-DPAT treatment. Figure 4 shows examples of the effects of 8-OH-DPAT on UPP activity during spontaneous reflex bladder contractions in rats of both sexes after α-bungarotoxin treatment. After neuromuscular blockade treatment of rats, EUS-EMG activity was completely eliminated. However, UPP type 3 (a decrease in the baseline of UPP) during reflex bladder contractions was still observed in both sexes (Fig. 4, A and B). Subsequently, in male rats, 8-OH-DPAT treatment markedly elevated the UPP baseline of the voiding phase from 16.7 ± 0.35 to 19.67 ± 0.38 cmH2O (P < 0.05; Fig. 4, A compared with C). However, in female rats, the UPP baseline of the voiding phase was significantly reduced from 15.6 ± 0.35 to

Table 1. Effects of 8-OH-DPAT (0.3 mg/kg iv) and WAY-100635 (0.1 mg/kg iv) on isovolumetric IVP in male and female rats

<table>
<thead>
<tr>
<th></th>
<th>Maximum Amplitude of Bladder Contraction, cmH2O</th>
<th>Duration of Bladder Contraction, cmH2O</th>
<th>Area of Bladder Contraction, cmH2O</th>
<th>Frequency of Bladder Contraction, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>54.1 ± 3.37</td>
<td>36.77 ± 1.82</td>
<td>1.895 ± 85</td>
<td>1.40 ± 0.15</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>66.74 ± 3.10*</td>
<td>31.09 ± 1.32*</td>
<td>2.154 ± 95*</td>
<td>1.78 ± 0.12*</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>47.06 ± 0.90*</td>
<td>39.07 ± 1.64*</td>
<td>1.631 ± 79*</td>
<td>0.94 ± 0.10*</td>
</tr>
<tr>
<td><strong>Female rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50.07 ± 2.50*</td>
<td>25.33 ± 0.96*</td>
<td>1.347 ± 66*</td>
<td>1.27 ± 0.13</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>49.74 ± 2.51†</td>
<td>30.38 ± 1.68†</td>
<td>1.674 ± 71†</td>
<td>1.66 ± 0.09†</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>48.62 ± 2.73†</td>
<td>20.58 ± 1.20†</td>
<td>1.063 ± 82†</td>
<td>0.68 ± 0.07†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 male rats and 10 female rats. 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; isovolumetric IVP, intravesical pressure under isovolumetric conditions. *Statistically significant difference from the control value of male rats, P < 0.05; †statistically significant difference from the control value of female rats, P < 0.05.
13.97 ± 0.40 cmH2O by the drug (P < 0.05; Fig. 4, B compared with D).

**Effects of 8-OH-DPAT and α-bungarotoxin on LPP.** LPP testing was used to examine effects of the 5-HT receptor agonist on urethral resistance in rats of both sexes. To quantify the contribution of urethral striated and smooth muscles to urethral resistance, 8-OH-DPAT and α-bungarotoxin were intravenously administered in turn to the animals. LPP results are shown in Table 4. Before any drug intervention, the average LPP value in male rats was 1.5-fold higher than that in female rats (63.3 ± 4.2 vs. 41.8 ± 2.2 cmH2O), suggesting that urethral resistance in male rats was significantly higher than that in female rats. Subsequently, the first dose of 8-OH-DPAT treatment significantly increased the LPP value by ~20% in male rats, whereas the LPP value in female rats only slightly increased after rats received the drug. Two hours after 8-OH-DPAT treatment, LPP values were again measured (the second control), and values in both sexes had returned to values close to the first control values.

Subsequently, α-bungarotoxin was administered to rats to paralyze the urethral striated muscles. The effects usually lasted for 3–5 h. The LPP test was conducted 20–30 min after α-bungarotoxin treatment. Results showed that LPP values in both male and female rats significantly decreased with the drug to 77.4% and 86.4% of their initial control values, respectively (Table 4). Approximately 30–40 min after α-bungarotoxin treatment, 8-OH-DPAT was again administrated to rats. In male rats, 8-OH-DPAT significantly reversed the LPP value from 77.4% to 86.4% of its initial control, whereas in female rats, the drug only slightly elevated the LPP value from 86.4% to 90.3% of its initial value.

### DISCUSSION

Numerous studies (5, 6, 15, 19, 23, 28, 43, 46) have reported that the regulatory functions of 5-HT receptors in the LUT may be mediated by supraspinal and spinal sites. In the present study, we provide histological and functional findings suggesting the possibility of serotonergic mechanisms below the supraspinal level that control urethral functions. Our results revealed that serotonergic mechanisms of 5-HT1A receptors...
demonstrated no significant sexual differences in the regulation of the urinary bladder and EUS-EMG activity. However, significant sexual differences in the control of urethral activity were found. This sex-based difference was demonstrated in UPP measurements and in LPP testing. In addition, the IHC distribution of 5-HT1A receptors in cross-sections of spinal cord specimens showed some sexual differences. At the L6–S1 segments of the spinal cord, the areas of the IML (parasympathetic preganglionic neurons) and DLN (pudendal motoneurons) in male rats had greater numbers of expressed 5-HT1A receptors compared with those of female rats. However, at the L1–L2 segments, areas of sympathetic preganglionic neurons in male rats had fewer 5-HT1A receptors compared with those of female rats (Table 5). These IHC findings might be partially correlated with discrepancies in urethral activity between the sexes.

In this study, activation of 5-HT1A receptors with 8-OH-DPAT produced an excitatory effect on bladder activity in rats of both sexes, as evidenced by increases in the area and frequency of bladder contractions (Table 1). In contrast, ex-

Table 4. Effects of 8-OH-DPAT (0.3 mg/kg iv) and α-bungarotoxin (0.1 mg/kg iv) on LPP using a vertical-tilt table method in spinal cord-transected male and female rats

<table>
<thead>
<tr>
<th></th>
<th>First control</th>
<th>8-OH-DPAT</th>
<th>Second control</th>
<th>α-Bungarotoxin</th>
<th>α-Bungarotoxin With 8-OH-DPAT</th>
</tr>
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<tbody>
<tr>
<td>Male rats</td>
<td></td>
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<tr>
<td>LPP, cmH2O</td>
<td>63.3 ± 4.2</td>
<td>76.3 ± 2.4*</td>
<td>63.8 ± 4.5</td>
<td>49.0 ± 2.1*</td>
<td>54.8 ± 1.4*†</td>
</tr>
<tr>
<td>Percentage of first control</td>
<td>100.0 ± 6.7</td>
<td>120.5 ± 3.8</td>
<td>100.8 ± 1.9</td>
<td>77.4 ± 3.3</td>
<td>86.6 ± 2.2</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LPP, cmH2O</td>
<td>41.8 ± 2.2</td>
<td>45.7 ± 2.4</td>
<td>42.3 ± 2.1</td>
<td>36.1 ± 2.4*</td>
<td>37.8 ± 2.4*</td>
</tr>
<tr>
<td>Percentage of first control</td>
<td>100.0 ± 5.3</td>
<td>109.2 ± 5.6</td>
<td>101.2 ± 3.4</td>
<td>86.4 ± 5.8</td>
<td>90.3 ± 5.8</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 male rats and 6 female rats. LPP, leak point pressure. *P < 0.05, significant difference in male or female rats compared with their corresponding first control; †P < 0.05, significant difference in male or female rats compared with their corresponding value of α-bungarotoxin treatment.
ently opposite effects were observed in both sexes with WAY-100635 treatment. These results were consistent with those of previous studies (19, 23, 28, 46) showing that intracerebroventricular or intrathecal administration of these 5-HT agents produced excitatory and inhibitory effects on bladder activity, respectively. In addition, our results demonstrated that 5-HT1A receptors were markedly expressed at the lumbosacral spinal cord (preganglionic neurons) in rats of both sexes. These results confirm that supraspinal and spinal 5-HT1A receptors are involved in reflex bladder activity.

Enhancement of the guarding reflex (continence reflexes) by 5-HT1A receptor agonists was observed in rats of both sexes. However, the effects of serotonergic mechanisms on urethral continence reflexes exhibited some similarities and differences between the sexes in the present experiments. The amplitude of EUS-EMG during the bladder continence phase (the period of bladder relaxation) was significantly enhanced by 8-OH-DPAT in rats of both sexes (Table 3), which suggests that the drug promotes urinary continence. This assertion was also supported by the fact that 8-OH-DPAT increased the urethral resistance
in both sexes, as evidenced by the detection of a ~9–20% increase in LPP values in rats (Table 4). In addition, EUS motoneurons are located in the DLN (34, 41), and our results demonstrated that immunoreactivity of 5-HT1A receptors at the DLN was clearly observed in rats of both sexes (Fig. 5, B and D). These results suggest that 5-HT1A receptors directly act on the bladder-to-somatic pathway to facilitate continence reflexes in rats of both sexes.

Enhancement of the urethral continence reflex through the activation of urethral smooth muscles was also prominently detected in male rats with 5-HT1A receptor agonists, but this was not observed in female rats. This was demonstrated by the fact that the average LPP value was significantly increased by 8-OH-DPAT in male rats after neuromuscular blockade with α-bungarotoxin treatment, but the LPP value was not affected by the drug in female rats under a condition of neuromuscular blockade (Table 4). Enhancement of urethral continence through smooth muscle contraction in male rats may result from two mechanisms. First, 8-OH-DPAT might have activated reflexes in the lumbosacral parasympathetic pathways that project to the pelvic cholinergic nerves and induced urethral smooth muscle contraction. This parasympathetic cholinergic excitatory pathway to the urethra has been reported to exist in male rats but has not been identified in female rats (18, 21, 22). In addition, our histological results revealed that parasympathetic preganglionic neurons in male rats more densely express 5-HT receptors compared with those in female rats (Table 5). On the basis of these sexual differences, we concluded that the pelvic cholinergic pathway could be an important contributor to the continence reflex of urethral smooth muscle in male rats.

However, we could not exclude another possibility, that 5-HT receptors enhanced smooth muscle continence by means of regulating lumbar sympathetic reflex pathways projecting to the hypogastric nerves in male rats (1). This notion was supported by a previous study (16) that indicated that pharmacological activation of 5-HT receptors may facilitate the bladder-to-sympathetic reflex. Our histological findings also showed that the expression of 5-HT1A receptors at locations of sympathetic preganglionic neurons (DGC and IML) showed significant sexual differences (Table 5), suggesting that the contribution of serotonergic mechanisms to the sympathetic continence reflex may be a sexual difference. However, the histological findings in relation to the mechanism of 5-HT1A receptors in the regulation of sympathetic efferent pathways require further investigation.

The significant increase in the UPP baseline of the voiding phase in male rats and the decrease in female rats indicated that urethral activity during the voiding phase in rats after 8-OH-DPAT treatment also exhibited sexual differences (Table 2). These sexual differences in UPP responses have also been demonstrated in rats under a condition of neuromuscular blockade (Fig. 4) and in previous studies (25, 26) by electrical stimulation of pudendal or hypogastric nerves in rats. These results confirm that the sexual dimorphism in UPP originates from a difference in urethral smooth muscle activity. However, possible mechanisms of sexual dimorphism in the control of urethral smooth muscles are still controversial. In male rats, the elevated UPP baseline during the voiding phase can be attributed to facilitation of the sympathetic lumbar or parasympathetic lumbosacral reflexes by 8-OH-DPAT. The reflexes project along two possible routes: 1) pelvic cholinergic nerves or 2) hypogastric adrenergic nerves. First, the serotonergic mechanism regulates lumbosacral reflexes through the pelvic cholinergic route because previous study (22) has indicated that UPP was measured in male rats under a condition of neuromuscular blockade. UPP (which results from urethral smooth muscle contraction) is markedly suppressed by atropine and hexame-

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**Table 5. Average optical density in areas of the spinal gray matter related to neural control of the urethra in L1–L2 and L6–S1 segments in male and female rats**

<table>
<thead>
<tr>
<th>Afferent Neurons (Dorsal Horn Area)</th>
<th>Sympathetic Preganglionic Neurons</th>
<th>Parasympathetic Preganglionic Neurons (Intermediolateral Neuron Area)</th>
<th>Pudendal Motoneurons (Dorsolateral Nucleus Area)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dorsal Gray Commissure Area</td>
<td>Intermediolateral Neuron Area</td>
<td></td>
</tr>
<tr>
<td>Male rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1–L2</td>
<td>0.0223 ± 0.0082</td>
<td>0.0167 ± 0.0075</td>
<td>N/A</td>
</tr>
<tr>
<td>L6–S1</td>
<td>0.0300 ± 0.0063</td>
<td>N/A</td>
<td>0.0146 ± 0.0020</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1–L2</td>
<td>0.0285 ± 0.0043</td>
<td>0.0255 ± 0.0048*</td>
<td>N/A</td>
</tr>
<tr>
<td>L6–S1</td>
<td>0.0263 ± 0.0095</td>
<td>N/A</td>
<td>0.0081 ± 0.0054*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 male rats and 6 female rats. Average optical density is the total optical densities of all pixels (in relative units)/total pixel area (in μm²). N/A, not applicable. *P < 0.05, statistically significant difference compared with the corresponding value in male rats.
thionium. However, it is unaffected by administration of an α₁-adrenergic blockade agent (prazosin) or hypogastric nerve transaction, suggesting that the enhancement of urethral smooth muscle contractions during the micturition reflex in male rats is mediated by activation of the parasympathetic cholinergic pathway rather than the sympathetic pathway. Although Kakizaki and colleagues asserted that the dominance of urethral smooth muscle activity during the micturition reflex occurs through the parasympathetic cholinergic pathway, another study (26) showed that elevation of urethral pressure by hypogastric nerve stimulation is inhibited by an α₁-adrenergic blockade agent (prazosin) and an adrenergic neuron blocker (guanethidine). Therefore, this study suggested that the elevated urethral pressure mediated by hypogastric adrenergic nerves is another possibility. In contrast, in female rats, the predominant relaxation of the urethral smooth muscle response during the voiding reflex is caused by 5-HT agents that control spinal reflexes through nitric oxide mechanisms. This notion has been supported by previous studies (3, 22).

**Perspectives and significance.** In this study, 5-HT expression density (receptor numbers) in the spinal cord exhibited some sexual differences, although the expression area in the spinal cord was generally similar in rats of both sexes. In addition, sexually dimorphic urethral activity induced by 8-OH-DPAT was also observed in rats. These histological and functional findings suggest that the spinal reflex pathways of 5-HT₁A receptors that control urethral activity in rats of both sexes are likely to involve all spinal efferent pathways that project to the urethra. Previous studies (18, 21, 22, 25, 26) have indicated that partial efferent terminals in sympathetic, parasympathetic, or somatic nerves might release different neurotransmitters that control urethral smooth muscles in male and female rats. Thus, it will be of interest to verify the possible spinal efferent pathways and their neurotransmitters that control urethral activity mediated by 5-HT₁A receptors in rats of both sexes in future studies.

Pharmacotherapy for SUI is presently a matter of great interest. Serotonergic agents have been successfully used to treat SUI in women, but no pharmacotherapy for men is available. In this study, sexually dimorphic urethral activity was detected in rats after 8-OH-DPAT treatment. This may be clinically relevant. For patients with LUT obstruction, drugs such as α₁-adrenoceptor antagonists could exhibit different effects in men and women (30) because the prostate is predominantly innervated by sympathetic nerves situated along the proximal urethra in men. However, whether the sexually dimorphic effects of 5-HT agents in animals translate to humans is unclear. Nevertheless, this possibility warrants further exploration. The present study contributes to our understanding of the roles of 5-HT₁A receptors in physiological and IHC properties of urethral smooth muscles in male and female rats, and the findings may be a reference for the development of pharmacotherapy for SUI in men.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


**AUTHOR CONTRIBUTIONS**

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