Influence of serotonergic mechanisms on the urine flow rate in male rats

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Fan WJ, Chen SC, Hsieh TH, Lai CH, Lin YS, Peng CW, Kou YR. Influence of serotonergic mechanisms on the urine flow rate in male rats. Am J Physiol Regul Integr Comp Physiol 307: R1239–R1250, 2014. First published September 14, 2014; doi:10.1152/ajpregu.00160.2014.—This study extensively examined the role of a 5-HT1A receptor in controlling voiding function in anesthetized male rats. A simultaneous recording of the intravesical pressure (IVP), external urethral sphincter (EUS)-electromyography (EMG), and urine flow rate (UFR) during continuous cystometry was used. 8-Hydroxy-2-(di-n-propylaminotetralin (8-OH-DPAT), a 5-HT1A receptor agonist, significantly improved the voiding efficiency, as detected by increases in the evoked contraction amplitude, EUS burst period, and silent period, and decreases in the volume threshold, pressure threshold, and residual volume. Interestingly, the UFR during voiding was reduced by 8-OH-DPAT, as evidenced by decreases in the maximal UFR and mean UFRs of the voiding period, spike duration, and interspike interval. Conversely, treating rats with WAY-100635, a 5-HT1A antagonist, produced effects opposite to those produced by 8-OH-DPAT. These findings suggest that 8-OH-DPAT improved the voiding efficiency by enhancing the detrusor contractile activity and prolonging EUS burst period, which would compensate for the lower UFR, resulting from urethral smooth muscle contractions and longer EUS silent periods during voiding. The present study contributes to our understanding of the role of 5-HT1A receptors in controlling the urine flow rate in male rats.

Intravesical pressure; external urethral sphincter-electromyography; 8-hydroxy-2-(di-n-propylaminotetralin; WAY-100635; voiding efficiency

SEROTONIN (5-HYDOXYTRYPTAMINE, 5-HT) comprises 14 structurally different 5-HT receptors in mammalian species, including seven subfamilies (5-HT1 to 5-HT7) (28). A number of 5-HT receptor subfamilies, particularly 5-HT1A receptors, located at nerve terminals are involved in the function of the lower urinary tract (LUT) (38). Indeed, these 5-HT receptors-related nerve terminals are distributed in areas of the spinal cord containing afferent and efferent components of LUT neural control centers (8, 15, 19, 24, 38). Thus, 5-HT receptors appear to modulate all of the pathways involved in the control of reflex bladder and urethral sphincter activity, including the parasympathetic, sympathetic, and somatic pathways (13).

The 5-HT1A receptor is one of the most extensively investigated agents in functions of the LUT in various animal pharmacological experiments, including rats, guinea pigs, and cats (28). However, contradictory results have generated various interpretations of the functions of this receptor subtype in different animal species. Because the rat model has now gained greater popularity as the main species for investigating functions of the LUT, the effects of a 5-HT1A receptor agonist on regulating urine storage and micturition reflexes have been extensively investigated in female rats (14, 20–22, 35). However, few studies have explored these pharmacological effects in male animals. Moreover, our recent studies demonstrated that 8-OH-DPAT, a 5-HT1A receptor agonist, elevated the urethral pressure (resistance) during the voiding reflex in male rats (15). This finding raised the possibility of the UFR in male rats being decreased by 8-OH-DPAT. Therefore, the primary goal of this study was to examine the role of the 5-HT1A receptor in controlling the voiding function in male rats. Serotonergic receptors were activated or blocked by systemic administration of 8-OH-DPAT or WAY-100635 (a 5-HT1A receptor antagonist), respectively. Simultaneous recordings of the intravesical pressure (IVP), external urethral sphincter (EUS)-electromyography (EMG), and UFR were utilized to extensively investigate the role of serotonergic mechanisms in controlling voiding functions. The results indicated that activation of 5-HT1A receptors by 8-OH-DPAT significantly reduced the urine flow rate (UFR) during voiding, but it substantially elevated the voiding efficiency. Conversely, WAY-100635 produced effects opposite to those produced by 8-OH-DPAT.

MATERIALS AND METHODS

All animal care and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Taipei Medical University.

General preparations. Eighteen male Sprague-Dawley rats (276–300 g) were used in this study. All rats were anesthetized with urethane (1.2 g/kg sc). The body temperature was maintained at 36–38°C with a recirculating water blanket. The trachea was cannulated to facilitate respiration. In addition, the femoral vein was catheterized for fluid and drug administration. However, a few rats 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.

The urinary bladder was exposed via a midline abdominal incision and a polyethylene (PE) tube 60 (1.0 mm ID and 1.5 mm OD) was inserted into the bladder lumen for bladder pressure measurements. The bladder end of the PE tube was heated to form a collar and then passed through a small incision at the apex of the bladder dome. The
tube was secured with a purse-string suture, and the abdominal wall was closed with nylon sutures. The PE tube was, in turn, connected via a 3-way stopcock to an infusion pump for filling with physiological saline and to a pressure transducer for monitoring the IVP. Then two insulated silver wire electrodes (0.05 mm in diameter) with exposed tips were inserted into the lateral sides of the midurethra for EUS-EMG recordings. An ultrasonic flow probe (Probe #ME2PXMN; Transonic Systems, Ithaca, NY), connected to a flow meter (TS410; Transonic Systems) was used to measure the UFR. The flow probe was placed around the most distal part of the urethra (31, 39).

Physiological investigations. Rats underwent simultaneous recordings of IVP, EUS-EMG, and UFR during continuous transvesical cystometry that usually began 3–4 h after induction of anesthesia. After manually emptying the bladder, transvesical cystometry was performed at an infusion rate of 0.12 ml/min with physiological saline at room temperature (5, 26). The urethra was open, allowing elimination of fluid during micturition. The infusion pump was turned off after two or three voiding contractions. All recorded data were first amplified and sampled at 10 kHz (Biopac MP 150; BIOPAC Systems, Goleta, CA), and the recorded EUS-EMG was further filtered by a 3–3000-Hz band-pass filter.

Researchers who analyzed the IVP, EUS-EMG, and UFR were blinded to the status of the rats. Various IVP parameters were measured as previously reported (5, 23, 26): 1) the volume threshold, the minimum infused volume of saline sufficient to induce the first voiding contraction; 2) the pressure threshold, the pressure required to induce voiding contractions; 3) contraction amplitude, the maximal pressure during voiding; 4) evoked contraction amplitude, the contraction amplitude minus the pressure threshold; 5) bladder compliance, the ratio of the volume threshold to the pressure threshold; 6) residual volume, the volume of saline withdrawn through the intravesical catheter after voiding; 7) voided volume, the micturition volume threshold minus the residual volume; and 8) voiding efficiency, the ratio of the voided volume to the micturition volume threshold. In addition, as described in earlier papers (5, 25), three EUS-EMG parameters were determined, including the burst period, silent period, and active period, as shown in Fig. 1.

To analyze the UFR, several parameters were measured: 1) the maximal UFR, the maximal value of the UFR during the voiding period; 2) mean UFR of the voiding period; 3) spike duration, the duration of a spike; 4) interspike interval, the interval between two successive spikes; 5) mean UFR of the spike duration, the average value of the UFR of all spike durations; and 6) mean UFR of the interspike interval, the average value of all interspike intervals.

Drug administration. 8-OH-DAP and WAY-100635 (both from Sigma, St. Louis, MO) were dissolved in saline. To assess the roles of 5-HT1A receptors in voiding function, 8-OH-DAP (0.3 mg/kg iv) and WAY-100635 (0.1 mg/kg iv) were administered to rats at intervals of no less than 1 h. The drug dose was in accordance with dosages determined in previous studies (8, 10, 22). The first post-treatment urodynamic examinations were completed within 20–45 min following drug administration because of the short half-life of 8-OH-DAP (14, 22). To further determine the independent effect of 8-OH-DPAT or WAY-100635 on urethral smooth muscle activity, some rats received an intravenous injection of α-bungarotoxin (0.4 mg/kg, dissolved in saline) 30–40 min prior to WAY-100635 treatment. Subsequently, those rats were further treated with 8-OH-DPAT, at ~2–3 h after α-bungarotoxin administration. The effects of α-bungarotoxin generally last for 3–6 h (21). The IVP, EUS-EMG, and UFR were simultaneously recorded both before and after administration of these drugs.

Statistical analysis. This study presents all data as means ± SD. One-way ANOVA was used to compare parameters obtained from the IVP, EUS-EMG, and UFR recordings. ANOVA was followed by Tukey’s honestly significant difference post hoc test paired comparisons (SigmaStat, SPSS, Chicago, IL), and a value of P < 0.05 was considered significant in all analyses. In addition, a Pearson correlation test was used to analyze the timing relationship between recordings.

RESULTS

Typical patterns of the IVP, EUS-EMG, and UFR. Typical IVP, EUS-EMG, and UFR recordings during the continuous transvesical infusion of saline in urethane-anesthetized male rats are depicted in Fig. 1A. When the volume threshold was reached, the bladder contracted and voiding occurred. Subsequent contractions occurred at earlier times during filling than the first contraction, because the residual volume after the first contraction was added to the infused volume. The EUS exhibited low-amplitude tonic activity during the initial filling phase and between micturition contractions; meanwhile, no urine flow was detected in the UFR measurement. However, during bladder contractions, EUS activity markedly increased in amplitude, which coincided with the occurrence of a urine flow event. Detailed features of EUS-EMG activity during a single micturition contraction clearly presented a long burst period, as shown in Fig. 1B. Burst discharges in the burst period showed clusters of high-frequency EMG activity (corresponding to an active period) separated by periods of quiescence (corresponding to a silent period), as shown in Fig. 1C. In addition, the EUS burst period was accompanied by IVP and UFR, which were both superimposed upon a series of high-frequency oscillations (Fig. 1, B and C). Relationships among the IVP, EUS, and UFR recordings during the burst period were modeled by four critical IVP points, and the oscillatory IVP was classified into two waveform templates (i.e., small and large waves), as shown in Fig. 2A. Small IVP waves typically appeared at point a and lasted to point c; subsequently, a large wave continued at point c and ended at point a’. At the peak of the small wave (point b), the EUS-EMG indicated the occurrence of an active period (correlation value R = 0.87, obtained from 12 rats), and this active period usually ceased at the end of the small wave (point c; R = 0.84). The oscillatory UFR was only composed of a single waveform template, which was divided into spike durations and interspike intervals (Fig. 1C). The ascending periods of the spike duration rhythmically coincided with the appearance of the EUS active period, and correlation values of the initial and end points of this ascending period to IVP points b and c were 0.91 and 0.89, respectively. Subsequently, the descending period of spikes was generally distributed within the EUS silent period. The peak of spike durations usually coincided with the endpoint of the active period, and a high correlation between the peak and IVP point c was detected (R = 0.92).

Effects of 8-OH-DPAT and WAY-100635 on the IVP. Effects of the intravenous administration of 8-OH-DPAT (0.3 mg/kg iv) and WAY-100635 (0.1 mg/kg iv) on control of the IVP, EUS-EMG, and UFR activity were examined in anesthetized male rats (n = 12). Table 1 summarizes all IVP parameters obtained from rats before and after 5-HT drug treatment. 8-OH-DPAT significantly reduced the volume threshold and pressure threshold for inducing a reflex bladder contraction, while it significantly increased the evoked contraction amplitude (Fig. 3; Table 1). In addition, compliance of the bladder contraction, i.e., the ratio of the volume threshold to the pressure threshold, was significantly decreased by the drug, indicating an enhancement of bladder micturition reflexes.
These results suggest that the 5-HT1A receptor agonist markedly facilitated bladder emptying, and the voiding efficiency was subsequently improved from 69.4% to 85.8% \((P < 0.05; \text{Table 1})\). Conversely, treatment of rats with WAY-100635, a 5-HT1A receptor antagonist, produced effects opposite to those produced by 8-OH-DPAT (Fig. 3; Table 1). The voiding efficiency was significantly reduced from 69.4% to 35.1% \((P < 0.05)\).

Effects of 8-OH-DPAT and WAY-100635 on the EUS-EMG. The EUS exhibited low-amplitude tonic activity during the initial filling phase and between micturition contractions, while large amplitudes were detected by the EUS-EMG during the

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Fig. 1. Typical patterns of the intravesical pressure (IVP; top), external urethral sphincter-electromyogram (EUS-EMG; middle), and urine flow rate (UFR; bottom) were recorded in an anesthetized male rat. A: bladder micturition contractions were induced by a constant rate of intravesical saline infusion, which was accompanied by large amplitudes of EUS-EMG and UFR activity. Asterisks indicate micturition contractions. B: burst period (BP) in the EUS-EMG can be clearly observed in the expanded view of one micturition contraction in A. The BP was accompanied by the IVP and UFR, both of which were superimposed with a series of high-frequency oscillation waves. The UFR recording was quantified by the maximal and mean UFRs during the voiding period. C: individual EUS bursts were composed of active periods and silent periods, as shown in the expanded BP portion (bracket) in B. Oscillatory UFR waves correlated with EUS-EMG bursts, which were divided into spike durations and interspike intervals.
bladder voiding phase (Fig. 3A). Although the general pattern of the EUS-EMG during continuous cystometry was similar in rats before and after 8-OH-DPAT or WAY-100635 treatment (Fig. 3), there were marked quantifiable differences during the voiding phase. Table 2 summarizes EUS-EMG measurements in rats before and after 8-OH-DPAT or WAY-100635 treatment. Compared with control data, 8-OH-DPAT significantly increased the duration of the burst period, whereas it was markedly reduced by WAY-100635 (Fig. 4; Table 2). Similarly, 8-OH-DPAT and WAY-100635 treatment dramatically prolonged and shortened the silent period, respectively (middle traces in Fig. 5; Table 2). However, the active period was not significantly affected by WAY-100635 or 8-OH-DPAT treatment in rats.

Table 1. Effects of 8-OH-DPAT (0.3 mg/kg iv) and WAY-100635 (0.1 mg/kg iv) on the intravesical pressure during continuous cystometry in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume Threshold, ml</th>
<th>Pressure Threshold, cmH2O</th>
<th>Contraction Amplitude, cmH2O</th>
<th>Evoked Contraction Amplitude, cmH2O</th>
<th>Bladder Compliance, ml/cmH2O</th>
<th>Voided Volume, ml</th>
<th>Residual Volume, ml</th>
<th>Voiding Efficiency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.27 ± 0.18</td>
<td>17.21 ± 1.09</td>
<td>43.63 ± 1.10</td>
<td>26.42 ± 0.73</td>
<td>0.73 ± 0.08</td>
<td>0.88 ± 0.15</td>
<td>0.39 ± 0.09</td>
<td>69.4 ± 5.2</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>0.66 ± 0.08*</td>
<td>12.33 ± 1.11*</td>
<td>42.12 ± 1.30</td>
<td>29.79 ± 0.78*</td>
<td>0.54 ± 0.09*</td>
<td>0.57 ± 0.09*</td>
<td>0.09 ± 0.03*</td>
<td>85.8 ± 4.9*</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>1.84 ± 0.15*</td>
<td>22.72 ± 1.61*</td>
<td>46.69 ± 1.76*</td>
<td>23.97 ± 1.06*</td>
<td>0.82 ± 0.04*</td>
<td>0.65 ± 0.18</td>
<td>1.20 ± 0.21*</td>
<td>35.1 ± 10.0*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n = 12 male rats. 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)-tetralin. *Statistically significant difference from the control value, P < 0.05. Bladder compliance is the ratio of the volume threshold to the pressure threshold.

Fig. 2. Characteristics of the IVP (top), EUS-EMG (middle), and UFR (bottom) during the EUS burst period. Traces in B are expansions of traces in the dashed-line box in A. The oscillatory IVP was composed of small and large waves, which were characterized by four critical pressure points a to d. The small IVP wave typically ranged from points a to c, and subsequently the large wave ranged from points c to a'. At the peak of the small wave (point b), the initiation of EUS occurred in an active period (AP) of the EUS-EMG; and the AP ended at point c. In the meantime, the UFR measurement exhibited a rapidly increasing flow rate during the AP. Note that the peak of the ascending UFR was exactly located at the endpoint of the AP or IVP point c, and at the IVP point c, the UFR was immediately transformed into a descending curve that was distributed within the EUS silent period.
Effects of 8-OH-DPAT and WAY-100635 on the UFR. Cystometric results showed that the appearance of UFR events in rats after 8-OH-DPAT or WAY-100635 treatment remained synchronized with the occurrence of the EUS burst period, as shown in bottom traces in Figs. 3 and 4. Several UFR parameters were obtained from rats both before and after 5-HT drug treatment, as tabulated in Table 3. Experimental results showed that the maximal UFR and mean UFRs of the voiding period, spike duration, and interspike interval were all decreased by 8-OH-DPAT, which implied that the UFR was as a whole reduced by the 5-HT1A receptor agonist. However, the total length of oscillatory flow was markedly elongated by the drug, which was approximately the same length as its corresponding EUS burst period (Fig. 4). Importantly, 8-OH-DPAT significantly reduced the spike duration, whereas the drug significantly increased the interspike interval (lower traces in Fig. 5B;
increased to 12.8 while significant urine flow activity reemerged during reflex volume threshold for inducing reflex bladder contractions, WAY-100635 was administered to rats 30–40 min after significant UFR activity was detected during transvesical cystometry, but EUS-EMG and UFR activities were both nearly completely eliminated (Fig. 6B). The voiding efficiency during continuous cystometry in rats after neuromuscular blockade treatment was reduced from 12.8% in rats after 8-OH-DPAT to 1.3% in rats after WAY-100635 treatment, and subsequent 8-OH-DPAT during voiding would largely improve bladder contractility and decrease the urethral cross-sectional diameter or area, thereby reducing the UFR during voiding. In addition, reduction of the volume threshold might have resulted from the 5-HT1A receptor agonist facilitating the micturition switching circuit in the pontine micturition center (20, 40) or enhancing bladder afferent input or afferent processing in the spinal cord (3, 22). In this way, reduction of the volume threshold accompanied by increased bladder contractions by 8-OH-DPAT during voiding would largely improve bladder emptying.

The urethral smooth muscle activity modulated by 5-HT drug possibly accounts for the reduction in the UFR. According to Poiseuille’s law (1), the rate and resistance at which a fluid flows through a pipe are positively and inversely proportional to the pipe diameter raised to the fourth power, respectively. Although the urethra is more complex, as the urethra is not a rigid pipe and has elastic components, the urethral cross-sectional diameter or area remains a critical factor dominating the UFR. Our recent study demonstrated that 8-OH-DPAT significantly elevated the urethral pressure during voiding in male rats after neuromuscular blockade treatment (15), which implies that 8-OH-DPAT may facilitate urethral smooth muscle contractions and decrease the urethral cross-sectional diameter, thereby reducing the UFR during voiding. In addition, in the present study, we further proved that WAY-100635 significantly increased the voiding efficiency from 0.7% to 12.8% in rats after α-bungarotoxin treatment, and subsequent 8-OH-DPAT treatment again reduced the voiding efficiency to close to 0% (Fig. 6). Thus, the combined data suggested that

Table 2. Effects of 8-OH-DPAT (0.3 mg/kg iv) and WAY-100635 (0.1 mg/kg iv) on external urethral sphincter-electromyography activity during continuous cystometry in male rats

<table>
<thead>
<tr>
<th></th>
<th>Burst Period, s</th>
<th>Silent Period, ms</th>
<th>Active Period, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.6 ± 0.6</td>
<td>108 ± 5.5</td>
<td>35.7 ± 3.4</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>11.3 ± 1.3*</td>
<td>155.0 ± 9.7*</td>
<td>37.7 ± 3.6</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>3.2 ± 0.3*</td>
<td>85.0 ± 3.2*</td>
<td>36.2 ± 3.7</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n = 12 male rats. *Statistically significant difference from the control value; P < 0.05. Silent and active periods denote the average durations of quiescent and tonic external urethral sphincter-electromyography activities during the burst period shown in Fig. 1.

Table 3). Conversely, opposite effects were detected in rats after WAY-100635 treatment.

Characteristics of micturition contractions during the burst period. Relationships among IVP, EUS-EMG, and UFR during the EUS burst period in rats after 5-HT drug treatment were examined in detail, as shown in Fig. 5. Similar to the control IVP, four critical pressure points in the oscillatory pattern of IVP in rats after 8-OH-DPAT were still readily detected, in which the oscillatory IVP was divided into small and large waves. However, WAY-100635 markedly altered the oscillatory pattern to a single-wave pattern (Fig. 5C), and the four critical IVP points that appeared in the control data were not recognized in any micturition contractions (n = 72 of 72 contractions across 12 rats). The initial point of the EUS active period in the control condition was approximately at the IVP point b, but this timing relationship was altered in rats after 8-OH-DPAT, in that point b was detected earlier than the appearance of the active period (n = 72 of 72 voiding reflexes across 12 rats; Fig. 5B). Interestingly, the spike duration was longer than its interspike interval in the control condition, whereas, after 8-OH-DPAT treatment, the interspike duration became longer than its spike duration (Table 3; Fig. 5).

Effects of 5-HT drug treatment in rats after neuromuscular blockade. Our results demonstrated that the UFR was reduced by 8-OH-DPAT but was elevated by WAY-100635 treatment (Table 3). To further determine the independent roles of urethral striated and smooth muscles in the UFR and voiding efficiency in rats after 5-HT drug treatment, some rats (n = 6) were treated with α-bungarotoxin. Figure 6 shows examples of the independent effects of WAY-100635 and 8-OH-DPAT during continuous cystometry in rats after α-bungarotoxin treatment. After neuromuscular blockade treatment, spontaneous reflex bladder contractions still appeared during transvesical cystometry, but EUS-EMG and UFR activities were both nearly completely eliminated (Fig. 6B). The voiding efficiency in rats after α-bungarotoxin treatment was reduced from 70.1 ± 7.2% to 0.7 ± 0.5% (P < 0.05). Subsequently, WAY-100635 was administered to rats 30–40 min after α-bungarotoxin treatment. The drug significantly increased the volume threshold for inducing reflex bladder contractions, while significant urine flow activity reemerged during reflex bladder contractions, and the voiding efficiency significantly increased to 12.8 ± 7.4% (P < 0.05). After the same rat was further treated with 8-OH-DPAT at ~2.5 h after α-bungarotoxin treatment, reflex bladder contractions still existed, but no significant UFR activity was detected during transvesical cystometry (Fig. 6D), and the voiding efficiency was again reduced to close to the zero level (0.4 ± 0.3%).

DISCUSSION

This study shows that activation of 5-HT1A receptors in male rats with 8-OH-DPAT, a 5-HT1A agonist, generally reduced the UFR during micturition contractions, as evidenced by significant decreases in the maximal UFR and mean UFRs of the voiding period, spike duration, and interspike interval (Table 3). However, the voiding efficiency during continuous cystometry was not reduced but was improved by the drug (Fig. 4; Table 1). Conversely, treatment of rats with WAY-100635, a 5-HT1A antagonist, produced effects opposite to those produced by 8-OH-DPAT. Although numerous studies (2, 5, 6, 8, 18) reported that 8-OH-DPAT significantly improved the voiding function in female rats, to the best of our knowledge, our study is the first to report on the effects of the 5-HT drug on regulating the UFR in male rats.

One important question emerging from this study is why 8-OH-DPAT significantly improved the voiding efficiency but the UFR during voiding was substantially reduced rather than increased by the drug. This contradiction can be explained by three possibilities, including 1) enhanced bladder reflex activity, 2) facilitated urethral smooth muscle contractions, and 3) altered the pattern of urethral striated muscle activity during the voiding period by 8-OH-DPAT treatment. First, the enhanced voiding efficiency by 8-OH-DPAT was attributed to enhancement of the bladder reflex activity, as evidenced by an increase in the bladder-evoked contraction amplitude and a decrease in the volume threshold for induction of bladder contractions by the drug (Table 1). The increase in the evoked contraction amplitude represents improvements in the bladder contractile ability for bladder evacuation during voiding. In addition, reduction of the volume threshold might have resulted from the 5-HT1A receptor agonist facilitating the micturition switching circuit in the pontine micturition center (20, 40) or enhancing bladder afferent input or afferent processing in the spinal cord (3, 22). In this way, reduction of the volume threshold accompanied by increased bladder contractions by 8-OH-DPAT during voiding would largely improve bladder emptying.

The urethral smooth muscle activity modulated by 5-HT drug possibly accounts for the reduction in the UFR. According to Poiseuille’s law (1), the rate and resistance at which a fluid flows through a pipe are positively and inversely proportional to the pipe diameter raised to the fourth power, respectively. Although the urethra is more complex, as the urethra is not a rigid pipe and has elastic components, the urethral cross-sectional diameter or area remains a critical factor dominating the UFR. Our recent study demonstrated that 8-OH-DPAT significantly elevated the urethral pressure during voiding in male rats after neuromuscular blockade treatment (15), which implies that 8-OH-DPAT may facilitate urethral smooth muscle contractions and decrease the urethral cross-sectional diameter, thereby reducing the UFR during voiding. In addition, in the present study, we further proved that WAY-100635 significantly increased the voiding efficiency from 0.7% to 12.8% in rats after α-bungarotoxin treatment, and subsequent 8-OH-DPAT treatment again reduced the voiding efficiency to close to 0% (Fig. 6). Thus, the combined data suggested that
urethral smooth muscle activity plays an important role in regulating the UFR in male rats.

On the other hand, alteration of urethral striated muscle activity is another possible explanation of this contradiction. 8-OH-DPAT significantly prolonging the EUS silent period could be another factor reducing the UFR. The EUS burst period, which represents the relaxation and opening of the outlet, is essential to achieve efficient voiding (9, 11) in rats, and, particularly, urine release is mainly promoted by phasic EUS contractions (active period). In the present study, 8-OH-DPAT markedly prolonged the duration of EUS relaxation (silent period) but did not alter the active period (Table 2). Therefore, the longer silent period would decrease the average UFR of the burst period. Contrarily, the total EUS burst period was significantly prolonged to ~2-fold that of the control by 8-OH-DPAT (Fig. 4, Table 2). This long EUS burst period...
represents a longer duration of voiding for emptying bladder urine, which would largely compensate for the lower UFR caused by urethral smooth muscle contractions and longer silent periods. Taken together, a decreased bladder volume threshold, an enhanced detrusor contractile ability, and a lengthened EUS burst period induced by activation of 5-HT receptors would all contribute to significantly improving the voiding efficiency regardless of the lower UFR during voiding.

In the present study, administration of 8-OH-DPAT in male rats significantly reduced the bladder volume threshold, increased the detrusor contractile ability, prolonged the durations of the EUS burst and active periods, and subsequently improved the voiding efficiency, which were all consistent with results in females (2, 5, 8, 18). EUS burst activity is necessary for efficient bladder emptying in rats; this assertion was demonstrated by the EUS paralysis with α-bungarotoxin signif-

Fig. 5. A: effects of 8-OH-DPAT and WAY-100635 on relationships of the IVP (top), EUS-EMG (middle), and UFR (bottom) during the EUS burst period. Four critical pressure points in the oscillatory IVP in the rat after 8-OH-DPAT treatment (B) were still readily detected, which was similar to results of the control A; however, WAY-100635 significantly altered the basic pattern of the oscillatory IVP (C), and therefore, it was difficult to distinguish the four critical pressure points observed in the control IVP. Note that 8-OH-DPAT also significantly increased the silent period of the EUS-EMG and the interspike interval of the UFR, but WAY-100635 produced effects opposite to those produced by 8-OH-DPAT.
cantly reducing the voiding efficiency from ~77–65% to ~30–50% in female rats (8, 26). Interestingly, in the present study, almost no urine was released during continuous cystometry by male rats under a condition of EUS paralysis, and the average voiding efficiency was dramatically reduced from 70.1% to ~0% (Fig. 6B). These findings imply that the lack of EUS burst activity seemed to have a larger impact on the voiding efficiency in male than that in female rats. This difference could be attributed to the fact that some sexual differences exist in the urethral anatomy and function (11, 15, 27, 29, 41). For example, male rats generally have a longer urethra and thicker EUS overlaying the urethra compared with female rats (11, 27, 29), and the bladder outlet resistance in male rats is significantly higher than that in female rats (15).

With these sexual differences, male rats would be expected to need a higher evacuation energy to produce efficient bladder emptying. It is noteworthy that 8-OH-DPAT significantly elongated the length of the EUS silent period, but the amplitudes or durations of individual oscillatory waves (spike duration) in the UFR did not concomitantly increase (Fig. 5B). Meanwhile, although the interspike interval was simultaneously prolonged during the long EUS active period (Fig. 5B; Table 3), the mean flow rate of this interval was not elevated by the drug, but dropped to close to the zero level, indicating that almost no urine flow occurred during the long interspike interval. This phenomenon can be explained by the urethral smooth muscle contraction being activated by 8-OH-DPAT, in which the urethral resistance for urine evacuation might have been elevated and thus only induced a short-term duration of urine flow (a short spike duration; Table 3). Conversely, WAY-100635 dramatically relaxed the urethral smooth muscle, which was accompanied by a short EUS silent period (Fig. 5C) and led to a high speed of continuous oscillatory urine flow during EUS burst activity (Fig. 4C). Thus, all of these results demonstrated that, in male rats, the short duration of oscillatory waves (short spike durations) in the UFR was mainly associated with a urethral smooth muscle contraction induced by 8-OH-DPAT.

For UFR measurements, a discontinuous-like pattern of urine flow was detected, and this characteristic of UFR recordings was similar to that in previous studies by Streng et al. (30, 32, 33). However, the pattern of EUS-EMG in our study completely differed from that of Streng et al. This is because the approach used for EUS-EMG recording by Streng et al. (30, 32) was a monopolar suction electrode method, and that in our study, the approach was a bipolar wire electrode method. This discrepancy made it difficult to compare EUS-EMG data from our and previous studies. However, the bipolar wire approach compared with the monopolar approach commonly has a more restricted recording area and directional sensitivity of the motor unit of action potentials from the background but is more likely to be affected by movement artifact noises and usually requires further processing via a digital filter. In the present study, a 3–3,000-Hz band-pass filter was used to reduce artifact noises, which should not have significantly altered the properties of the original EUS-EMG signal. This is because the frequency of the EUS-EMG signal in rats is mainly located at 200–600 Hz (4), and, thus, this band-pass filter should not have distorted the original EUS-EMG signals. Hence, our bipolar recording approach should be a useful method for observing fine details of EUS activity during voiding.

Relationships of the IVP and UFR with EUS activity were also examined in rats before and after drug treatment. From our EUS-EMG recordings, the exact timing of the EUS contraction and relaxation was readily distinguished by an active period and a silent period, respectively. Interestingly, the initial point of the EUS active period coincided with the starting point of the UFR spike duration (Fig. 5A), while the endpoint of the active period was usually located at the peak of the spike duration or the IVP point c. Note that these timing relationships were not altered by administration of 5-HT receptor agonist or antagonist (Fig. 5, B and C), which suggests that phasic EUS contractions are one of the main mechanisms producing the energy (force) that initiates oscillatory urine flow.

One limitation of the present study was the use of rats to investigate LUT functions. Although rats are widely used for physiological and pharmacological studies of the LUT (13), rats (and dogs) exhibit phasic patterns of EUS activity during voiding, in contrast to the (complete) relaxation of the sphincter observed in humans (and cats). Phasic EUS activation is essential to efficient voiding in rats (26), as evidenced by the present results of EUS paralysis with α-bungarotoxin significantly reducing the voiding efficiency. However, relaxation of the EUS promotes complete bladder emptying in humans. Therefore, it is not clear whether the present results that 5-HT1A activation altered the flow rate and voiding efficiency by increasing bladder contractility and smooth muscle-mediated urethral contractions will translate to humans (males), where the neural control of EUS activity differs from that in the rat.

**Perspectives and Significance**

Serotonin may have different functions in the central nervous control of the LUT due to species differences. For example, in cats, 5-HT receptor activation by 8-OH-DPAT inhibits bladder activity and facilitates EUS continence activity under conditions of a bladder irritated with acetic acid (37). Contrarily, in rats, the drug has a facilitative effect on bladder activity and promotes emptying EUS activity (5). Several studies indicated that bulbospinal projections from raphe neurons in the brain stem might mediate inhibitory
Fig. 6. Independent effects of 8-OH-DPAT and WAY-100635 on the IVP (top), EUS-EMG (middle), and UFR (bottom) during continuous cystometry in rats under a condition of paralyzed urethral striated muscles. The voiding efficiency (VE) was measured in the control condition (A), following administration of α-bungarotoxin (0.4 mg/kg iv; B), following WAY-100635 treatment at ~0.5 h after administration of α-bungarotoxin (C), and following 8-OH-DPAT treatment at ~2.5 h after administration of α-bungarotoxin (D).
control over voiding function (7, 12, 34). 5-HT1A autoreceptors are involved in negative feedback control of 5-HT release from raphe neurons (16, 17, 21). In rats, administration of WAY-100635 increases the firing rate of raphe neurons by blocking 5-HT1A inhibitory autoreceptors, and the 5-HT is largely secreted onto 5-HT2C receptors, which activate an inhibitory interneuron to inhibit the parasympathetic drive to the bladder (12, 28). However, how the spinal 5-HT1A receptor pathway integrates into this postulated pathway remains unknown; and thus, modulation of LUT functions by 8-OH-DPAT or WAY-100635 is generally considered to be mediated through 5HT1A autoreceptors located in the raphe nucleus in rats. In cats, studies indicated that serotonin acts at various sites in the spinal cord to modulate LUT functions, including direct inhibition of parasympathetic preganglionic neurons and suppression of the processing of afferent input from the bladder, and suppression of inhibitory interneuronal input to sphincter motoneurons (12, 36). Thus, in contrast with the serotonin mechanism in the brain stem of rats, direct activation of inhibitory 5HT1A postsynaptic receptors may be more involved in the control of micturition in cats. To date, it is not clear whether that is the case in the serotonin control of LUT in humans due to limited clinical data. Nevertheless, the serotonergic mechanism in controlling LUT functions in humans warrants further exploration.

**REFERENCES**


