Role of 5-HT<sub>1A</sub> receptors in control of lower urinary tract function in anesthetized rats

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Cheng C, de Groat WC. Role of 5-HT<sub>1A</sub> receptors in control of lower urinary tract function in anesthetized rats. Am J Physiol Renal Physiol 298: F771–F778, 2010. First published December 30, 2009; doi:10.1152/ajprenal.00266.2009.—The role of 5-hydroxytryptamine (5-HT) 1A (5-HT<sub>1A</sub>) receptors in lower urinary tract function was examined in urethane-anesthetized female Sprague-Dawley rats. Bladder pressure and the external urethral sphincter electromyogram (EUS EMG) activity were recorded during continuous-infusion transvesical cystometrograms (TV-CMGs) to allow voiding and during transurethral-CMGs (TU-CMGs) which prevented voiding and allowed recording of isovolumetric bladder contractions. 8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a 5-HT<sub>1A</sub> receptor agonist, decreased volume threshold (VT) for initiating voiding and increased contraction amplitude (CA) during TV-CMGs but decreased CA during TV-CMGs. 8-OH-DPAT prolonged EUS bursting as well as the intrabursting silent periods (SP) during voiding. N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamine trihydrochloride (WAY-100635), a 5-HT<sub>1A</sub> antagonist, increased VT, increased residual volume, markedly decreased voiding efficiency, decreased the amplitude of micturition contractions recorded under isovolumetric conditions, and decreased the SP of EUS bursting. These results indicate that activation of 5-HT<sub>1A</sub> receptors by endogenous 5-HT lowers the threshold for initiating reflex voiding and promotes voiding function by enhancing the duration of EUS relaxation, which should reduce urethral outlet resistance.

micturition; external urethral sphincter; urinary bladder; electromyogram; serotonin

The localization of nerve terminals containing serotonin (5-hydroxytryptamine; 5-HT) in sympathetic, parasympathetic (6), and urethral sphincter motor nuclei (27) in the lumbosacral spinal cord has focused attention on the role of 5-HT as a neurotransmitter in the central neural pathways controlling lower urinary tract function (9, 11, 25, 28). Pharmacological studies in animals have revealed that administration of 5-HT receptor agonists or antagonists modulates reflex bladder and urethral sphincter activity (1, 2, 12, 14, 15, 17, 19, 20, 30–32, 34, 36). These effects are mediated by activation of multiple 5-HT receptors, which are distributed at various sites in the central pathways controlling the lower urinary tract (9, 12, 16, 21, 28, 29, 37).

The effect of 5-HT on bladder and sphincter activity varies in different species. For example, in the cat, activation of 5-HT<sub>1A</sub> receptors inhibits the parasympathetic excitatory input to the urinary bladder (14, 15, 27, 31, 36). On the other hand, in the rat, activation of 5-HT<sub>1A</sub> receptors enhances reflex activity of the bladder and sphincter (1, 2, 12, 20), whereas block of 5-HT<sub>1A</sub> receptors suppresses bladder and sphincter activity, suggesting that endogenous 5-HT exerts a tonic influence on the neural pathways controlling the lower urinary tract (1, 9, 17, 19, 26, 32).

In rats, 5-HT<sub>1A</sub> receptors may regulate urine storage as well as voiding by facilitating two types of external urethral sphincter (EUS) activity (1, 2, 12). Administration of a 5-HT<sub>1A</sub> agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), enhances tonic EUS activity that occurs during bladder filling. This would be expected to promote urinary continence. 8-OH-DPAT also enhances bursting activity, which should promote efficient voiding. In rats with an intact neuraxis, the facilitation of tonic EUS activity is due to activation of 5-HT<sub>1A</sub> receptors in the spinal cord, whereas the facilitation of bursting activity is dependent on supraspinal pathways involving the pontine micturition center (2). The facilitation of EUS bursting is eliminated by acute transection of the thoracic spinal cord but recovers in chronic spinal cord-injured rats presumably due to reorganization of voiding reflexes in spinal segments caudal to the injury (2).

The present experiments, which were conducted in rats with an intact neuraxis, utilized simultaneous recordings of intravesical pressure and EUS electromyographic (EMG) activity to evaluate the contribution of 5-HT<sub>1A</sub> receptors to bladder-EUS coordination and voiding efficiency. Receptors were activated or blocked, respectively, by systemic administration of 8-OH-DPAT, a receptor agonist, or WAY-100635, a 5-HT<sub>1A</sub> receptor antagonist. The results indicate that activation of 5-HT<sub>1A</sub> receptors by endogenous 5-HT decreases the micturition volume threshold and produces changes in EUS EMG activity that facilitate voiding.

MATERIALS AND METHODS

General preparation. Fifty-three female Sprague-Dawley rats (weight 230–300 g) were used in this study. The animals were anesthetized with a subcutaneous injection of urethane (1.2 g/kg). The trachea was cannulated to facilitate respiration. The femoral vein was catheterized for fluid and drug administration. Body temperature was maintained between 36 and 38°C by a heat lamp. Most animals breathed spontaneously; however, a few were artificially ventilated after intravenous injection of pancuronium bromide (Organon), a neuromuscular blocking agent, which was used to block EUS activity during the experiment.

Transvesical cystometrograms. The urinary bladder was exposed via a midline abdominal incision. Two fine insulated silver wire electrodes (0.05-mm diameter) with exposed tips were inserted into lateral sides of the midurethra, where muscle fibers of the EUS were identified. The recorded EUS EMG was attributed to striated muscle because the activity was eliminated after neuromuscular blockade with pancuronium bromide (3). A polyethylene (PE) tube (1.0-mm inner diameter) was inserted into the bladder via a transvesical route and connected to a 300-ml calibration bag for_TV-CMGs. A 0.5-mm PE catheter was inserted into the urethra for TU-CMGs.
Veterans General Hospital, approved the protocol used in this study.Shown to affect bladder activity but not blood pressure in the rat (5).

WAY-100635 was administered 1 h after vehicle. Doses of drugs the effect of this drug had worn off before the administration of the short half-life of 8-OH-DPAT in the rat (12, 20), it is assumed that and WAY-100635 (0.1 mg/kg iv) at intervals of at least 1 h. Based on course of this study.

MO) were dissolved in saline. Drug solutions were administered after of 15 cmH2O was defined as the micturition volume threshold. Three saline sufficient to induce bladder contractions exceeding a pressure of 15 cmH2O; 4) residual volume, the volume of saline withdrawn through the intravesical catheter after voiding; and 5) voiding efficiency, the ratio between voiding volume (volume threshold minus residual volume) and volume threshold. The EUS EMG activity analysis was blinded to the status of the rats. As described in an earlier paper (4), various EUS EMG parameters were measured including average interval of the bursting duration, silent period, active period, total silent period in each voiding, and the ratio of bursting duration to contraction duration. All parameters were calculated with the aid of Acknowledge software (Biopac Systems). Computed data were compiled in spreadsheets using Excel (Microsoft). EUS EMG activity was displayed on a storage oscilloscope and recorded on VCR tape and a paper recorder along with bladder pressure.

Transurethral CMG. A PE tube (0.76-mm inner diameter, 1.22-mm outer diameter) was inserted into the bladder through the urethra and tied in place by a ligature around the urethral orifice. By means of a three-way stopcock, the catheter was connected to a pressure transducer to record the bladder pressure isovolumetrically and to a syringe for bladder infusion. The catheter system was filled with 0.9% saline. After emptying of the bladder, a CMG was performed by filling with a constant infusion (0.123 ml/min) of saline. The infusion pump was turned off after the onset of rhythmic bladder contractions. For isovolumetric recording, ureters were tied distally, cut, and the proximal stumps were cannulated and drained externally. The volume of saline sufficient to induce bladder contractions exceeding a pressure of 15 cmH2O was defined as the micturition volume threshold. Three or four CMGs were performed in each animal after vehicle or each drug.

Drugs. 8-OH-DPAT and WAY-100635 (both from Sigma, St. Louis, MO) were dissolved in saline. Drug solutions were administered after control recordings and vehicle administration just before the start of a CMG. Two different experimental protocols were employed in the course of this study. Protocol 1 was conducted with drugs administered in the following sequence: vehicle, 8-OH-DPAT (0.3 mg/kg iv), and WAY-100635 (0.1 mg/kg iv) at intervals of at least 1 h. Based on the short half-life of 8-OH-DPAT in the rat (12, 20), it is assumed that the effect of this drug had worn off before the administration of WAY-100635. However, this was confirmed by testing WAY-100635 (0.1 mg/kg iv) in the absence of 8-OH-DPAT in protocol 2, where WAY-100635 was administered 1 h after vehicle. Doses of drugs were selected based on results of previous experiments (5, 19). The dose of 8-OH-DPAT was selected to produce a consistent enhancement of bladder activity, while the dose of WAY-100635 has been shown to affect bladder activity but not blood pressure in the rat (5).

The Institutional Animal Care and Use Committee, Taichung Veterans General Hospital, approved the protocol used in this study.

Statistical analysis. The results are given as means ± SE. For comparisons between values obtained before and after drug administration, one-way ANOVA was used for comparisons between vehicle and 5-HT1A agonist or antagonist treatment and was followed by a post hoc least significance difference test. P < 0.05 was considered statistically significant.

RESULTS

Effect of 8-OH-DPAT and WAY-100635 on bladder activity during transvesical CMGs. With the urethra outlet open, to allow intravesical fluid to be evacuated during voiding, various parameters of bladder activity were assessed, including volume threshold, contraction amplitude, contraction duration, postvoid residual volume, and voiding efficiency before and after serial injection of vehicle, 8-OH-DPAT (0.3 mg/kg iv), and WAY-100635 (0.1 mg/kg iv). Vehicle did not significantly change any parameters. In protocol 1, volume threshold and contraction amplitude were significantly decreased (P < 0.05) by 20–30% after administration of 8-OH-DPAT (Fig. 1B), while contraction duration, residual volume and voiding efficiency were not significantly changed. WAY-100635 administered after 8-OH-DPAT significantly increased volume threshold (Fig. 1C), residual volume, and decreased voiding efficiency beyond the vehicle control levels (Fig. 1A, Table 1). In protocol 2, WAY-100635 was administered following vehicle administration. WAY-100635 significantly increased volume threshold and residual volume, and decreased voiding efficiency as noted in experiments performed using protocol 1 (Table 2).

Effect of 8-OH-DPAT and WAY-100635 on EUS EMG activity during transvesical CMGs. During continuous infusion CMGs before the onset of voiding, two types of EUS EMG activity were detected (Figs. 1 and 2). Most animals exhibited consistent, low amplitude, tonic EUS EMG activity during the filling phase; and in some rats, this tonic activity increased gradually as the infusion volume approached the micturition volume threshold. Just prior to voiding, phasic EUS EMG activity was noted in some experiments (Fig. 2C). During a bladder contraction, the EUS EMG activity markedly increased and consisted of an initial period of tonic activity followed by a bursting pattern of activity characterized by clusters of high-frequency spikes (active periods) and separated by periods of quiescence (silent periods) (Fig. 2). After vehicle treatment, the total interval of the average bursting duration was 4.60 ± 1.45 s, and the average silent period was much longer (0.14 ± 0.03 s) than the average active period (0.07 ± 0.01 s).

Although the basic pattern of EUS EMG activity during micturition was similar after either 8-OH-DPAT or WAY-100635 treatment, there were marked quantitative differences. 8-OH-DPAT enhanced tonic EUS EMG activity between voids (Figs. 1B and 3B), and WAY-100635 suppressed this activity (Figs. 1C and 3C). 8-OH-DPAT markedly increased the average silent period (0.23 ± 0.06 vs. 0.14 ± 0.03 s, P < 0.05) and total bursting duration (7.75 ± 1.54 vs. 4.60 ± 1.45 s, P < 0.05) and the total silent period (5.75 ± 1.31 vs. 3.03 ± 1.01 s, P < 0.05), but did not affect the duration of the average active period (0.08 ± 0.01 vs. 0.07 ± 0.01 s) (Table 2, Fig. 3B). WAY-100635 shortened the average silent period (0.10 ± 0.02 s) but did not significantly change other parameters (Fig. 3C). The percentage of the time during a bladder contraction (indicated by contraction duration) occupied by EUS EMG bursting (i.e., duration of the bursting duration/contraction duration) was in-
creased (~33%) but not significantly after 8-OH-DPAT treatment (Table 3). The effects of WAY-100635 were similar in experiments using protocols 1 and 2 where the drug was administered after 8-OH-DPAT and vehicle, respectively (Table 4).

Effect of pancreuronium bromide on bladder activity during transvesical CMGs. Administration of pancreuronium totally suppressed EUS EMG activity and altered CMG parameters presumably by eliminating the EUS regulation of the urethral

Table 1. Parameters of bladder activity in transvesical CMGs after serial administration of vehicle, 8-OH-DPAT, and WAY-100635 in urethane-anesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>VT, ml</th>
<th>CA, cmH2O</th>
<th>CD, min</th>
<th>RV, ml</th>
<th>VE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 18)</td>
<td>0.73 ± 0.14</td>
<td>32.92 ± 6.38</td>
<td>0.40 ± 0.10</td>
<td>0.17 ± 0.05</td>
<td>71.6 ± 12.9</td>
</tr>
<tr>
<td>Agonist (8-OH-DPAT; 0.3 mg/kg)</td>
<td>0.52 ± 0.13*</td>
<td>26.66 ± 4.89*</td>
<td>0.47 ± 0.11</td>
<td>0.06 ± 0.03</td>
<td>86.1 ± 10.6</td>
</tr>
<tr>
<td>Antagonist (WAY-100635; 0.1 mg/kg)</td>
<td>1.55 ± 0.36†</td>
<td>36.74 ± 6.47†</td>
<td>0.36 ± 0.10†</td>
<td>1.05 ± 0.31†</td>
<td>29.3 ± 22.9*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; CMGs, cystometrograms; VT, volume threshold; CA, contraction amplitude; CD, contraction duration; RV, residual volume; VE, voiding efficiency = VT – RV/VT; *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (post hoc least significance difference test). †P < 0.05 indicates a statistically significant difference compared with 8-OH-DPAT treatment (post hoc least significance difference test).
outlet. After injection of pancuronium, the volume threshold and residual volume increased and voiding efficiency significantly decreased (60%) even though contraction amplitude and contraction duration were not changed (Table 5). In these animals, 8-OH-DPAT decreased volume threshold and residual volume but did not significantly change voiding efficiency. WAY-100635 increased volume threshold and residual volume and further impaired voiding efficiency (Table 5).

## Effect of 8-OH-DPAT and WAY-100635 on bladder activity during transurethral CMGs.

Various parameters of the bladder activity, including basal pressure, volume threshold, contraction amplitude, contraction duration, and intercontraction intervals, were measured during transurethral CMGs. Table 2 shows the parameters of bladder activity in transvesical CMGs after vehicle and WAY-100635 treatment in urethane-anesthetized rats.

### Table 2. Parameters of bladder activity in transvesical CMGs after vehicle and WAY-100635 treatment in urethane-anesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>VT, ml</th>
<th>CA, cmH₂O</th>
<th>CD, min</th>
<th>RV, ml</th>
<th>VE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 11)</td>
<td>0.42 ± 0.14</td>
<td>31.45 ± 3.18</td>
<td>0.39 ± 0.10</td>
<td>0.11 ± 0.04</td>
<td>73.5 ± 7.8</td>
</tr>
<tr>
<td>Antagonist (WAY-100635: 0.1 mg/kg)</td>
<td>0.98 ± 0.24*</td>
<td>33.40 ± 5.01</td>
<td>0.41 ± 0.08</td>
<td>0.62 ± 0.35*</td>
<td>39.2 ± 22.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (post hoc least significance difference test).

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Fig. 2. Bladder (top traces) and EUS EMG activity (bottom traces) recorded during a continuous-transvesical infusion CMG in an anesthetized rat. A: intravesical pressure and EUS EMG activity were relatively stable during the filling phase. A reflex bladder contraction, indicated by an abrupt, large increase in bladder pressure, was accompanied by large-amplitude EUS EMG activity. B: same recording indicated by asterisk in A shown at faster time scale. The bracket in B indicates the recording period in C, and the bracket in C indicates the recording period in D at a faster time scale. Note the decline in intravesical pressure during EUS EMG bursting in B and C, which indicates the period of voiding. C: tonic EUS EMG activity precedes the large rise in intravesical pressure and shifts to a bursting pattern at the peak of bladder contraction before the onset of voiding. Small oscillations in intravesical pressure coincide with each burst of EUS EMG activity. D: recordings in C shown at very fast time scale showing individual EUS EMG bursts composed of active (AP) and silent periods (SP; brackets) and the small fluctuations in intravesical pressure accompanying each burst. Vertical calibration, intravesical pressure (in cmH₂O); horizontal calibration, time (in minutes or seconds); Inf, start of saline infusion.
interval were evaluated after infusing of saline into the bladder under isovolumetric conditions to elicit rhythmic bladder contractions (n = 12). Because the urethral outlet was ligated, voiding efficiency and postvoid residual volume were not measured in these animals. The average volume threshold for evoking a micturition reflex significantly decreased (60%) after 8-OH-DPAT injection, but increased (55%) after WAY-100635 (Table 6). 8-OH-DPAT significantly increased contraction amplitude (Fig. 4B), and WAY-100635 decreased the amplitude to a level below the vehicle control (Fig. 4C, Table 4). The intercontraction interval was slightly but not significantly reduced by 8-OH-DPAT (Fig. 4B) but significantly increased by 50% after WAY-100635 administration (Fig. 4C, Table 6). Basal pressure was unaffected by the different treatments.

Table 3. Parameters of EUS EMG activity after serial administration of vehicle, 8-OH-DPAT, and WAY-100635 in urethane-anesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>SP, s</th>
<th>AP, s</th>
<th>BD, s</th>
<th>TSP, s</th>
<th>BD/CD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 18)</td>
<td>0.14 ± 0.03</td>
<td>0.07 ± 0.01</td>
<td>4.60 ± 1.45</td>
<td>3.03 ± 1.01</td>
<td>21.05 ± 10.21</td>
</tr>
<tr>
<td>Agonist (8-OH-DPAT; 0.3 mg/kg)</td>
<td>0.23 ± 0.06*</td>
<td>0.08 ± 0.01</td>
<td>7.75 ± 1.54*</td>
<td>5.75 ± 1.31*</td>
<td>28.74 ± 9.25</td>
</tr>
<tr>
<td>Antagonist (WAY-100635; 0.1 mg/kg)</td>
<td>0.10 ± 0.02†</td>
<td>0.08 ± 0.01</td>
<td>3.45 ± 2.10†</td>
<td>1.90 ± 1.15†</td>
<td>15.07 ± 5.87†</td>
</tr>
</tbody>
</table>

Values are means ± SE, EUS EMG, external urethral sphincter electromyogram. Silent (SP) and active period (AP) denote the average duration of quiescent and tonic EUS EMG activity during the bursting period shown in Fig. 2. TSP, total duration of the silent periods during each void; BD, bursting duration; CD, contraction duration. *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (post hoc least significance difference test). †P < 0.05 indicates a statistically significant difference compared with 8-OH-DPAT treatment (post hoc least significance difference test).
**Table 4. Parameters of EUS EMG activity after vehicle and WAY-100635 treatment in urethane-anesthetized rats**

<table>
<thead>
<tr>
<th></th>
<th>SP, s</th>
<th>AP, s</th>
<th>BD, s</th>
<th>TSP, s</th>
<th>BD/CD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 11)</td>
<td>0.12 ± 0.02*</td>
<td>0.07 ± 0.01*</td>
<td>5.23 ± 1.67</td>
<td>3.39 ± 1.07</td>
<td>23.23 ± 9.25</td>
</tr>
<tr>
<td>Antagonist (WAY-100635; 0.1 mg/kg)</td>
<td>0.10 ± 0.01*</td>
<td>0.07 ± 0.01*</td>
<td>5.23 ± 1.50</td>
<td>3.09 ± 0.89</td>
<td>21.75 ± 7.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (post hoc least significance difference test).

**DISCUSSION**

An analysis of the effects of a 5-HT$_{1A}$ receptor agonist and antagonist on urinary bladder and EUS EMG activity in the female rat revealed that serotonergic mechanisms regulate urine storage as well as voiding. 8-OH-DPAT, a 5-HT$_{1A}$ agonist, enhanced tonic EUS EMG activity that occurred during the period between voids, an effect that would promote urine storage. On the other hand, 8-OH-DPAT reduced the bladder volume threshold for initiating reflex micturition, increased the amplitude of reflex bladder contractions recorded under isovolumetric conditions, and enhanced EUS EMG bursting activity during reflex micturition. These effects would be expected to facilitate voiding. WAY-100635, a 5-HT$_{1A}$ receptor antagonist produced effects opposite to those produced by 8-OH-DPAT, indicating that it blocked physiological regulatory mechanisms that were activated by endogenously released 5-HT. Thus serotonergic mechanisms involving 5-HT$_{1A}$ receptors (1, 2, 12, 36) and possibly other 5-HT receptor subtypes that have been identified in previous studies (9, 16, 21, 28, 29) appear to play an important role in autonomic and somatic neural pathways controlling multiple lower urinary tract functions.

It is reasonable to attribute most of the lower urinary tract effects of 8-OH-DPAT and WAY-100635 to actions on the central nervous system because intrathecal or intracerebroventricular injections of these agents have excitatory and inhibitory effects, respectively, on reflex bladder activity (17, 19, 20, 38). In addition, intravenous administration of WAY-100635 does not alter the bladder contractions induced by peripheral nerve stimulation (19), indicating that its effects are due to an action on the central nervous system. However, a direct excitatory effect of 8-OH-DPAT on the bladder cannot be excluded because a recent study showed that this agent can enhance spontaneous contractions of isolated bladder strips prepared from rats with chronic bladder outlet obstruction (22).

A reduction in the micturition volume threshold raises the possibility that 8-OH-DPAT might act by several mechanisms, including 1) an enhancement of bladder afferent input or afferent processing in the spinal cord (2, 20) or 2) a facilitation of the micturition switching circuit in the pontine micturition center (17, 38). The 8-OH-DPAT enhancement of the amplitude of bladder contractions recorded with a transurethral catheter under isovolumetric conditions is consistent with a facilitatory action on the micturition reflex pathway. However, during transvesical CMGs with the urethral outlet open and the bladder able to empty, 8-OH-DPAT decreased contraction amplitude by >20%. This decrease could reflect an inhibitory effect on the parasympathetic outflow to the bladder but is most likely due to a facilitation of EUS bursting and a decrease in urethral outlet resistance which negates the facilitatory effect on bladder contractions.

WAY-100635 prominently increased the micturition volume threshold, increased residual volume, and reduced voiding efficiency during transvesical CMGs. These effects are attributable to an action on multiple physiological mechanisms that involve endogenously released 5-HT. The doubling of the volume threshold without changing the contraction amplitude from control levels suggests that tonic activation of 5-HT$_{1A}$ receptors regulates the set point for micturition in the urethane-anesthetized rat but may not influence the efferent limb of the micturition reflex. However, during transurethral CMGs with the outlet closed, WAY-100635 did decrease the amplitude of the micturition contractions, raising the possibility that the efferent parasympathetic pathway to the bladder was suppressed by the drug. This effect in combination with a suppression of EUS bursting activity most likely accounts for the decrease in voiding efficiency produced by WAY-100635. The failure of WAY-100635 to change peak voiding pressure during voiding with the outlet open is probably due to the increased outlet resistance occurring in response to the reduction in EUS EMG bursting (4). WAY-100635 reduced the average silent period, indicating that activation of 5-HT$_{1A}$ receptors by endogenous 5-HT is necessary for bladder-sphincter coordination and efficient voiding.

To evaluate the role of EUS activity in voiding efficiency, a neuromuscular blocking agent (pancuronium) was administered to paralyze the EUS muscle. This agent did not change the volume threshold or bladder contraction amplitude but markedly increased residual volume and reduced voiding efficiency by >50%, 8-OH-DPAT decreased the volume threshold and residual volume measured after pancuronium treatment, and WAY-100635 had the opposite effect, indicating that these

**Table 5. Parameters of bladder activity in transvesical CMGs after serial administration of vehicle, pancuronium bromide, 8-OH-DPAT, and WAY-100635 in urethane-anesthetized rats**

<table>
<thead>
<tr>
<th></th>
<th>VT, ml</th>
<th>CA, cmH$_2$O</th>
<th>CD, min</th>
<th>RV, ml</th>
<th>VE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 12)</td>
<td>0.69 ± 0.15</td>
<td>24.12 ± 4.03</td>
<td>0.37 ± 0.06</td>
<td>0.16 ± 0.07</td>
<td>77.2 ± 7.2</td>
</tr>
<tr>
<td>Pancuronium bromide (1.5 mg/kg)</td>
<td>0.85 ± 0.17</td>
<td>25.59 ± 5.82</td>
<td>0.31 ± 0.06</td>
<td>0.59 ± 0.17†</td>
<td>30.4 ± 14.2‡</td>
</tr>
<tr>
<td>Agonist (8-OH-DPAT; 0.3 mg/kg)</td>
<td>0.57 ± 0.15*</td>
<td>22.56 ± 5.39</td>
<td>0.33 ± 0.07</td>
<td>0.33 ± 0.11*</td>
<td>37.1 ± 18.2</td>
</tr>
<tr>
<td>Antagonist (WAY-100635; 0.1 mg/kg)</td>
<td>1.58 ± 0.26‖</td>
<td>27.64 ± 6.66</td>
<td>0.37 ± 0.08</td>
<td>1.26 ± 0.23‡*</td>
<td>19.0 ± 13.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 indicates a statistically significant difference compared with pancuronium bromide administration (post hoc least significance difference test). †P < 0.05 indicates a statistically significant difference compared with 8-OH-DPAT treatment (post hoc least significance difference test). ‡P < 0.05 indicates a statistically significant difference compared with vehicle treatment (post hoc least significance difference test).
Table 6. Parameters of bladder activity in transurethral CMGs after serial administration of vehicle, 8-OH-DPAT, and WAY-100635 in urethane-anesthetized rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VT, ml</th>
<th>Base, cmH2O</th>
<th>CA, cmH2O</th>
<th>CD, min</th>
<th>ICI, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 12)</td>
<td>0.80 ± 0.29</td>
<td>14.77 ± 1.95</td>
<td>50.94 ± 5.70</td>
<td>0.62 ± 0.15</td>
<td>0.81 ± 0.20</td>
</tr>
<tr>
<td>Agonist (8-OH-DPAT; 0.3 mg/kg)</td>
<td>0.33 ± 0.10*</td>
<td>14.90 ± 3.73</td>
<td>58.03 ± 7.56*</td>
<td>0.66 ± 0.23</td>
<td>0.68 ± 0.19</td>
</tr>
<tr>
<td>Antagonist (WAY-100635; 0.1 mg/kg)</td>
<td>1.26 ± 0.31†</td>
<td>14.10 ± 2.68</td>
<td>42.86 ± 3.15*†</td>
<td>0.65 ± 0.20</td>
<td>1.02 ± 0.16*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Base: basal pressure; ICI: intercontraction interval. *P < 0.05 indicates a statistically significant difference compared with vehicle treatment (post hoc least significance difference test). †P < 0.05 indicates a statistically significant difference compared with 8-OH-DPAT treatment (post hoc least significance difference test).

Effects of the two drugs are due to changes in the autonomic control of the bladder and not to changes in EUS activity.

The present experiments complement and extend previous studies which revealed that 8-OH-DPAT enhances and WAY-100635 suppresses short-latency and long-latency EUS reflex activity evoked by stimulation of afferent axons in the pelvic nerve (1). It was proposed that these two reflexes are mediated by different central pathways and have different functions. The short-latency reflex is organized in the spinal cord and is mediated by a pathway that generates tonic EUS EMG activity during bladder filling. The long-latency reflex is dependent on supraspinal mechanisms and is related to bursting EUS EMG activity occurring during micturition. Because NMDA and AMPA glutamatergic mechanisms contribute to the two pelvic-EUS reflexes (1), it is probable that a convergence of 5-HT and glutamatergic pathways is necessary for the regulation of EUS activity.

Other studies showed that reflex EUS activity and urethral closure mechanisms in the rat can be evoked during sneezing (18, 23, 24). This response, which is presumably involved in the maintenance of urinary continence during increases in intra-abdominal pressure, is mediated by supraspinal inputs to EUS motoneurons. Pharmacological experiments revealed that the sneeze-evoked responses were enhanced by duloxetine, a serotonin-norepinephrine reuptake inhibitor and suppressed by an NMDA glutamatergic antagonist or by an α2-adrenoceptor antagonist (13, 23). The effect of duloxetine was also suppressed by an α1-adrenoceptor antagonist but not by a nonselective 5-HT receptor antagonist (13, 23). It was concluded that serotonergic, noradrenergic, and glutamatergic excitatory pathways as well as noradrenergic inhibitory pathways are important contributors to the supraspinal sneeze-evoked EUS response. These studies indicate that the function of the EUS is dependent on a complex interaction between multiple neurotransmitters at spinal and supraspinal levels. Because the 8-OH-DPAT-evoked facilitation of EUS bursting activity also occurs in rats after chronic transection of the spinal cord at the midthoracic level, it is clearly dependent on activation of 5-HT1A receptors in the lumbosacral spinal cord (2, 12).

On the other hand, the modulation of bladder activity by 5-HT1A agonists and antagonists could be mediated by actions at supraspinal as well as spinal sites because these drugs are effective when injected intrathecally (19, 26) or intracerebroventricularly (17, 20, 26, 38) as well as intravenously (2, 9, 19, 26, 30). Bladder activity is regulated by multiple excitatory central neurotransmitter mechanisms (glutamatergic, noradrenergic, dopaminergic, and serotonergic) as well as GABAAergic and glycine receptor-mediated mechanisms (10, 11). It is believed that glutamate is the essential excitatory transmitter and that other neurotransmitters such as 5-HT and norepinephrine exert their effects by modulating glutamatergic transmission (9, 11).

A similar interaction between glutamatergic transmission and monoaminergic modulatory mechanisms has been proposed to explain the reflex control of the EUS motoneurons and the action of duloxetine in the cat spinal cord (7, 33, 35), although in this species 5-HT2 rather than 5-HT1A receptors are thought to be involved (8, 33). The clinical efficacy of duloxetine to enhance EUS function and reduce stress urinary incontinence (33) suggests that monoaminergic-glutamatergic interactions may be important for promoting urinary continence in humans.
In summary, the present results indicate that activation of 5-HT1A receptors by exogenous administration of a selective receptor agonist or by endogenously released 5-HT facilitates reflex voiding by lowering the volume threshold for initiating the micturition reflex and by enhancing the relaxation of the external urethral sphincter, as evidenced by the increased silent period during external urethral sphincter bursting.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


