Effect of a 5-HT1A Receptor Agonist (8-OH-DPAT) on External Urethral Sphincter Activity in a Rat Model of Pudendal Nerve Injury

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Abstract

Although serotonergic agents have been used to treat patients with stress urinary incontinence, the characteristics of the external urethral sphincter (EUS) activity activated by 5-HT receptors have not been extensively studied. This study examined the effects of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, on the EUS electromyography (EUS-EMG) and resistance of the urethra in a rat model with bilateral pudendal nerve injury (BPNI). Two measurements were utilized to assess the effects of the drug on bladder and urethral functions: the simultaneous recordings of transvesical pressure under isovolumetric conditions (isovolumetric IVP) and urethral perfusion pressure (UPP), and the simultaneous recordings of IVP during continuously isotonic transvesical infusion with an open urethra (isotonic IVP) and EUS-EMG. This study also evaluated the urethral continence using leak point pressure (LPP) testing. The UPP and LPP measurements of BPNI rats reveal that 8-OH-DPAT significantly increased urethral resistance during the bladder storage phase, yet decreased resistance during the voiding phase. The entire EUS burst period was significantly prolonged, within which the average silent period increased and the frequency of burst discharges decreased. 8-OH-DPAT also improved the voiding efficiency, as evidenced by the detection of decreases in the contraction amplitude and residual volume, with increases in contraction duration and voided volume. These findings suggest that 8-OH-DPAT not only improved continence function, but also elevated the voiding function in a BPNI rat model.

Keywords: External urethral sphincter, 8-OH-DPAT, urethral perfusion pressure, EMG, intravesical pressure, burst period, pudendal nerve, leak point pressure
INTRODUCTION

Stress urinary incontinence (SUI) is one of the most common forms of incontinence among middle-aged women (42). SUI is associated with a decrease in urethral resistance (26) resulting from general diseases, surgical trauma, and childbirth. Childbirth by vaginal delivery is the primary epidemiological factor, as it injures the pudendal nerves, pelvic floor muscles, and connective tissues that help to maintain continence (17). Previous researchers have discussed a bilateral pudendal nerve injury (BPNI) rat model for studying SUI and inefficiency in voiding (35). Results show that EUS-EMG activity recovered 6 weeks after the complete bilateral transection of the pudendal nerves.

Serotonergic agents such as Duloxetine are often used to treat patients with SUI (20). However, recent studies have continued investigating the effects of serotonergic ligands on the bladder activity in rats. Serotonin (5-hydroxytryptamine, 5-HT) comprises 14 structurally different 5-HT receptors in mammalian species, including seven subfamilies (5-HT_1 to 5-HT_7) (38). A number of 5-HT receptors (5-HT_1, 5-HT_2, and 5-HT_3 receptors) are involved in the function of the lower urinary tract (LUT). These 5-HT receptors and terminals are located in areas of the spinal cord containing afferent and efferent components of LUT neural control centers (5, 12, 22, 31, 45). These receptors appear to modulate all of the pathways involved in the control of micturition, including the parasympathetic, sympathetic, and somatic pathways (18).

The 5-HT_1A receptor is one of the most extensively investigated agents in various animal pharmacological experiments, including rats, guinea pigs, and cats (38). However, contradictory results have generated various interpretations of the functions of this receptor subtype in different animal species. Previous cat experiments provided a general understanding of the involvement of 5-HT in the control of micturition (43). For example, 8-hydroxy-2-(di-n-propylamino)tetralin (8-
OH-DPAT) significantly increased both the bladder volume threshold and inter-contraction interval in cat experiments, thereby moderately inhibiting bladder activity. The bladder-inhibitory effect was also reported in humans, but exhibited the exact opposite effect of 8-OH-DPAT in rats (34, 38, 41). Because the rat model has now gained greater popularity as the main species for investigating urine storage and micturition reflexes, it is essential to precisely define the role of 5-HT₁A receptors in the regulatory function of the LUT in rats.

Although many studies investigated the role of 5-HT₁A receptors in the regulatory function in the LUT in rats, most of those studies just focused primarily on the measurements of urodynamic changes, including changes in the volume threshold, bladder pressure, voided volume, and residual volume (14, 23, 34, 41, 48). Few studies have assessed the effect of the 5-HT₁A receptor agonist on EUS activity or urethral function (12, 44). Therefore, the electrophysiological effects of 5-HT on EUS activity require further investigation.

This study evaluated a recently-developed BPNI rat model to examine the role of the 5-HT₁A receptor in controlling EUS activity during the micturition reflex. Systemic administration of 8-OH-DPAT, a 5-HT receptor agonist, or WAY-100635, a 5-HT₁A receptor antagonist activated or blocked 5-HT₁A receptors, respectively. Two different measurement methods assessed the effects of the drug on bladder and urethral functions: 1) the simultaneous recordings of transvesical pressure under isovolumetric conditions (isovolumetric IVP) and urethral perfusion pressure (UPP), and 2) the simultaneous recordings of transvesical pressure during continuously isotonic transvesical infusion with an open urethra (isotonic IVP) and EUS-EMG. The leak point pressure (LPP) test was also used to evaluate the resistance of the urethral continence.
MATERIALS AND METHODS

Experimental preparation

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. Female Sprague-Dawley rats (n=48) weighing 290–360 g were used in this study. The rats were divided into two equal groups: the normal control (NC) and BPNI groups. The NC rats received a sham operation that did not damage the pudendal nerves, while BPNI rats underwent bilateral pudendal nerve transection. A description of the surgical procedure appears in a previous study (35). After the 6-week recovery period, all rats were anesthetized with urethane (1.2 g/kg, s.c.). The femoral vein was catheterized for fluid and drug administration, and the body temperature was maintained at 36–38 °C with a heating lamp.

Two different preparations were used to quantify the effects of drug on bladder and urethral functions. The first series of experiments (NC and BPNI, n=6 in each group) involved simultaneous recordings of isovolumetric IVP and UPP. This approach permitted the functional separation of bladder and urethral activity without the risk of surgical damage to the innervation in the bladder neck region. The procedure for simultaneous recordings of isovolumetric IVP and UPP was performed as previously reported (2). The bladder and proximal urethra was exposed via a midline abdominal incision. A custom-designed triple-lumen catheter (8 Fr tube) containing two polyethylene catheters of different sizes (PE-160 and PE-50) connected to a pipette tip was then inserted through the bladder dome, and the pipette tip was placed securely in the bladder neck. The outer lumen was connected to a pressure transducer for isovolumetric IVP monitoring. The middle lumen was connected to an infusion pump for continuous saline infusion to urethra. The inner
lumen (PE-50) was connected to a pressure transducer for monitoring the UUP. The abdomen was then closed with sutures. After surgery, the bladder was filled with 0.7–1.0 mL of normal saline via the outer lumen of the catheter, and the isovolumetric IVP was recorded throughout the experiment. The urethra was perfused with saline (0.075 mL/min) using an infusion pump under an open urethra condition. The isovolumetric IVP and UPP were recorded simultaneously and the experimental drug was given during the recording.

The second series of experiments (NC and BPNI, n=12 in each group) was conducted with simultaneous recordings of isotonic transvesical infusion of intravesical pressure with an open urethra (isotonic IVP) and EUS-EMG. The transvesical infusion was performed at an infusion rate of 0.123 ml/min with saline, and the urethra was opened allowing elimination of fluid during micturition. The procedure for simultaneously recording isotonic IVP and EUS-EMG was performed as reported previously (13, 35, 36).

All recorded data were first amplified and sampled at 3 kHz (Biopac MP 150, BIOPAC Systems). Several isovolumetric IVP and UPP parameters were measured, including the frequency of isovolumetric bladder contraction, the maximum amplitude of reflex bladder contraction, the baseline UPP between reflex bladder contractions, and the baseline of high frequency oscillations (baseline HFOs) in UPP during reflex bladder contractions (Fig. 1) (2, 46).

Various cystometric parameters were calculated during the simultaneous recordings of isotonic IVP and EUS-EMG (13, 35): (1) the volume threshold, i.e., the volume of saline sufficient to evoke the first voiding contraction; (2) the inter-contraction interval, i.e., the interval between two bladder contractions; (3) the contraction amplitude, i.e., the maximal pressure during voiding; and (4) the bladder
contraction duration during voiding. The urodynamic parameters of the residual volume, voided volume, voiding efficiency, and critical pressure were also obtained. The residual volume was the volume of saline withdrawn through the intravesical catheter after micturition. The collection of fluid was facilitated by manually pressing on the abdominal wall. The voided volume represented the volume threshold minus the residual volume, and voiding efficiency was the ratio between the voided volume and volume threshold. The critical pressure was the amplitude initially appearing at high frequency oscillation waves in IVP (Fig. 2C).

The analysis of EMG activity was blinded to the status of the rats. Various EUS-EMG parameters were measured: (1) the burst period, the duration of the burst discharges, defined as the interval between the point at which tonic EMG is converted into burst discharge and the point at which EMG is converted into tonic EMG (Fig. 2); (2) the silent period, the duration of quiescence between two clusters of high-frequency spikes, defined as the interval between the point at which high-frequency spikes are converted into low-frequency waves and the point at which low-frequency waves are converted into high-frequency spikes; and (3) the active period, the duration of the high-frequency EMG spikes separated by periods of quiescence, defined as the interval between the point at which low-frequency waves are converted into high-frequency spikes and the point at which high-frequency spikes are converted into low-frequency waves. The number of silent periods in each bladder contraction was also calculated, and the ratio between the number of silent periods and its bladder contraction duration represented the frequency of burst discharges. The burst period, silent period, and active period were measured using the “Delta T” function of Acknowledge software (BIOPAC Systems). All data were obtained and averaged from three micturition contractions for each animal.
Computed data were compiled in spreadsheets using Excel (Microsoft, WA, USA).

**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 on bladder activity and outlet resistance, the rats with preparation 1 were, in turn, administrated with 8-OH-DAPT (0.3 mg/kg, i.v.) and WAY-100635 (0.1 mg/kg, i.v.) at intervals of no less than 1 h, while rats with preparation 2 were only treated with 8-OH-DAPT (0.3 mg/kg, i.v.). The drug dose was in accordance with dosages determined in previous studies (12, 14, 28). The dose of 8-OH-DPAT was set to produce a consistent enhancement of bladder activity, while the dose of WAY-100635 was set to affect bladder activity but not blood pressure (14). The rats underwent urodynamic and EUS-EMG examinations before and after administration of the drug. The first post-treatment urodynamic and EUS-EMG examinations were completed within 20~45 min following drug administration, due to the short half-life of 8-OH-DAPT (21, 28).

**LPP testing**

Several NC and BPNI rats ($n=6$ in each group) were used to conduct the LPP tests and quantify the urethral resistance. LPP values were measured in all rats prior to, and 5~10 min following 8-OH-DPAT treatment. Prior to the tests, the spinal cord was acutely transected at the T9-T10 level, and the cystometric recording was performed as described above. Spinal cord transection eliminates reflex bladder activity in response to increasing intravesical pressure, but it does not interfere with the spinal continence reflexes of the bladder neck and urethra (24). LPPs were measured as described previously (8, 15, 35). LPP tests were conducted in all BPNI rats before and after successive bilateral pelvic (branch to iliococcygeous/pubococcygeous muscles) and bilateral hypogastric neurotomies to
determine the contribution of different nerves to urethral outlet resistance. The effects of drug on LPPs were assessed 20~30 min after each transection.

**Statistical analysis**

This study presents all data as the means ± SD. One-way ANOVA was used to compare the parameters obtained from isovolumetric and isotonic IVPs, UPP, EUS-EMG, and LPP. ANOVA was followed by Student-Newman Keuls post hoc test using SigmaStat (SPSS, Chicago, IL), and a value of \( p < 0.05 \) was considered significant in all analyses.
RESULTS

*Effect of 8-OH-DAPT and WAY-100635 on isovolumetric IVP and UPP in NC and BPNI rats*

Experimental results show that in both NC and BPNI rats, isovolumetric contraction of bladder was associated with urethral relaxation. The HFOs of urethral pressure were present during urethral relaxation in both the control and BPNI rats (Fig. 1, control rat with no drug treatment). Although the basic pattern of isovolumetric IVP and UPP was similar in NC and BPNI rats with 8-OH-DPAT or WAY-100635 treatment, there were marked quantized differences. Table 1 summarizes all of the isovolumetric IVP and UPP parameters obtained from NC and BPNI rats before and after drug treatment.

The rats showed several significant changes following bilateral pudendal nerve injury, including decreases in the baseline UPP and the baseline HFOs (compared to NC rat, *p*<0.05). No significant differences were observed in isovolumetric IVP measurements, indicating that urethral resistance during continence and voiding were both reduced and bladder activity was unaffected (Table 1). 8-OH-DPAT intravenously administrated to BPNI rats significantly increased the baseline UPP and the frequency and duration of isovolumetric bladder contraction and decreased the baseline HFOs, compared to rats prior to treatment. This implies that 8-OH-DPAT markedly enhanced urethral resistance during continence and the isovolumetric bladder activity, while significantly decreasing urethral resistance during voiding. Similar effects appeared in NC rat with 8-OH-DPAT treatment. Conversely, WAY-100635 administrated after 8-OH-DPAT reduced the baseline UPP and the frequency and duration of isovolumetric bladder contraction and
increased the baseline HFOs in both NC and BPNI rats (Table 1). However, some parameters in BPNI rats did not reach statistical significance.

**Typical pattern of isotonic IVP and EUS-EMG in NC rats before drug treatment**

Figure 2 depicts the typical isotonic IVP and EUS-EMG measurements in NC rats with no treatment. The rats exhibited micturition contractions during isotonic intravesical infusion of saline. EUS-EMG exhibited low-amplitude tonic activity during the initial filling phase of the IVP or between micturition contractions, but the activity markedly increased in amplitude during bladder contractions. The single micturition contraction of the bladder includes three phases of isotonic IVP (29): phase 1, the rising phase, defined as the duration between the point at which a curve begins rising sharply and the point at which high-frequency oscillation waves begin; phase 2, high-frequency oscillation (HFO) phase, defined as the duration between the points at which the high-frequency oscillation waves begin and end; and phase 3, rebound and falling phase, defined as the duration between the point at which the high-frequency oscillation waves end and the point at which the falling curve flattens (Fig. 2B). A long period of bursting on EUS-EMG appeared during each micturition contraction, which lasted from the end of phase 1 throughout the entire duration of phase 2, as shown in Fig. 2C. The burst period (BP) was characterized by HFO waves in IVP, and could be divided into non-voiding and voiding burst activities (Fig. 2C). The non-voiding burst activity in phase 1 usually appeared for a short period (~0.87 s) and was not accompanied by any oscillation waves in IVP. However, the voiding burst activity in phase 2 usually lasted for 3~5 s and was accompanied by a series of HFOs superimposed on the IVP. Burst discharges in the BP 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. 2D.
Effect of 8-OH-DAPT on isotonic IVP in NC and BPNI rats

Figure 3 shows typical isotonic IVP examples of NC and BPNI rats before and after 8-OH-DPAT treatment (0.3 mg/kg, i.v.). Figure 4 compares the measured bladder activity parameters between NC and BPNI rats before and after drug administration. Rats after bilateral pudendal nerve injury the bladder contraction duration was significantly increased, while the bladder contraction amplitude and the voiding efficiency were reduced compared to NC rats (Fig. 4B, 4C, and 4G). The lower voiding efficiency appeared to significantly increase residual volume and decrease the intercontraction interval (Fig. 4D and 4F), because at a constant filling rate the bladder again reached the contraction threshold volume in less time.

Subsequently administering 8-OH-DPAT to BPNI rats significantly increased the bladder contraction duration and the voided volume, while decreasing the residual volume (Fig. 4C, 4E, and 4F), which in turn dramatically increased voiding efficiency (Fig. 4G). This increase in the voiding efficiency led to an apparent increase in intercontraction intervals (Fig. 3D compared to 3C), but the latter was not significant (Fig. 4D). NC rats exhibited similar effects on cystometric parameters after 8-OH-DPAT treatment, including a significant reduction in residual volume and contraction amplitude, and a significant increase in the contraction duration and voiding efficiency (Fig. 4). Note that the critical pressure for the onset of HFOs in BPNI rats was significantly lower than that in NC rats, and this was further reduced by 8-OH-DPAT treatment in either NC or BPNI rats (Fig. 4H).

Effect of 8-OH-DAPT on EUS-EMG in NC and BPNI rats

In all BPNI rats 6 weeks after pudendal nerve injury, large-amplitude EUS-EMG activity was still found during isotonic IVP recording (Fig. 5C). Similar to the pattern of EUS-EMG activity in NC rats (Fig. 5A), three phases of EUS-EMG
activity during micturition contractions could still be recognized in BPNI rat (Fig. 5C). In phase 1, EUS-EMG gradually increased in amplitude. The burst type of EUS-EMG began at the end of phase 1 (Fig. 2C, 5C) and continued throughout the entire phase 2 of IVP. In phase 3, EUS-EMG revealed tonic-type activity.

The amplitude of tonic EUS-EMG activity during the bladder-filling phase significantly increased by 10%~15% in BPNI rats after 8-OH-DPAT treatment. Drug treatment significantly altered the pattern of EUS burst activity (Fig. 5D). In phase 1, almost the entire tonic EUS-EMG transformed to burst activity. The EUS-EMG exhibited non-voiding burst activity because it was not accompanied by any HFO waves in isotonic IVP (Fig. 5D) or urination. During phase 2, the entire period of the burst discharges was significantly prolonged, which paralleled the occurrence of HFO waves in IVP. Observations of the urethral outlet showed that urine was emitted in a long stream-like pattern during this phase. Similar to the EUS-EMG results prior to drug administration, the EUS-EMG changed to tonic-type activity during phase 3.

Table 2 presents the EUS-EMG measurements in NC and BPNI rats before and after 8-OH-DPAT (0.3 mg/kg, i.v.) treatment. Several abnormal EUS-EMG parameters appeared in BPNI rats compared to NC rats, including decreases in the silent periods in phases 1 and 2 and increases in the frequency of burst discharges in phase 2. However, no significant difference appeared in the average burst period or the active period in either phase 1 or 2 (Table 2). Conversely, BPNI rats after 8-OH-DPAT treatment exhibited significant increases in the burst period and the silent period in both phases 1 and 2 and significant decreases in the frequency of burst discharges in both phases 1 and 2, but phases 1 and 2 of the active periods were not affected by the drug. Interestingly, similar effects on the EUS-EMG parameters were found in NC rats after 8-OH-DPAT administration (Table 2). In both NC and BPNI
rats with drug treatment, longer durations of the silent period in phase 2 were accompanied by smaller-amplitude HFO waves in IVP, compared to those in NC and BPNI rats before drug treatment, respectively (Fig. 6B and 6D compared to Fig. 6A and 6C, respectively).

**Effect of 8-OH-DAPT on LPP in NC and BPNI rats**

Figure 7 shows that the LPPs decreased significantly after 6 weeks of pudendal nerve injury, compared to NC rats ($p<0.05$). The average LPP of NC rats after 8-OH-DPAT treatment (0.3 mg/kg, i.v.) increased only slightly, but the drug treatment significantly increased the average LPP of BPNI rats. LPP measurements were further conducted in BPNI rats following acute successive transection of the bilateral pelvic nerves (PN transection) and bilateral hypogastric nerves (HN transection). Results indicate that transecting the bilateral pelvic nerves slightly decreased the average LPP value (22.80±2.74 cmH2O) compared to the value (25.85±2.92 cmH2O) before nerve transection. Subsequent transection of the bilateral hypogastric nerves further decreased the LPP value (18.40±1.29 cmH2O, $p<0.05$). However, the LPP value significantly increased with 8-OH-DPAT treatment in BPNI rats with either PN transection or subsequent HN transection.

**DISCUSSION**

This study shows that the activation of 5-HT1A receptors in BPNI rats with 8-OH-DPAT, a 5-HT1A agonist, significantly increased urethral outlet resistance during the storage phase and decreased resistance during the voiding phase, as evidenced by an increase in the baseline UPP and a decrease in the baseline HFOs. Similarly, LPP testing revealed an improvement in the urethral continence following treatment with 8-OH-DPAT. Conversely, rats treated with WAY-100635, a 5-HT1A antagonist,
produced effects opposite to those produced by 8-OH-DPAT. Importantly, 8-OH-DPAT significantly improved the voiding function by altering the pattern of EUS-EMG burst activity and bladder activity during continuously isotonic transvesical infusion. The results of 8-OH-DPAT treatment included marked decreases in the frequency of EMG burst discharges, bladder contraction amplitude, and residual volume, and significant increases in the burst period, silent period, bladder contraction duration, and voiding efficiency. All results indicate that 8-OH-DPAT significantly improved functions related both to continence and micturition in this BPNI model, although it did not recover bladder functions to a normal level.

Previous researchers have discussed the mechanisms involved in recovering EUS-EMG activity 6 weeks after BPNI (35). In summary, this recovery might have been due to the sprouting of adjacent motor nerves (e.g., the muscular branch of pelvic nerve) into the denervated EUS or regeneration of pudendal motor axons. Previous research showed that the EUS-EMG activity during cystometric measurements originates from striated muscles rather than smooth muscles, because it was eliminated after acute nerve transection or administering a neuromuscular blocking agent (11, 35). The present results confirm that 8-OH-DPAT significantly increased the amplitude of EUS-EMG by approximately 10%~15% during the filling phase. This is consistent with the effect of the 5-HT1A agonist on urethral function enhancing EUS activity during the storage phase of the micturition cycle in normal rats and in clinical patients (6, 10, 40). However, the EUS-EMG activity during the voiding phase was altered by 8-OH-DPAT, for which no report has appeared in any clinical studies. This discrepancy may be attributed to several factors. First, the effect of the drug on EUS activity may differ between species. This possibility has been found between cats and rats (38, 43). In addition, new neural pathways might appear
following injury to the pudendal nerve, which could influence the effects of 8-OH-DPAT on the EUS.

8-OH-DPAT (0.3 mg/kg, i.v.) significantly altered the patterns associated with burst activity. The complete change in EUS-EMG during phase 1 could not be attributed to a drug overdose. This is because the change was produced in anesthetized normal rats even with low doses (0.03 mg/kg) of 8-OH-DPAT (unpublished results). In phase 1, nearly the entire tonic EUS-EMG was transformed into burst activity; however, the rising IVP was not accompanied by any HFO waves. This could be explained by the possibility that 8-OH-DPAT has a significant excitatory effect, triggering the EUS burst center in spinal cord, causing the appearance of EUS burst activity prior to the opening of bladder neck/internal sphincter innervated by hypogastric nerve. Previous studies have indicated that 8-OH-DPAT acts on least at two discrete sites of the central nervous system (CNS): in spinal cord and at the supraspinal level (28). In addition, recent research has shown that the burst activity of EUS-EMG in intact rats is generated in the segment of the spinal cord between T8–9 and L3–4 (10). Similarly, the burst activity of periurethral striated muscles for the ejaculation reflex also appeared in male rats in the L3–4 spinal segment (16, 47). These findings imply that 8-OH-DPAT has a significant excitatory effect on somatic pathways in the spinal cord, triggering the early generation of EUS burst activity during the voiding phase in BPNI rats.

Approximate to the point at which phase 1 enters phase 2, the bladder neck/internal sphincter and urethral smooth muscle were significantly inhibited by the drug, as demonstrated by a decrease in the baseline HFOs. Meanwhile, an increase in IVP immediately exceeded the urethral resistance (i.e. the critical pressure), whereupon HFO waves occurred in IVP and urine flow in the urethra with a simultaneous decreased in the amplitude of bladder contractions. Upon entering phase
EUS-EMG was converted from burst to tonic pattern without accompanying HFO waves in the isotonic IVP, indicating that the external urethral sphincter was closed whereas detrusor muscle remained continuously relaxed.

One question emerging from this study is why BPNI produced a substantial reduction in leak point pressure without improving the efficiency of voiding. This contradiction could be explained by the denervation of EUS in BPNI rats, reducing urethral resistance during the bladder storage phase, as evidenced by decreases in the baseline UPP and the LPP. However, other urethral structures generate sufficient resistance to maintain continence. For example, the bladder neck/internal sphincter innervated by the hypogastric nerve, the urethral smooth muscle and iliococcygeus/pubococcygeus muscles innervated by the pelvic nerve, and the viscoelastic properties of the outlet could all resist the increase in intravesical pressure during the bladder filling phase (19, 25, 32, 33). Without a decrease in the volume threshold and the absence of passive urine dripping during the filling phase of the cystometry in rats after BPNI confirm this assumption.

Lower voiding efficiency in rats with BPNI could be attributed to an impairment of EUS burst activity. Electrophysiological studies have shown that the EUS-EMG in normal control rats exhibits a 4~8-Hz burst pattern during voiding (27, 29). The EUS-EMG bursts, and particularly those occurring during the silent period in phase 2 of isotonic IVP, represent the relaxation and opening of the outlet, which is essential to achieving efficient voiding (13). In this study, rats with pudendal nerve injury showed a decrease in the duration of the silent period (to 66% that of NC rats) and an increase in the frequency of EUS-EMG bursts, with a subsequent decrease in the duration of urethral opening leading to inefficient voiding. This assumption is consistent with previous reports that the suppression of external urethral sphincter activity through
pharmacologic blocking, such as pancuronium bromide (12) or alpha-bungarotoxin (49), reduced voiding efficiency.

On the other hand, results of present study indicate that 8-OH-DPAT increased both the voiding efficiency and leak point pressure (LPP) in BPNI rats. The increase in voiding efficiency could be caused by several mechanisms, including 1) the facilitation of the micturition switching circuit in the pontine micturition center (23, 50); 2) or the enhancement of bladder afferent input or afferent processing in the spinal cord (9, 28). These assumptions are supported by the fact that 8-OH-DPAT increased the frequency of isovolumetric IVP and decreased the volume threshold under isotonic IVP. In addition, the drug produced an increase in both the duration of isovolumetric and isotonic bladder contractions, indicating a facilitatory action on the pelvic efferent pathways; or 3) an enhancement of EUS burst and a decrease in urethral outlet resistance during voiding, as evidenced by a detected increase in the duration of EUS silent period (118% that of NC rats) and a decrease in the baseline HFOs following treatment with 8-OH-DPAT. The decrease in baseline HFOs implies that 8-OH-DPAT enhanced the relaxation of urethral smooth muscle, facilitating the flow of urine through the urethra.

The LPP decreased in rats following chronic injury to the pudendal nerve; however, it was elevated by 17% following treatment with 8-OH-DAPT. These results imply that the bladder-to-pudendal reflex pathway might partially recover from pudendal nerve injury and activation of 5-HT_{1A} receptors with 8-OH-DPAT enhanced this continence reflex, elevating urethral outlet resistance. The increase in urethral outlet resistance was also demonstrated by increases in the baseline UPP and amplitude of EUS-EMG (10%~15%) during the bladder filling. The increase in baseline UPP suggests that treatment with 8-OH-DPAT might enhance the closure of
the bladder neck/internal sphincter and the contraction of urethral smooth muscle innervated by pelvic nerves (1).

In the LPP experiment, bilateral transection of the hypogastric and/or pelvic nerves (to the iliococcygeous/pubococcygeous muscles) further decreased LPP values (Fig. 7). Interestingly, treatment with 8-OH-DAPT significantly increased the LPP values, even in chronic BPNI rats which had undergone acute bilateral transection of the hypogastric and pelvic nerves. These results suggest that bladder-to-somatic, bladder-to-pelvic (nerve to iliococcygeous/pubococcygeous muscles), and bladder-to-hypogastric reflexes all contribute to the guarding reflex induced by a passive increase in intravesical pressure in a chronic BPNI rat model.

The durations of bladder contraction and EUS burst were both extended by 8-OH-DPAT. This raises the possibility that a neural pathway exists, linking the bladder to EUS activity during micturition reflex. Such a pathway would be modulated via several transmission routes. First, the micturition switch located in the pons might be activated by 8-OH-DPAT simultaneously delivering excitatory output to both the sacral parasympathetic nucleus and the spinal EUS pattern generator. The other possible route would be the simultaneous enhancement of urethral sensory feedback to both the bladder and EUS by 8-OH-DPAT amplifying the duration of micturition. This has been proven using urethral anesthesia with lidocaine or by transection of pudendal afferents significantly reducing the durations of bladder contractions and EUS burst simultaneously (36). Conversely, the stimulation of urine flow to the urethra can excite pudendal afferent nerves and in turn facilitate a bladder contraction reflex (3, 4). Thus, urethral afferent feedback is necessary both to augment contractions in the bladder and pattern the EUS activity into alternating bursts and quiescence.
The durations of isovolumetric and isotonic bladder contraction were both increased by 8-OH-DAPT, supporting the contention that the drug enhanced the contractile ability of the bladder, thereby improving voiding efficiency. However, compared to NC rats, BPNI rats without treatment with drugs exhibited an increase in the duration of bladder contractions and a slight elongation in burst period with a decrease in voiding efficacy. This apparent conflict can be explained by changes in EUS activity. Although the average burst period and duration of contractions increased in rats following injury to the pudendal nerve, the duration of the silent period within the burst period was shortened (Table 2). Because the silent period in phase 2 of IVP represents the relaxation and opening of the bladder outlet to achieve efficient voiding, relaxing and opening the bladder outlet for shorter durations reduced voiding efficiency.

On the other hand, 8-OH-DPAT increased the baseline UPP of isovolumetric IVP but decreased the volume threshold of isotonic IVP. These results appear contradictory; however, they could be explained by the involvement of 8-OH-DPAT in the modulation of continence and micturition reflexes. During the bladder filling phase, 8-OH-DPAT elevated the urethral resistance via the enhancement of the bladder-somatic and bladder-hypogastric reflex. Meanwhile, the drug also enhanced bladder afferent activity, acting on the micturition switching circuit, thereby reducing the bladder volume threshold for triggering micturition.

Treatment with 8-OH-DPAT also reduced the amplitude of bladder contractions and the critical pressure at the onset of HFOs during isotonic IVP in NC and BPNI rats (Fig. 4B and 4H). These decreases may be the results of a reduction in urethral resistance during the voiding phase rather than the drug inhibiting the contractile ability of the bladder. Simultaneous recordings of isovolumetric IVP and UPP confirmed this possibility, indicating that both NC and BPNI rats exhibited a lower
baseline of HFOs in UPP and a higher frequency of isovolumetric bladder contraction after 8-OH-DPAT treatment, without changes in the amplitude of isovolumetric bladder contractions (Table 1). Reduced urethral resistance during the voiding phase might have been the result of drug-enhanced relaxation of the urethral smooth muscle or an extension of the silent period of the EUS burst. This explanation is indirectly supported by the fact that the shorter urethra and thinner EUS overlaying the urethra of female rats might easily cause lower urethral outlet resistance during the opening of the urethra (7, 37, 39). Therefore, it could be concluded that treatment with 8-OH-DPAT leads to marked decreases in the amplitude of bladder contractions and the critical pressure at the onset of HFOs resulting from the reduction in the urethral resistance rather than the bladder activity.

The phasic relaxation of EUS may reduce urethral resistance. Our results show that 8-OH-DPAT increased EUS burst activity in phase 1; however, the baseline UPP was not reduced but increased by the drug. This conflict could be explained by the appearance of EUS burst prior to the opening of bladder neck/internal sphincter, and the sustained activation of urethral smooth muscle by 8-OH-DPAT until the opening of the bladder neck/internal sphincter. Urethra resistance increased by the activation of urethral smooth muscle might exceed the decrease introduced by the phasic relaxation of EUS, leading to an increase in baseline UPP.

In summary, this study showed that the activation of 5-HT$_{1A}$ receptors by the systemic administration of 8-OH-DPAT improves urethral continence by elevating the storage phase of urethral outlet resistance. Further, the drug increased bladder voiding efficiency by lowering the voiding phase of urethral resistance and enhancing the bladder and EUS-EMG burst activity.
**Perspectives and Significance**

In this study, EUS-EMG activity reemerged but was abnormal in rats 6 weeks after BPNI, and NC and BPNI exhibited a number of differences in EUS activity after 8-OH-DPAT treatment (Table 2). This increased the possibility that the neural pathways controlling the EUS might be altered. This is clinically relevant because, for patients with stress urinary incontinence (SUI) induced by pudendal nerve damage, drugs such as Duloxetine could exhibit different effects (30). This is due to the fact that “new” neural pathways might be controlling the EUS, and these pathways might have different responses to 5-HT receptor activation. However, further research would be needed to confirm these possibilities.

**ACKNOWLEDGMENTS**

This study was supported by grants from the National Science Council, Taiwan, R.O.C. (NSC97–2314-B-038–047-MY2 and NSC98–2221-E-038–005-MY2), and Taipei Medical University (NTUT-TMU-100-15).
REFERENCES


Figure legends

**Fig. 1**: Patterns of intravesical pressure under isovolumetric conditions (isovolumetric IVP) (top trace) and urethral perfusion pressure (UPP) (bottom trace) in an anesthetized rat without any treatment. The isovolumetric contraction of bladder was associated with relaxation of the urethra, and high-frequency oscillations (HFOs) of urethral pressure appearing during urethral relaxation. The recordings were quantified by the baseline UPP, the baseline HFOs, the maximum amplitude of reflex bladder contraction, the duration of reflex bladder contraction, as well as the frequency of isovolumetric bladder contraction.

**Fig. 2**: Typical patterns of isotonic transvesical pressure under continuous transvesical infusion (isotonic IVP) (top traces) and external urethral sphincter (EUS)-electromyography (EMG) (bottom traces) recorded in an anesthetized rat. (A) The bladder micturition contractions were induced by a constant rate (0.123 ml/min) of intravesical saline infusion, which was accompanied by large-amplitude EUS-EMG activity. Asterisks indicate micturition contractions. Micturition contraction could be divided into the three phases of bladder activity in (B). The burst period (BP) in the EUS-EMG is readily apparent in (C), which lasted from the end of phase 1 throughout the entire duration of phase 2. The BP included non-voiding and voiding BP, which were located in phases 1 and 2, respectively. The voiding BP was accompanied by the IVP superimposed with a series of high-frequency oscillations (HFOs), but the non-voiding BP did not cause any oscillation waves in the IVP. The critical pressure was defined as the point between phase 1 and 2 of IVP. (D) Individual bursts consisted of active and silent periods.

**Fig. 3**: Comparison of the transvesical pressure under isotonic transvesical infusion (isotonic IVP) (0.123 ml/min) among normal control (NC) and bilateral pudendal nerve-injured (BPNI) rats before (A and C) and after (B and D) 8-OH-DPAT (0.3
mg/kg) treatment. Arrows indicate the beginning of the saline infusion. Compared to NC rats, a slight increase was observed in the volume threshold (VT); but a significant decrease was noted in the inter-contraction interval (ICI) in BPNI rats. Drug treatment decreased the VT but increased the ICI in BPNI rats.

**Fig. 4:** Summary of parameters related to bladder activity including (A) volume threshold, (B) maximal bladder contraction amplitude, (C) bladder contraction duration, (D) inter-contraction interval, (E) voided volume, (F) residual volume, (G) voiding efficiency, and (H) critical pressure in normal control (NC) rats (n=12), and bilateral pudendal nerve-injured (BPNI) rats (n=12) before and after 8-OH-DPAT treatment (0.3 mg/kg, i.v.). An asterisk (*) indicates a significant difference (p<0.05) compared to NC rats before drug administration. Statistically significant differences between BPNI rats between before and after treatment are marked with a pound sign (#) (p<0.05).

**Fig. 5:** Comparison of the external urethral sphincter (EUS)-electromyographic (EMG) activity (bottom traces) in the three phases of the isotonic transvesical pressure (isotonic IVP) (top traces) among normal control (NC) and bilateral pudendal nerve-injured (BPNI) rats before (A and C) and after (B and D) 8-OH-DPAT (0.3 mg/kg) treatment. Right traces are expansions of the section in brackets in the left traces. Comparing the durations of phases before and after 8-OH-DPAT treatment in NC and BPNI rats, the durations of non-voiding burst activity were both elongated in phase 1, and the durations of burst activity were both elongated in phase 2. In phase 3, the EUS-EMG converted to tonic EUS-EMG either before or after drug treatment.

**Fig. 6:** Comparison of the amplitude of high-frequency oscillation (HFO) waves (top traces) and external urethral sphincter (EUS)-electromyography (EMG) (bottom traces) in phase 2 of isotonic transvesical pressure (isotonic IVP) among normal
control (NC) and bilateral pudendal nerve-injured (BPNI) rats before (A and C) and after (B and D) 8-OH-DPAT (0.3 mg/kg) treatment. Compared to NC rats, the number of silent periods (SPs) increased in BPNI rats, but no significant change appeared in the amplitude. In either NC or BPNI rats, the amplitude of the bladder HFO waves and the number of SPs were significantly reduced after drug administration, and the duration of the SP increased.

**Fig. 7:** Effects of 8-OH-DPAT (0.3 mg/kg, i.v.) treatment on the leak point pressure (LPP) in normal control (NC) \(n=6\), and bilateral pudendal nerve-injured (BPNI) rats \(n=6\). Additional LPP tests were conducted in BPNI rats before and after acute selective transection of the bilateral pelvic nerves innervating the iliococcygeous/pubococcygeous muscles (PN transection) and bilateral hypogatic nerves (HN transection). * Significant difference \((p<0.05)\) compared to BPNI rats before nerve transection and drug treatment. A significant difference \((\# p < 0.05)\) appeared in NC or BPNI rats between before and after drug administration.
**A**

NC rat

**Isotonic IVP (cmH\textsuperscript{2}O)**

30

0

1 min

**B**

NC rat after 8-OH-DAPT

**Isotonic IVP (cmH\textsuperscript{2}O)**

30

0

1 min

**C**

BPNI rat

**Isotonic IVP (cmH\textsuperscript{2}O)**

30

0

1 min

**D**

BPNI rat after 8-OH-DAPT

**Isotonic IVP (cmH\textsuperscript{2}O)**

30

0

1 min
A

Isotonic IVP (cmH$_2$O)

EUS-EMG (mV)

100 ms

NC rat

B

Isotonic IVP (cmH$_2$O)

EUS-EMG (mV)

100 ms

NC rat after 8-OH-DPAT

C

Isotonic IVP (cmH$_2$O)

EUS-EMG (mV)

100 ms

BPNI rat

D

Isotonic IVP (cmH$_2$O)

EUS-EMG (mV)

100 ms

BPNI rat after 8-OH-DPAT
Before drug

After 8-OH-DPAT

LPP (cmH2O)

<table>
<thead>
<tr>
<th></th>
<th>NC rat</th>
<th>BPNI rat</th>
<th>BPNI rat with PN transection</th>
<th>BPNI rat with PN and HN transections</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPP (cmH2O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before drug</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>After 8-OH-DPAT</td>
<td></td>
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</table>
Table 1: Effects of 8-OH-DPAT (0.3 mg/kg, i.v.) and WAY-100635 (0.1 mg/kg, i.v.) on isovolumetric intravesical pressure (isovolumetric IVP) and urethral perfusion pressure (UPP) in NC and BPNI rats

<table>
<thead>
<tr>
<th></th>
<th>Frequency of Bladder Contraction, Hz</th>
<th>Max. Amplitude of Bladder Contraction, cmH₂O</th>
<th>Duration of Bladder Contraction, cmH₂O</th>
<th>Baseline UPP, cmH₂O</th>
<th>Baseline HFOs in UPP, cmH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NC rat (n=6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1.16 (±0.27)</td>
<td>54.00 (±5.00)</td>
<td>23.00 (±0.34)</td>
<td>21.61 (±0.46)</td>
<td>15.95 (±0.59)</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>1.45* (±0.17)</td>
<td>54.32 (±2.72)</td>
<td>25.56* (±0.91)</td>
<td>22.75* (±0.46)</td>
<td>13.62* (±0.53)</td>
</tr>
<tr>
<td>WAY-100635</td>
<td>0.73* (±0.08)</td>
<td>54.99 (±2.76)</td>
<td>20.63* (±0.94)</td>
<td>18.48* (±0.83)</td>
<td>17.10* (±0.55)</td>
</tr>
<tr>
<td><strong>BPNI rat (n=6)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.34 (±0.16)</td>
<td>56.40 (±6.10)</td>
<td>23.42 (±0.46)</td>
<td>18.36* (±0.45)</td>
<td>14.50* (±0.41)</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>1.74 #,§ (±0.09)</td>
<td>57.10 (±6.57)</td>
<td>26.03 # (±0.94)</td>
<td>21.28 #,§ (±0.80)</td>
<td>12.90 # (±0.58)</td>
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<tr>
<td>WAY-100635</td>
<td>1.165 ¥, (±0.15)</td>
<td>55.10 (±6.10)</td>
<td>20.10 # (±0.93)</td>
<td>17.53 # (±0.67)</td>
<td>15.25 #, ¥ (±0.50)</td>
</tr>
</tbody>
</table>

Values are Mean±SD. * Statistically significant difference from the control value of NC rat, p < 0.05.
# Statistically significant difference from the control value of BPNI rat, p < 0.05.
§ Statistically significant difference from the value of NC rat after 8-OH-DPAT, p < 0.05.
¥ Statistically significant difference from the value of NC rat after WAY-100635, p < 0.05.
Table 2: Effects of 8-OH-DPAT (0.3 mg/kg, i.v.) on EUS-EMG activity in NC and BPNI rats

<table>
<thead>
<tr>
<th></th>
<th>Frequency of Burst Discharge, Hz</th>
<th>NC rat (n=12)</th>
<th></th>
<th>BPNI rat (n=12)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Burst Period, Silent Period, Active Period, Number of Silent Period</td>
<td>Phase 1</td>
<td>Phase 2</td>
<td>Phase 1</td>
<td>Phase 2</td>
</tr>
<tr>
<td></td>
<td>sec</td>
<td>msec</td>
<td>msec</td>
<td>msec</td>
<td>m sec</td>
</tr>
<tr>
<td>Control</td>
<td>0.87</td>
<td>3.92</td>
<td>69.1</td>
<td>98.5</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td>(±0.37)</td>
<td>(±0.43)</td>
<td>(±4.6)</td>
<td>(±3.3)</td>
<td>(±3.8)</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>3.71*</td>
<td>8.57*</td>
<td>125.2*</td>
<td>171.6*</td>
<td>99.4</td>
</tr>
<tr>
<td></td>
<td>(±0.91)</td>
<td>(±1.48)</td>
<td>(±8.9)</td>
<td>(±10.3)</td>
<td>(±3.9)</td>
</tr>
<tr>
<td>Control</td>
<td>0.67</td>
<td>3.94</td>
<td>59.7*</td>
<td>65.1*</td>
<td>103.0</td>
</tr>
<tr>
<td></td>
<td>(±0.31)</td>
<td>(±0.45)</td>
<td>(±6.0)</td>
<td>(±5.4)</td>
<td>(±5.0)</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>8.67 #</td>
<td>6.72 #</td>
<td>115.9 #</td>
<td>116.2 #</td>
<td>126.9 §</td>
</tr>
<tr>
<td></td>
<td>(±3.74)</td>
<td>(±1.12)</td>
<td>(±7.5)</td>
<td>(±8.9)</td>
<td>(±27.3)</td>
</tr>
</tbody>
</table>

Frequency of burst discharge is represented as the ratio between the number of silent period and its burst period. Values are Mean±SD. * Statistically significant difference from the control value of NC rat, p < 0.05. # Statistically significant difference from the control value of BPNI rat, p < 0.05. § Statistically significant difference from the value of NC rat after 8-OH-DPAT, p < 0.05.