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Serotonergic 5-HT$_{1A}$ receptor agonist (8-OH-DPAT) ameliorates impaired micturition reflexes in a chronic ventral root avulsion model of incomplete cauda equina/conus medullaris injury

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Abstract

Trauma to the thoracolumbar spine commonly results in injuries to the cauda equina and the lumbosacral portion of the spinal cord. Both complete and partial injury syndromes may follow. Here, we tested the hypothesis that serotonergic modulation may improve voiding function after an incomplete cauda equina/conus medullaris injury. For this purpose, we used a unilateral L5-S2 ventral root avulsion (VRA) injury model in the rat to mimic a partial lesion to the cauda equina and conus medullaris. Compared to a sham-operated series, comprehensive urodynamic studies demonstrated a markedly reduced voiding efficiency at 12 weeks after the VRA injury. Detailed cystometrogram studies showed injury-induced decreased peak bladder pressures indicative of reduced contractile properties. Concurrent external urethral sphincter (EUS) electromyography demonstrated shortened burst and prolonged silent periods associated with the elimination phase. Next, a 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), was administered intravenously at 12 weeks after the unilateral L5-S2 VRA injury. Both voiding efficiency and maximum intravesical pressure were significantly improved by 8-OH-DPAT (0.3-1.0 mg/kg). 8-OH-DPAT also enhanced the amplitude of EUS tonic and bursting activity as well as duration of EUS bursting and silent period during EUS bursting. The results indicate that 8-OH-DPAT improves voiding efficiency and enhances EUS bursting in rats with unilateral VRA injury. We conclude that serotonergic modulation of the 5-HT$_{1A}$ receptor may represent a new strategy to improve lower urinary tract function after incomplete cauda equina/conus medullaris injuries in experimental studies.

INTRODUCTION

Trauma to the lower thoracic and upper lumbar portions of the spine commonly results in injury of the sacral spinal cord and lumbosacral nerve roots. These lesions may present as a conus medullaris syndrome, which is characterized clinically by paralysis, sensory

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impairment, pain, as well as bladder, bowel, and sexual dysfunction (Maynard et al., 1997; Havton and Carlstedt, 2009). The neurological deficits are the most severe when the lesion is anatomically complete. A bilateral avulsion injury of the L5-S2 ventral roots in rats results in loss of parasympathetic inputs to the major pelvic ganglia and motoneuron denervation of the external urethral sphincter (EUS) muscle and therefore mimics many clinical features of a complete conus medullaris syndrome, including urinary retention and impaired micturition reflexes (Hoang et al., 2006; Chang and Havton, 2008). Traumatic lesions to the thoraco-lumbar spine may also result in an incomplete conus medullaris/cauda equina (CM/CE) injury with partial denervation of the lower urinary tract. However, studies on the effects of incomplete CM/CE injuries on lower urinary tract function in experimental models have been sparse.

Here, we first avulsed the left-sided L5-S2 ventral roots in female rats and performed cystometrogram (CMG) recordings and EUS electromyography (EMG) at 12 weeks post-operatively as an experimental model to study long-term effects of an incomplete CM/CE injury. The partial denervation of the lower urinary tract significantly reduced voiding efficiency. CMG studies showed that this compromise in lower urinary tract function was associated with a decreased peak bladder pressure during voiding. In addition, EUS EMG recordings showed shortened burst and prolonged silent periods during the elimination phase.

In an attempt to improve urinary function, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), a $5HT_{1A}$ receptor agonist, was next administered at 12 weeks after the VRA injury. A serotonergic approach to modulate lower urinary tract function was chosen, as the Onuf's nucleus as well as the sympathetic and parasympathetic nuclei of the lumbosacral spinal cord receive serotonergic innervation in multiple mammals (Mizukawa, 1980; Skagerberg and Björklund, 1985; Kojima et al., 1982, 1983). In rats, the $5HT_{1A}$ receptor subtype is associated with spinal cord regions implicated with the control of micturition (Thor et al., 1993).

The intravenous administration of 8-OH-DPAT restored normal voiding efficiency and improved both bladder contractions and EUS activity. We suggest that pharmacological activation of $5HT_{1A}$ receptors represents a new and potentially useful approach to augment functional micturition after incomplete CM/CE forms of spinal cord injury.

METHODS

Thirteen adult female Sprague-Dawley rats (180-230 g) were included in the study. The animals were divided into two groups: 1) A control group undergoing a laminectomy and dura opening (sham rats; n=6), and 2) an experimental group undergoing in addition a unilateral L5-S2 VRA injury (VRA rats; n=7). All animal procedures were carried out according to the standards established by the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). The experimental protocols were approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

Surgical procedures

A left sided L1-L4 laminectomy was performed in all rats, which were anesthetized using 2-2.5% isoflurane (Abbott Laboratories, IL, USA). The dura was opened under the visual guidance provided by a surgical microscope. In the VRA group, the left L5-S2 ventral roots were avulsed by applying constant traction with a pair of fine jeweler's forceps along the normal course of each individual root (Fig. 1A). The ventral roots were then deflected from the spinal cord to prevent any direct contact with the original injury site and any

spontaneous re-innervation. For all animals, a titanium mesh was placed over the laminectomy site to stabilize the vertebral column and protect the spinal cord from compression by the overlying paraspinous muscles (Nieto et al., 2005). The paraspinous muscles and skin were next sutured in layers, and all animals were allowed to recover. Post-operatively, Buprenex® (0.045 mg subcutaneously, Reckitt Benckiser Pharmaceutical Inc., VA, USA) was given every 12 hours for 48 hours to control any procedure-related pain. Trimethoprim/sulfamethoxazole oral suspension, USP (40mg/200mg per 5 ml, Hi-tech Pharmacal Co., Inc., NY, USA) was added to the drinking water (1 ml of oral suspension per 100 ml drinking water) for 10 days post-operatively for the prevention of infections. Bladders were manually expressed two times per day for three days after surgery to monitor functional bladder impairments. No rat developed any signs of urinary retention.

Urodynamic recordings

All rats underwent a comprehensive urodynamic evaluation at 12 weeks postoperatively as a terminal procedure. The functional assessments of the lower urinary tract included CMG and EUS EMG recordings. For this purpose, urethane (1.2 g/kg, subcutaneous administration) was given 1 hour before the start of the surgical procedures. After confirming absence of the toe pinch reflex, each animal was placed on a water circulating heating pad (Gaymar Industries, Inc., NY, USA). A polyethylene catheter (PE-50, BD Intramedic, NJ, USA) filled with 0.9% normal saline was inserted into the jugular vein for i.v. administration of 8-OH-DPAT (Sigma, MO, USA). A midline incision was made over the lower abdominal area to expose the urinary bladder. A PE-50 was inserted into the bladder through an incision at the apex of the bladder dome. The catheter was then tied by surgical suture. A total of four 50 μm PFA-insulated platinum–iridium wire electrodes (A-M Systems, WA, USA) were placed in the EUS. Each pair of wire electrodes were placed bilaterally in the EUS, which is located at the mid-urethra along with the ventral vaginal artery. One electrode was inserted at 2 mm distal to the bladder neck. The second electrode was placed 3 - 5 mm distal to the first electrode. Bilateral EUS EMG activity was recorded by connecting the more caudally placed electrodes on both sides. Before placement, about 2-3 mm of the most distal portion of each electrode was stripped of its coating, hooked upon the tip of a 27-gauge needle, and inserted into the EUS. The needle was next withdrawn, and the electrode tip was left embedded in the muscle. The abdominal incision was closed, while allowing the bladder catheter and two sets of wire electrodes to exit. The other end of the bladder catheter was attached to a 3-way stopcock that was also connected to a programmable infusion pump (World Precision Instruments Inc., FL, USA) and a pressure transducer (Biopac Systems Inc., CA, USA). The infusion rate was set as 0.12 ml per min. The free ends of each pair of the EUS EMG electrodes were attached to alligator clips that were connected to an amplifier (EMG100C, Biopac Systems Inc., CA, USA). The high and low pass filters were set at 100 and 500 Hz, respectively. The gain was 1k. The EUS EMG recordings were performed using a pair of bipolar electrodes on both sides of the EUS. A ground electrode for the EMG recordings was placed in the abdominal wall.

Urodynamic analysis

Concurrent CMG and EUS EMG recordings of reflex micturition were obtained in each rat using established outcome measures (Maggi et al., 1986; Chang and Havton, 2008, 2010). CMG measurements included inter-contraction interval (ICI), maximum intravesical pressure (IVP), contraction duration, resting pressure and pressure threshold (Figs. 1B, C). The bladder was emptied before the start of each recoding. Additional urodynamic outcome measures were obtained and calculated, including voided volume, total bladder volume, and voiding efficiency (VE). The voided volume was collected and measured from each voiding bladder contraction. The total bladder volume was calculated as the ICI multiplied by the

infusion rate for each voiding cycle during continuous recordings. The VE was calculated as the ratio of voided volume over total volume.

Quantitative studies of the EUS EMG recordings included measurements of maximum amplitude during tonic and burst activity, frequency and duration of bursting, as well as duration of active and silent periods during bursting (Cheng and de Groat, 2004). The peak amplitude of EUS tonic and bursting activity as well as the other EMG measures were analyzed using AcqKnowledge 4 Software (Biopac Systems Inc., CA, USA).

Pharmacological studies

Urodynamic evaluations were performed after the i.v. administration of 8-OH-DPAT (0.1-1.0 mg/kg, Sigma, MO, USA) to sham (n = 5) and VRA (n = 6) rats. Increasing 8-OH-DPAT doses of 0.1, 0.3, 0.5, and 1.0 mg/kg were administered and accumulated in each rat at 15-minute intervals. Urodynamic recordings were repeated in 11 out of 13 rats after drug administration.

Statistical analysis

CMG and EUS EMG measurements were obtained from three consecutive voiding cycles and averaged from each recording before and after drug administrations in each animal. The t test was used to compare the measurements from the sham and VRA series without drug. Repeated t test was used to compare the pharmacological effects on the sham operated and VRA injured rats at each dose. Values are presented as mean ± SE. A value of $p < 0.05$ was considered statistically significant. We applied JMP® (SAS, NC, USA) for the statistical analyses and GraphPad Prism (La Jolla, CA, USA) for the graph demonstration.

RESULTS

Comprehensive urodynamic studies were performed to examine micturition reflexes at 12 weeks after a unilateral L5-S2 VRA injury (n=7) and in sham-operated animals, which served as controls (n=6). Combined CMG and EUS EMG studies were performed in all subjects to assess the long-term effects of a unilateral denervation of the major pelvic ganglia and EUS on lower urinary tract function.

Long-term effects of a unilateral VRA injury on voiding function and micturition reflexes

Urodynamic studies first determined the voided volume and total bladder volume to calculate the VE in all rats (see Methods for details). The VE was significantly reduced after the VRA injury (55 ± 2 %; $p < 0.01$; Fig. 1D) compared to corresponding measurements from sham-operated controls (76 ± 3 %). To investigate possible underlying mechanisms contributing to the impaired lower urinary tract function, detailed analyses of both CMG and EUS EMG recordings were next performed.

CMG recordings showed a significant decrease for the maximum IVP at 12 weeks after a unilateral VRA injury (38 ± 9 cm H_2O; $p < 0.05$) compared to the sham procedure (67 ± 7 cm H_2O; Fig. 2A). In addition, the resting pressure was significantly increased in the VRA series (14 ± 3 cm H_2O, $p < 0.05$) compared to shams (10 ± 3 cm H_2O; Fig. 2A). There was no detectable difference for pressure threshold, contraction duration, and ICI between VRA and sham operated rats.

EMG recordings from the EUS muscle were obtained concurrently with the CMG studies in rats of the VRA and sham series. All rats showed EUS tonic activity during the filling phase, and EUS bursting was demonstrated during the voiding phase (Figs. 1B, C). Bilateral EUS EMG recordings showed a significantly reduced duration of the voiding-associated EUS

bursting in the VRA series (3.2 ± 0.4 s; $p < 0.05$) compared to the sham operated series (6.1 ± 1.0 s) (Fig. 2A). However, there was no detectable difference for the bursting frequency between the VRA and sham operated rats.

EMG recordings were next obtained from the left and right sides of the EUS to study the effects of a unilateral L5-S2 VRA injury on EUS tonic and bursting activity on both the ipsi- and contralateral sides (Fig. 2B). The amplitude of EUS tonic activity was significantly larger in VRA injured rats on both the ipsilateral (0.091 ± 0.012 mV; $p < 0.05$) and contra-lateral (0.064 ± 0.008; $p < 0.05$) sides when compared to the corresponding EMG recordings from sham operated animals (0.038 ± 0.005 mV and 0.031 ± 0.002 mV, respectively, Fig. 2B). In addition, the amplitude of the EUS tonic activity was significantly larger on the ipsilateral side compared to the contralateral side in VRA injured rats ($p < 0.05$). There was no detectable side difference for the amplitude of EUS tonic activity in the sham operated series.

The amplitude of the EUS bursting was also significantly larger on both the ipsilateral (0.192 ± 0.037 mV; $p < 0.05$) and contralateral (0.121 ± 0.011 mV; $p < 0.05$) sides in VRA injured rats when compared to the corresponding EMG recordings from sham operated animals (0.112 ± 0.024 mV and 0.086 ± 0.005 mV, respectively, Fig. 2B). The amplitude of the EUS bursting was also significantly larger on the ipsilateral side compared to the contralateral side in VRA injured rats ($p < 0.05$). There was no side difference detected for the corresponding amplitudes of EUS bursting in the sham operated animals.

Next, we examined in more detail the composition of EUS bursting and determined the duration of both the active and silent periods on the ipsi- and contralateral sides in VRA injured and sham operated animals. The duration of the active period for the EUS bursting was not significantly different between the ipsilateral and contralateral sides within the VRA injured or sham operated series, and no difference was detected with regards to the duration of the active period when comparing the ipsi- or contralateral sides between the VRA injury and sham operated rats. However, the duration of the silent period of the EUS bursting was significantly longer on both the ipsilateral side (0.148 ± 0.031 ms; $p < 0.05$) and contralateral side (0.125 ± 0.019 ms; $p < 0.05$) in rats after the unilateral VRA injury when compared to the corresponding recordings in the sham operated series (0.098 ± 0.009 ms and 0.093 ± 0.006 ms, respectively, Fig. 2B). In addition, the duration of the silent period of the EUS bursting was significantly longer on the ipsilateral side compared to the contralateral side in VRA injured rats ($p < 0.05$).

Effects of 8-OH-DPAT on voiding function and micturition reflexes after a chronic unilateral VRA injury

After the initial set of urodynamic recordings were completed, we investigated the effects of intravenous administration of 8-OH-DPAT in rats of both the VRA injury series (n=6) and sham operated animals (n=5) at 12 weeks post-operatively. The additional urodynamic studies were performed after the administration of increasing doses of 8-OH-DPAT at 0.1, 0.3, 0.5, and 1.0 mg/kg. Comprehensive sets of urodynamic recordings were obtained within 15 minutes after each drug administration. Representative examples of urodynamic recordings from a VRA injured animal is shown in Fig. 3.

CMG recordings demonstrated a prominent effect of 8-OH-DPAT on micturition reflexes in the rats of the VRA injury group (Fig. 4). Specifically, voiding function was improved in the VRA series with a significant increase in VE from 54 ± 2 % (n=7) before drug administration to a VE of 70 ± 5 % (n=6) after the administration of 8-OH-DPAT at a dose of 0.3 mg/kg ($p < 0.05$). Similar significant improvements in VE were also demonstrated after the administration of 8-OH-DPAT at the higher doses of 0.5, and 1.0 mg/kg ($p < 0.05$).

The improved VE was associated with a significant increase in the duration of the ICI for all administered doses of 8-OH-DPAT compared to the corresponding CMG recordings without drug in the VRA injury series ($p < 0.05$). There was no detectable effect of 8-OH-DPAT on contraction duration, resting pressure, pressure threshold, or max IVP at any of the administered doses in the VRA injury series.

Bilateral EUS EMG recordings were performed to determine the bursting period and frequency of bursts associated with reflexive voiding both before 8-OH-DPAT administration and after increasing doses (0.1, 0.3, 0.5, and 1.0 mg/kg) of the drug (Fig. 5). The EUS bursting duration was significantly increased in both the VRA and sham series after 8-OH-DPAT administration at all doses compared to the same conditions before the drug was given ($p < 0.05$). In contrast, the frequency of bursts was significantly decreased after the administration of 8-OH-DPAT at all doses in both the VRA and sham series compared to the corresponding recordings before drug administration ($p < 0.05$).

Next, left and right sided EUS EMG recordings were obtained in rats of both the VRA and sham series before and after 8-OH-DPAT administration at increasing doses (Fig. 5). For each series, the EUS activity before and after drug administration was compared. In the VRA series, EUS EMG recordings showed an increase in the amplitude of the ipsilateral EUS tonic activity after 8-OH-DPAT administration at doses of 0.3 and 0.5 mg/kg ($p < 0.05$), but no drug effect was found for the contralateral EUS tonic activity. In the sham series, no drug effect was detected for EUS tonic activity on either side. The amplitude of ipsilateral EUS bursting was also increased in the VRA series by 8-OH-DPAT at doses of 0.1, 0.3, and 0.5 mg/kg, whereas the contralateral EUS bursting in the same animals showed an increase in amplitude only at doses of 0.3 and 0.5 mg/kg. The sham operated series showed increased amplitude of the ipsilateral EUS bursting after administration of 8-OH-DPAT at doses of 0.3, 0.5, and 1.0 mg/kg, whereas the amplitude of the contralateral EUS bursting in the same animals did not change in response to any doses of the administered drug.

The active period was increased in the VRA series on the ipsilateral side after administration of 8-OH-DPAT at a dose of 0.5 and 1.0 mg/kg iv. Also, in the VRA series, the silent period was prolonged by 8-OH-DPAT on both the ipsi- and contralateral sides at doses at doses of 0.3, 0.5, and 1.0 mg/kg. For comparison, the sham operated series showed no change in the duration of the active period after administration of any of the doses of 8-OH-DPAT, whereas the silent period was prolonged by the drug at doses of 0.3, 0.5, and 1.0 mg/kg.

DISCUSSION

Here, we show that a lesion, which is limited to a unilateral L5-S2 VRA injury in rats, resulted in impaired voiding function at 12 weeks post-operatively. Although no urinary retention was detected during the post-operative period in awake animals and no daily bladder expressions were indicated, detailed urodynamic studies, using cystometrogram and EUS EMG recordings under urethane anesthesia, showed a reduced VE and impaired micturition reflexes. Our urodynamic studies demonstrated impaired bladder contractile properties and altered EUS activation patterns, which may both have contributed to the overall reduced VE. However, the VE was significantly increased after the administration of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, with associated improved bladder contractions and EUS EMG activity in the VRA series.

Our cystometrogram studies showed a reduced max IVP and an increased RP at 12 weeks after the unilateral L5-S2 VRA injury. In light of the decreased VE, the increased RP may reflect an increased residual volume. In previous studies, a similar decrease in the maximum

IVP has been demonstrated at two weeks after a unilateral transection injury of the pelvic and hypogastric nerve in rats (Kwon et al., 2005). However, the unilateral pelvic and hypogastric nerve injury did not result in any detectable change in bladder capacity or residual volume (Kwon et a., 2005). Interestingly, in the present study, the partial denervation of the EUS after a unilateral L5-S2 VRA may have contributed to both the decreased bladder contractile properties and the increased residual volume, as a bilateral pudendal nerve injury in rats has been shown to decrease bladder contraction amplitudes during voiding and increase the residual volume at 6 weeks post-operatively (Peng et al., 2006; Chen et al., 2011).

The amplitude of both EUS tonic and bursting activity was increased at 12 weeks after the unilateral L5-S2 VRA injury. It is well established that partial denervation of skeletal muscle in experimental models result in collateral sprouting and an increase in the motor unit size (Edds, 1953; Luff et al., 1988; Rafuse and Gordon, 1992). In EMG studies of humans, a chronic partial denervation injury of skeletal muscle results in large motor unit potentials, which may be encountered in subjects with e.g. diabetes neuropathy, carpal tunnel syndrome, amyotrophic lateral sclerosis, and spinal muscular atrophy (Stålberg et al., 1996; Chichkova and Katzin, 2010; Mills, 2011). Although the literature on the degree of bilateral innervation of the EUS muscle is sparse, our findings of increased amplitude of EUS activity on both the ipsi- and contralateral sides in long-term studies after a unilateral L5-S2 VRA injury suggests that collateral sprouting of spared motor fibers with subsequent increase in motor unit size may take place bilaterally in the partially denervated EUS muscle. Furthermore, the increased gain of EUS tonic activity in VRA series may have resulted from the increased resting pressure in the presence of poor voiding efficiency.

In the VRA series, both EUS bursting and silent periods were affected. Interestingly, the amplitude of EUS bursting was increased in VRA series, possibly related to collateral sprouting in the periphery and increased motor unit size. Although the silent period was significantly increased on the ipsilateral side compared to the contralateral side in VRA series, the degree of the change was modest. This small change in silent period duration did not affect significantly the bursting frequency between the ipsilateral and contralateral sides.

Following a unilateral L5-S2 VRA injury, our EUS EMG recordings showed a reduced bursting period at 12 weeks post-operatively. Similar reductions of EUS bursting have been demonstrated after selective injury to the motor branch of the pudendal nerve. For instance, EUS EMG studies in rats showed that an acute transection of the unilateral pudendal nerve injury resulted in a significantly reduced bursting period in association with a reduced VE (Peng et al., 2008). Other studies have also noted a potential relationship between EUS bursting and VE. For instance, use of neuromuscular blocking agents resulted in absence of EUS bursting and resulted in a significant increase in the residual volume (Conte et al., 1991).

Multiple previous pharmacological studies have demonstrated that micturition reflexes may be modulated by 8-OH-DPAT, a 5-HT_{1A} receptor agonist, in neurologically intact and impaired rats (Lecci et al., 1992; de Groat, 2002; Chang et al., 2006, 2007; Cheng and de Groat, 2010, 2011). 8-OH-DPAT provides a stimulatory effect on the motility of the urinary bladder, demonstrated by an elicited micturition reflex when the urinary bladder in rats was filled to a subthreshold level (Lecci et al., 1992). EMG recordings from the rat EUS have shown increased spontaneous activity as well as increased evoked tonic activity and EUS bursting in association with decreased peak bladder pressure during comprehensive urodynamic recordings in both intact and chronically spinalized rat after 8-OH-DPAT (Chang et al., 2006, 2007; Cheng and de Groat, 2010). In the present study, the increased EUS activity during bladder infusion did not improve VE in the sham rats, possibly due to

decreased peak bladder pressure and prolonged ICI indicating an increased bladder capacity after 8-OH-DPAT. However, in VRA rats, the peak bladder pressure was not affected by 8-OH-DPAT, and the VE was improved in VRA rats after 8-OH-DPAT. Furthermore, in the diabetic rat, 8-OH-DPAT increased voided volume and reduced residuals, thereby improving voiding efficiency (Gu et al., 2012). Many of the above observed micturition effects of 8-OH-DPAT in the rat were reversed by the administration of the 5-HT_{1A} receptor antagonist, WAY-100635 (Testa et al., 1999; Kakizaki et al., 2001; Chang et al., 2007; Gu et al., 2012; Cheng and de Groat, 2011).

In the present study, we demonstrate that 8-OH-DPAT markedly increased VE after a VRA injury. The drug effect here was associated with a prolonged ICI as well as increased maximum amplitude and duration of EUS bursting. The direct effect of 8-OH-DPAT in VRA rats was to alter the pattern of EUS bursting, which promotes the bladder emptying. Indirectly, the augmented EUS bursting increased VE and prolonged ICI in responding to bladder emptying. The association of increased EUS bursting and improved VE after 8-OH-DPAT adds further support to the previous notion that rhythmic contractions and relaxations taking place in the EUS muscle are important for emptying the bladder efficiently in the rat (Maggi et al., 1986). In addition, Cheng and de Groat (2010) demonstrated a prolonged silent period and bursting duration during EUS bursting in 8-OH-DPAT treated rats. 8-OH-DPAT also suppressed the frequency of EUS bursting in intact rats and rats with bilateral pudendal nerve injury (Chen et al. 2011). These observations are consistent with our findings on the effect of 8-OH-DPAT in both sham and VRA operated rats.

Activation of 5-HT_{1A} receptor in the central nervous system with 8-OH-DPAT is known to enhance micturition reflexes (Lecci et al., 1992). The excitatory effect of 8-OH-DPAT on EUS bursting was identified between T8 and L4 spinal levels in rats with chronic spinal cord injury (Chang et al., 2007). Thus, in the present study, 8-OH-DPAT consistently induced EUS bursting and facilitated bladder emptying in VRA rats. The exact location for the effect of 8-OH-DPAT is not known. Since the spinal cord is anatomically intact, 8-OH-DPAT may have acted at the level of injury, at the more rostral segments putatively responsible for EUS bursting, or at the level of pontine micturition center.

We conclude that the unilateral L5-S2 VRA injury model in rats is a new and practical model for studies of the effects of incomplete cauda equina injuries on functional micturition. This experimental model is also useful for the evaluation of new experimental therapeutics, as autonomic and somatic motor functions may be evaluated both separately and together, as demonstrated by our CMG, EUS EMG recordings and VE evaluations. In addition, our studies demonstrate that 8-OH-DPAT, a 5-HT_{1A} receptor agonist, may reverse impaired micturition reflex function. Therefore, efforts aimed at increasing the activation of 5-HT_{1A} receptors may represent a potentially useful therapeutic approach for reversing lower urinary tract dysfunction after incomplete cauda equina injuries.

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A list of nonstandard abbreviations

8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)-tetralin
CM/CE	conus medullaris/cauda equina
CMG	cystometrogram
EMG	electromyography
EUS	external urethral sphincter
ICI	inter-contraction interval
IVP	intravesical pressure
VE	voiding efficiency
VRA	ventral root avulsion

Highlights

1. Chronic incomplete cauda equina injury causes poor lower urinary tract function
2. 5-HT_{1A} receptor agonist prolonged the relaxation of urethral sphincter
3. 5-HT_{1A} receptor agonist improves voiding efficiency after cauda equina injury

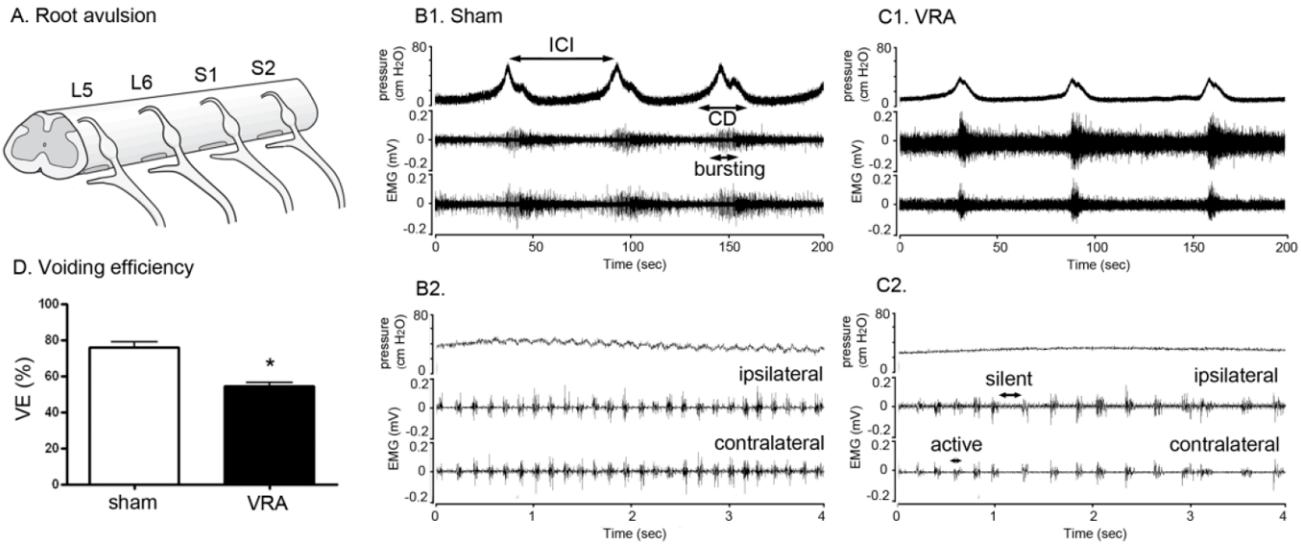

Fig. 1.

Anatomical schematic (A) showing the avulsion of L5-S2 ventral roots from the spinal cord. Representative examples of urodynamic recordings in sham (B1-B2) and VRA (C1-C2) animals. Upper tracings (B1 and C1) show the intravesicle pressure during the voiding cycles. Middle and bottom tracings (B1 and C1) respectively show the ipsilateral and contralateral external urethral sphincter (EUS) electromyogram (EMG) associated with voiding cycles. Faster time periods of EUS EMG tracings are shown from sham (B2) and VRA series (C2). Note marked reduction in voiding efficiency (VE) after VRA injury (D). CD: contraction duration. ICI: inter-contraction interval.

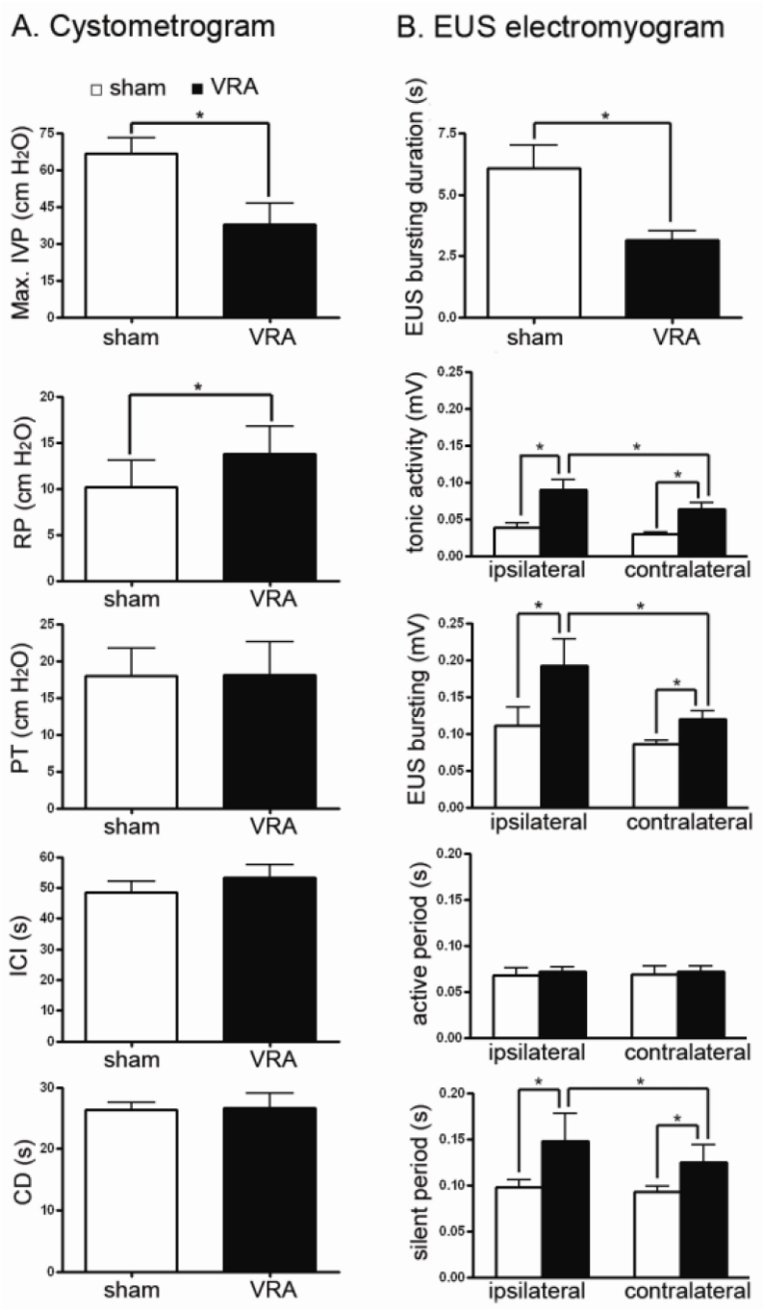

Fig. 2.

Statistical analysis of cystometrogram and EUS EMG recordings in sham and VRA series without drug administration. * indicates significant statistical differences at $p < 0.05$. RP: resting pressure. PT: pressure threshold. IVP: intravesicle pressure.

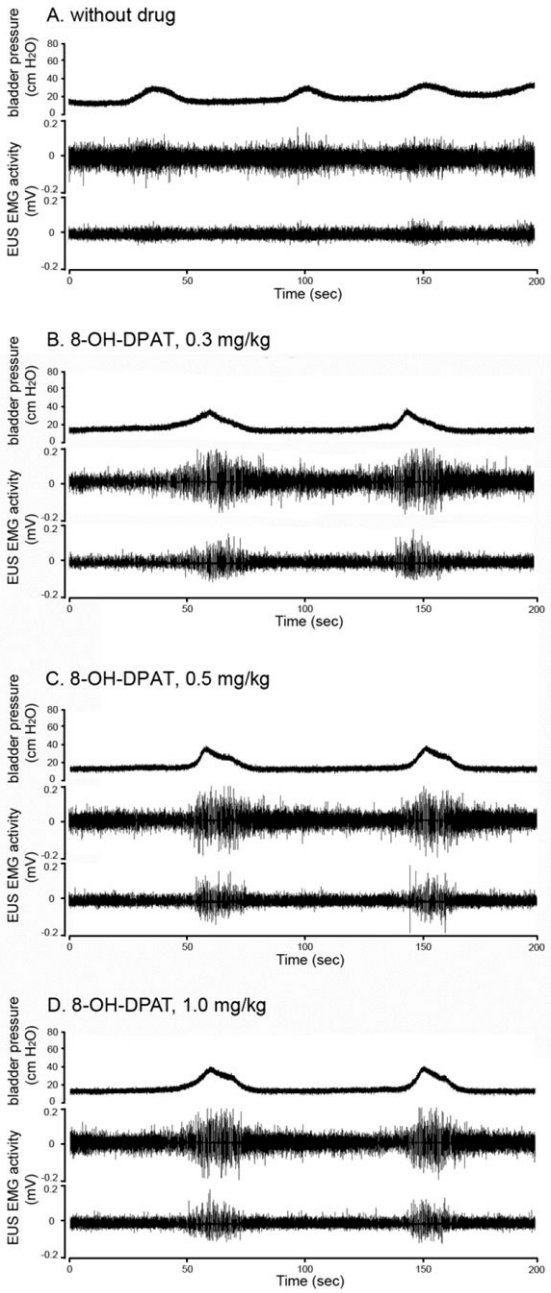

Fig. 3.

Representative examples of urodynamic recordings from a VRA injured animal before and after 8-OH-DPAT. Note that bladder contraction and EUS activation remain coordinated after the VRA injury. After 8-OH-DPAT, the contraction amplitude and duration of EUS bursting was increased.

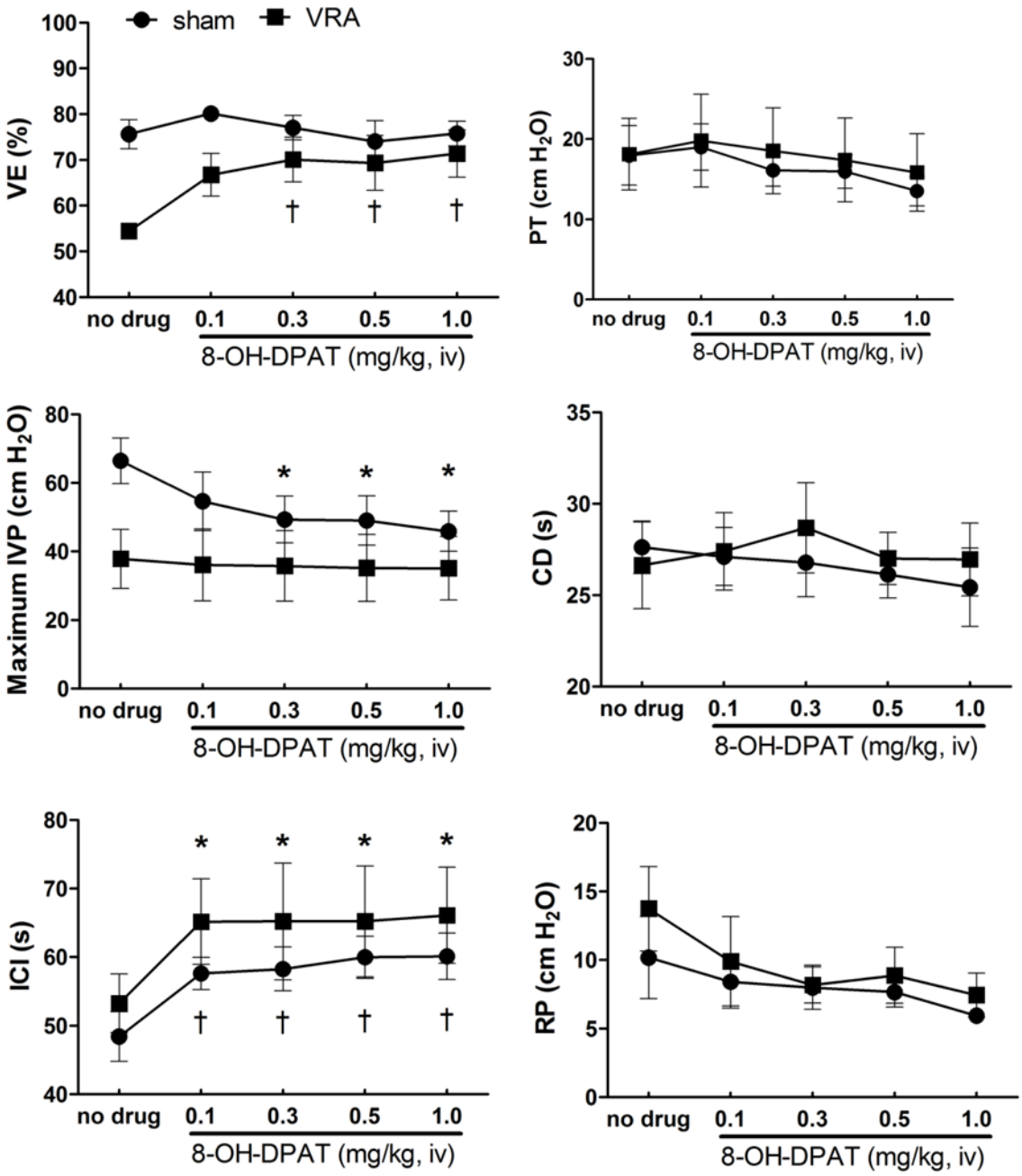

Fig. 4.

Dose response curve for 8-OH-DPAT effect on cystometrogram recordings in both sham and VRA series. * indicated the effect of 8-OH-DPAT in sham animals compared to the control condition without drug in the same group. † indicated the effect of 8-OH-DPAT in VRA animals significantly ($p < 0.05$) changed compared to the control condition without drug in the same group.

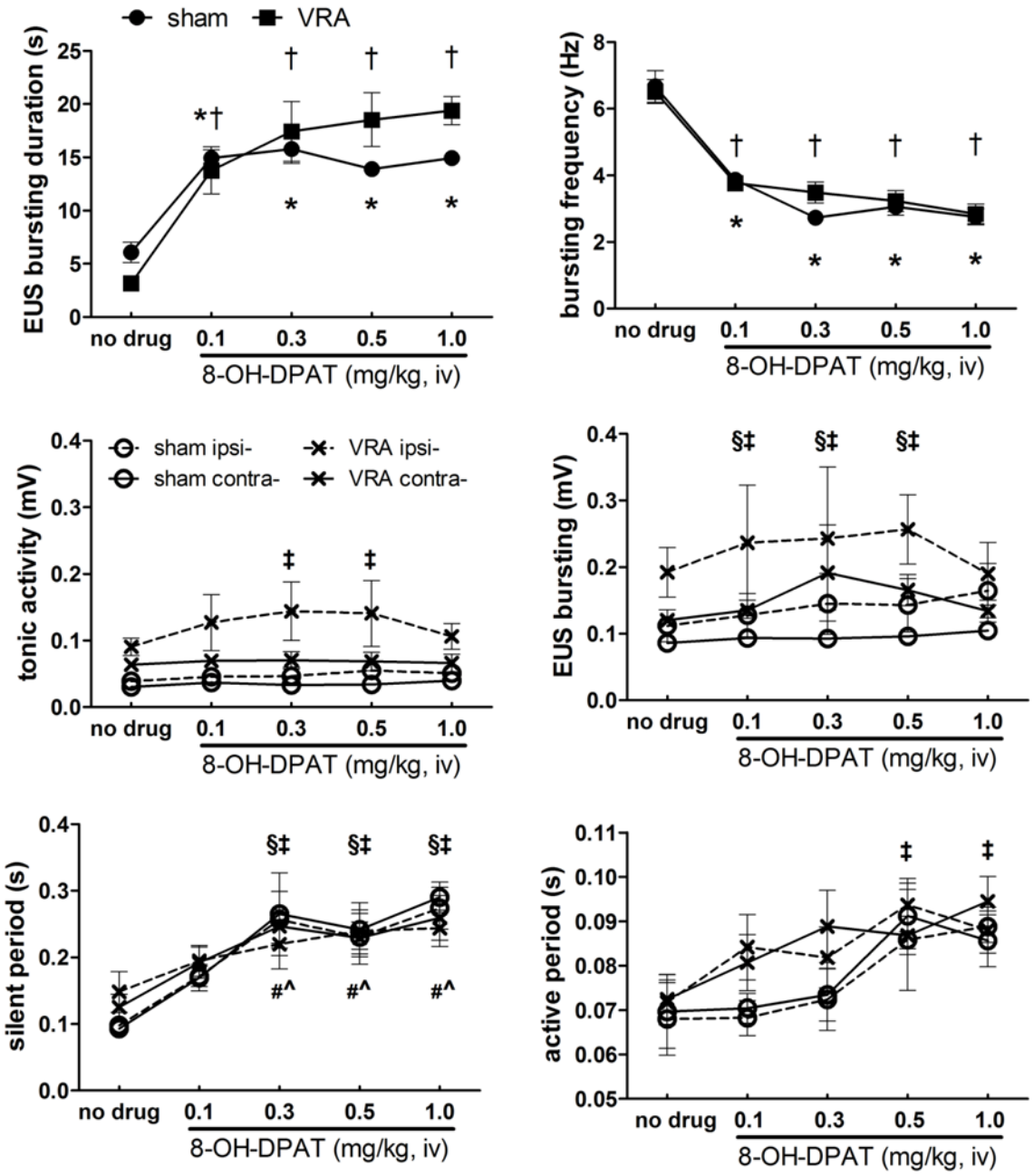

Fig. 5.

Dose response curve of 8-OH-DPAT effect on EUS EMG in both sham and VRA series. * indicates significant effect of 8-OH-DPAT in sham animals ($p < 0.05$) compared to the control condition without drug in the same group. † indicates significant effect of 8-OH-DPAT in VRA animals ($p < 0.05$) compared to the control condition without drug in the same group. ^ and # indicate significant change ($P < 0.05$) for shams on ipsilateral and contralateral sides, respectively, compared to the control condition without drug. ‡ and § indicate significantly change ($P < 0.05$) for VRA on ipsilateral and contralateral sides, respectively, compared to the control condition without drug.