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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 impairment, pain, as well as bladder, bowel, and sexual dysfunction (Havton and Carlstedt, 2009; Maynard et al., 1997). 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 (Chang and Havton, 2008; Hoang et al., 2006). Traumatic lesions to the thoracolumbar 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 postoperatively 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.

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.

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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 (Kojima et al., 1982, 1983; Mizukawa, 1980; Skagerberg and Björklund, 1985). 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. Postoperatively, Buprenex® (0.045 mg subcutaneously, Reckitt Benckiser Pharmaceutical Inc., VA, USA) was given every 12 h for 48 h to control any procedure-related pain. Trimethoprim/sulfamethoxazole oral suspension, USP (40 mg/200 mg 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 postoperatively for the prevention of infections. Bladders were manually expressed two times per day for 3 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 h 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 was 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.

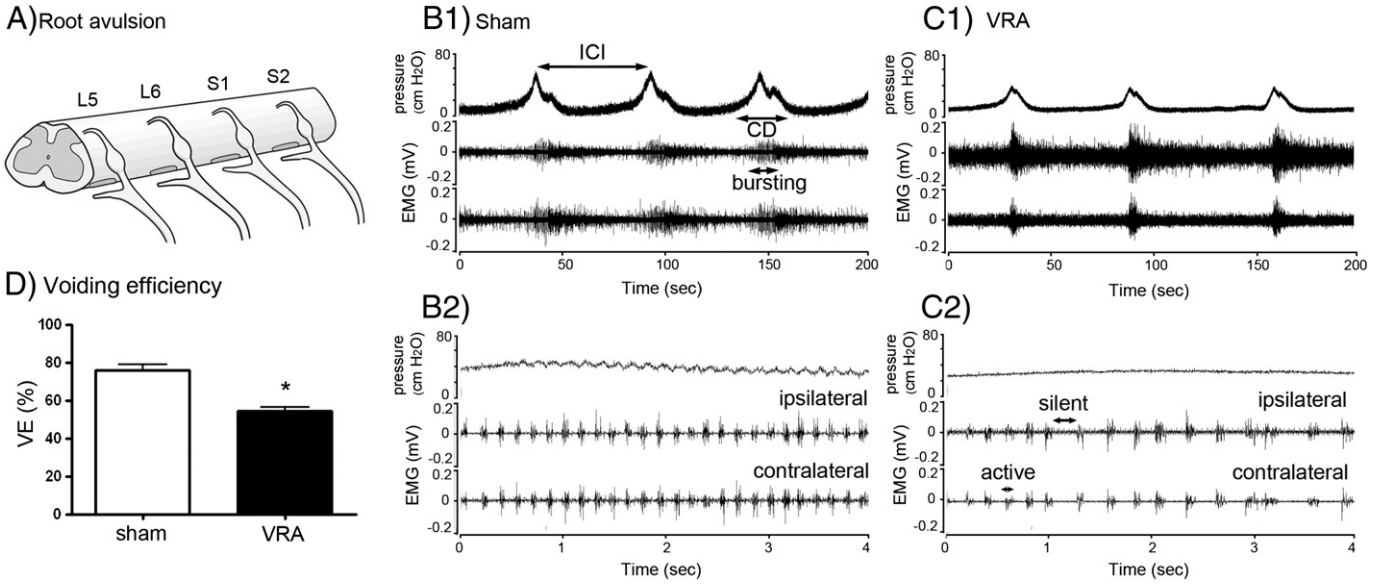

Fig. 1. Anatomical schematic (A) showing the avulsion of L5–S2 ventral roots from the spinal cord. Representative examples of urodynamic recordings in sham (B1–B2) and VRA (C1–C2) animals. Upper tracings (B1 and C1) show the intravesicle pressure during the voiding cycles. Middle and bottom tracings (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.