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Mechanisms by which the serotonergic system inhibits micturition in rats

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ABSTRACT

Aims: Serotonergic neurons and amino acid neurons are involved in the central nervous control of lower urinary tract function. We investigated the role of the serotonergic system in the central regulation of micturition, as well as the relationship between serotonergic neurons and amino acid neurons in the lumbosacral cord of rats.
Main methods: Under urethane anesthesia, bladder and urethral activity were recorded before and after intrathecal injection of serotonin (5-hydroxytryptamine: 5-HT), a 5-HT_{2A} receptor antagonist (ketanserin: KET), or KET + 5-HT by isovolumetric cystometry and measurement of the urethral pressure in intact rats and rats with hypogastric nerve transection (HGNT). Amino acid levels in the lumbosacral cord were also measured after intrathecal injection of 5-HT in intact rats.
Key findings: In intact rats, intrathecal injection of 5-HT transiently abolished rhythmic bladder contractions, decreased the maximal bladder contraction pressure, and increased the intravesical baseline pressure and the urethral baseline pressure. Intrathecal injection of KET + 5-HT also transiently abolished rhythmic bladder contractions. In HGNT rats, intrathecal injection of 5-HT transiently abolished rhythmic bladder contractions and increased the urethral baseline pressure. Intrathecal injection of 5-HT decreased the level of glycine in the lumbosacral cord.
Significance: The serotonergic system may be involved in blocking the afferent pathway of the micturition reflex, increasing sympathetic activity, and secondary promotion of urethral contraction through inhibition of glycinergic neurons in the lumbosacral cord. 5-HT_{2A} receptors may be involved in these effects on the bladder and urethra. Therefore, the serotonergic system may play a role of the maintenance of urine storage.

Introduction

Serotonergic neurons are located in the raphe nuclei of the brainstem and project their axons widely throughout the brain and spinal cord, including the dorsal horns of the spinal cord, the sympathetic nuclei, the parasympathetic nuclei, and the urethral sphincter motor nucleus (Onuf's nucleus) (Bowker et al. 1981; Thor et al. 1993; Helton et al. 1995; Xu et al. 2007). Onuf's nucleus has a major association with serotonin (5-hydroxytryptamine: 5-HT). Compared with other parts of the ventral horn in the sacral spinal cord, Onuf's nucleus shows dense staining for 5-HT in nerve terminals and also contains 5-$HT_{1A, B, C}$, 5-$HT_{2A, B, C}$ and 5-HT_{5A} receptors (Thor et al. 1993; Helton et al. 1995; Xu et al. 2007). Various studies have shown that 5-HT and its receptors are involved in the central control of lower urinary tract function (Helton et al. 1995; Sugaya et al. 1998; de Groat and Yoshimura 2001; de Groat 2002; Thor 2003: Ramage 2006). Electrical stimulation of the brainstem raphe nuclei, where 5-HT-containing neurons are located, inhibits rhythmic bladder contraction (Sugaya et al. 1998). S-norfluoxetine, a selective 5-HT reuptake inhibitor, was reported to increase bladder capacity and sphincter activity (Fuller et al. 1992; Wong et al. 1992). Therefore, 5-HT neurons appear to inhibit bladder activity. Among the various 5-HT receptors, 5-HT_{2A} receptors have been identified throughout the entire spinal cord, with a high level of expression in the sympathetic preganglionic cells, dorsal horn, and motoneurons (including the Onuf's nucleus) (Doly et al. 2004). Intravenous and intrathecal administration of a 5-HT_{2A} receptor agonist increases electromyographic activity in the external urethral sphincter (Mbaki and Ramage 2008).

Some amino acids are known to be important neurotransmitters and are involved in the central mechanisms regulating micturition and urine storage (Shapiro 1997). For example, glutamate is a major excitatory neurotransmitter that facilitates the micturition reflex (Mayer and Westbrook 1987). While γ-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the central nervous system and it inhibits the micturition reflex at the level of the lumbosacral cord (Igawa et al. 1993). Glycine is another important inhibitory neurotransmitter, and higher concentrations of glycine are found in the spinal cord than in supraspinal regions (Elekes et al. 1986). Therefore, both amino acid neurons and 5-HT neurons have a role in the central regulation of lower

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urinary tract function. However, the relations between these neurons involved in the regulation of lower urinary tract function are unknown. In order to clarify the role of 5-HT neurons in the central nervous control of lower urinary truct function, as well as the relations between 5-HT neurons and amino acid neurons, we examined the effects of intrathecal injection of 5-HT or a 5-HT_{2A} receptor antagonist (ketanserin: KET) on bladder and urethral activity in intact rats and rats with hypogastric nerve transection (HGNT rats). Amino acid levels in the lumbosacral cord were also investigated.

Materials and methods

Fifty-two female Sprague–Dawley rats weighing 200 to 250 g were used in this study. The study protocol was approved by the Institutional Animal Care and Use Committee of the University of the Ryukyus.

Isovolumetric cystometry and measurement of urethral pressure before and after intrathecal injection of drugs

Thirty-two rats were anesthetized with urethane (0.3 g/kg intraperitoneally and 0.9 g/kg subcutaneously, for a total dose of 1.2 g/kg). A lower abdominal incision was made and both ureters were transected, after which the distal ends were ligated. The bladder neck was also ligated to produce an isovolumetric state. A polyethylene catheter (PE-50) was inserted through the dome of the bladder for cystometry, and another polyethylene catheter (PE-50) was inserted into the urethra through the external urethral meatus for measurement of the urethral pressure. Laminectomy was performed at L3, and a polyethylene catheter (PE-10) was inserted into the subarachnoid space and was advanced to the level of L6-S1 spinal cord. In 8 rats (HGNT rats), the bilateral hypogastric nerves were transected at the level of the aortic bifurcation to block sympathetic activity.

Bladder and urethral activity were monitored via the bladder and urethral catheters connected to a pressure transducer and a pump for saline infusion through a three-way stopcock. The bladder was filled with physiological saline (0.05 ml/min) to above the threshold volume in order to induce isovolumetric rhythmic contractions. Physiological saline was also infused slowly (0.01 ml/min) and continuously into the urethral catheter to measure the urethral pressure. When rhythmic bladder contractions had been stable for at least 30 min, the interval between bladder contractions, the maximal contraction pressure of the bladder, the intravesical baseline pressure, and the urethral baseline pressure were measured. In intact rats, after bladder contractions had been stable for at least 30 min, physiological saline (5 μl), 5-HT (0.0001–1 μg in a volume of 5 μl injected every 15–30 min), KET (0.001–1 μg in a volume of 5 μl injected every 15–30 min), or KET + 5-HT (1 μg each in a volume of 5 μl; 5-HT was injected at 1 min after KET) was injected into the intrathecal catheter using a microsyringe (10 μl; Hamilton, Reno, NV), and the changes of bladder and urethral activity were recorded. Each agent was dissolved in physiological saline before administration and the intrathecal catheter was clamped for 1 min after injection. Bladder and urethral parameters were calculated and averaged for 15–30 min from the start of intrathecal injection of each agent, and the results were compared with the control recordings obtained for 30 min before injection of physiological saline. In HGNT rats, physiological saline (5 μl) and 5-HT (1 μg in a volume of 5 μl) were injected intrathecally. The changes of bladder and urethral activity were recorded and the results were compared with the control recordings obtained for 30 min before injection of physiological saline.

Measurement of amino acid levels in the lumbosacral cord after intrathecal injection of physiological saline or 5-HT in intact rats

In intact rats ($n = 20$) under urethane anesthesia (0.3 g/kg intraperitoneally and 0.9 g/kg subcutaneously, for a total dose of 1.2 g/kg), intrathecal injection of physiological saline (5 μl, control group, $n = 10$) or 5-HT (1 μg in a volume of 5 μl, 5-HT group, $n = 10$) was performed using a microsyringe. At 7 min after intrathecal injection, the rats were sacrificed, and the lumbosacral cord was immediately removed. This duration (7 min) was decided from the average of the duration of disappearance of bladder contraction after intrathecal injection of 5-HT. Then the cord was homogenized in cold water (1.0 ml/0.1 g tissue), and the homogenate was centrifuged at 15,000 rpm for 5 min. Heat denaturation of the supernatant was performed with 95 °C for 10 min and it was centrifuged with an Ultrafree C3 THK filter (Millipore, Bedford, MA, USA) for deproteinization. Then the samples were immediately stored − 80 °C until assay. The levels of four amino acids (glutamate, aspartate, GABA and glycine) were measured in each sample by a capillary electrophoresis system (Hewlett-Packard ^{3D}CE, Germany) using a basic anion buffer. Samples were injected at 50 mbar for 4 s and were separated at a constant voltage of − 30 kV at 30 °C in a capillary tube coated internally with polyamide-fused silica (Yokogawa Analytical Systems, Tokyo, Japan). The tube had a total length of 80.5 cm (an effective length of 72 cm) and an internal diameter of 75 μm.

Statistical analysis

Results are reported as the mean ± standard error (SE). Student's *t*-test was used for statistical analysis and $P < 0.05$ was considered to indicate statistical significance.

Results

Changes of bladder and urethral activity after intrathecal injection of 5-HT in intact rats

In intact rats, the interval between bladder contractions (1.5 ± 0.4 min), the maximal contraction pressure (40.5 ± 2.5 cm H_2O), the intravesical baseline pressure (14.0 ± 3.6 cm H_2O), and the urethral baseline pressure (16.1 ± 5.2 cm H_2O) were all stable (control) before injection of physiological saline. Urethral pressure decreased during bladder contraction. Intrathecal injection of physiological saline did not change any of the parameters of bladder and urethral activity. After intrathecal injection of 5-HT (0.01–1 μg), rhythmic bladder contractions were abolished and the time until the reappearance of contractions was 3–18 min (average: 7 min) (Fig. 1A). The duration for which bladder contractions were abolished had a tendency of dose dependent, but there was no statistically significant difference. The interval between bladder contractions after re-establishment was

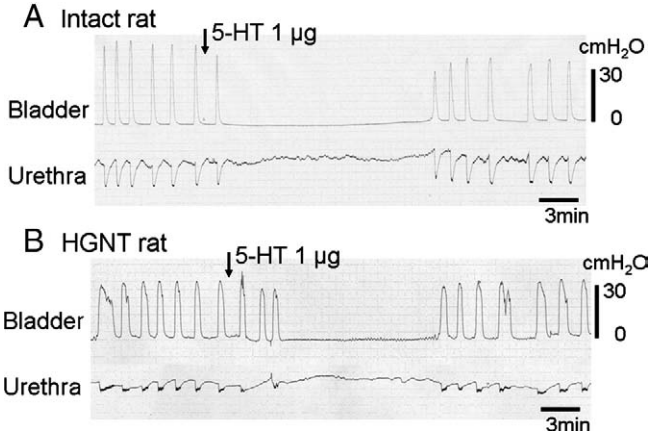

Fig. 1. Isovolumetric cystometry and urethral pressure before and after intrathecal injection of 5-HT in intact rats (A) and HGNT rats (B). Rhythmic bladder contractions were transiently abolished and urethral baseline pressure was increased after intrathecal injection of 5-HT in both intact rats (A) and HGNT rats (B).