Role of supraspinal and spinal α₁-adrenergic receptor subtypes in micturition reflex in conscious rats

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Yoshizumi M, Matsumoto-Miyai K, Yonezawa A, Kawatani M. Role of supraspinal and spinal α₁-adrenergic receptor subtypes in micturition reflex in conscious rats. Am J Physiol Renal Physiol 299: F785–F791, 2010. First published July 28, 2010; doi:10.1152/ajprenal.00553.2009.—α₁-Adrenergic receptor subtypes are widely distributed in the central nervous system and are involved in autonomic functions such as micturition. We investigated the presence and the role of supraspinal and/or spinal α₁-adrenergic receptors in modulating the micturition reflex in conscious female Wistar rats. The expression of α₁-adrenergic receptor subtypes in rat brain and lumbosacral spinal cord was studied using RT-PCR. Continuous-infusion cystometrograms were obtained in conscious rats, and α₁-adrenergic receptor antagonists were administered via intracerebroventricular or intrathecal routes. The mRNA expression of α₁A-, α₁B-, and α₁D-adrenergic receptors was detected in rat brain (midbrain and pons) and lumbosacral spinal cord (dorsal and ventral parts of spinal cord). In addition, intracerebroventricular injection of the α₁-adrenergic receptor antagonist tamsulosin (1–10 μg), the selective α₁A-adrenergic receptor antagonist silodosin (1–10 μg), and the selective α₁D-adrenergic receptor antagonist BMY 7378 (1–10 μg) significantly prolonged the intercontraction interval (ICI) but did not alter maximum voiding pressure (MVP). Although intrathecal injection of BMY 7378 (0.0001–10 μg) did not affect ICI, tamsulosin and silodosin prolonged ICI in a dose-dependent manner. MVP was significantly reduced by intrathecal injection of tamsulosin (10 μg) but not by silodosin or BMY 7378 (0.0001–10 μg). Supraspinal α₁A- and α₁D-adrenergic receptors are apparently important for the regulation of reflex-bladder activity in conscious rats. Noradrenergic projection from the brain stem to the lumbosacral spinal cord may promote the afferent limb rather than the efferent limb of the micturition reflex pathway via α₁A-adrenergic receptors. Cystometrogram; intracerebroventricular and intrathecal injections; tamsulosin; silodosin; BMY 7378

Micturition is mainly controlled by the autonomic nervous system through the spino-bulbospinal reflex via the micturition reflex center located on the rostral side of the pons, near the locus ceruleus; this is known as the pontine micturition center. In rats, the spinal micturition center is present in the lumbosacral spinal cord (L6–S1) and plays an important role in the regulation of the micturition reflex. It has been well established that sympathetic and/or parasympathetic nuclei in the lumbosacral cord receive inputs from noradrenergic neurons in the brain stem (2, 4), and these inputs, particularly from the locus ceruleus, have been implicated in the supraspinal control of micturition.

It has been reported that bladder contractions induced by electrical stimulation of the locus ceruleus are blocked by intrathecal injection of an α₁-adrenergic receptor antagonist, prazosin, in anesthetized cats (24, 25). Destruction of noradrenergic nuclei in the locus ceruleus by microinjection of 6-hydroxydopamine resulted a hypoactive bladder, and this effect was partially reversed by intrathecal injection of an α₁-adrenergic receptor agonist, phenylephrine (25). These studies suggest that α₁-adrenergic receptors in the lumbosacral cord are involved in the modulation of micturition function.

In anesthetized rats, Yoshiyama and de Groat (26) reported that intrathecal injection of a selective α₁A-adrenergic receptor antagonist, RS-100329, increased the frequency of bladder contraction, indicating that α₁-adrenergic receptors inhibit the spinal processing of afferent input from the urinary bladder. However, Jeong and Lee (9) found that intrathecal injection of an α₁-adrenergic receptor antagonist, tamsulosin, decreased the frequency of bladder contraction in anesthetized rats. Thus apparently contradictory actions have been documented, but these studies were conducted under anesthesia. Therefore, in the present study, by taking into account the influence of anesthesia, we investigated the effects of intracerebroventricular and intrathecal injection of selective α₁A-adrenergic receptor antagonists on the micturition reflex in conscious rats.

MATERIALS AND METHODS

Animals. Adult female Wistar rats, weighing 200–250 g, were used (age 13–18 wk, n = 30). Animals were housed with free access to standard food pellets and water and were maintained on a forced 12:12-h light-dark cycle at 22–24°C. Protocols for the experiments were previously approved by the Animal Research Committee, Akita University, and we followed the American Physiological Society guidelines for animal research.

Drugs. The following drugs were used: tamsulosin hydrochloride (Astellas Pharma, Tokyo, Japan), silodosin dihydrobromide (Kissei Pharmaceutical, Matsumoto, Japan and Daiichi Sankyo, Tokyo, Japan), and 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride (BMY 7378; Sigma, St. Louis, MO). Drugs were dissolved in sterilized artificial cerebrospinal fluid (CSF) containing (in μl): 7.4 NaCl, 0.19 KCl, 0.19 MgCl₂, and 0.14 CaCl₂. RT-PCR. The dorsal and ventral parts of the spinal cord (L5–S1), midbrain, and pons were dissected (n = 6, Fig. 1A). Total RNA was isolated from each area using ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer’s protocol. RNA samples were treated with DNase I (Sigma) at room temperature for 10 min to exclude artifacts from genomic DNA contamination. Synthesis of first-strand cDNA and PCR were performed using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) and TaKaRa ExTaQ HS (Takara Bio, Otsu, Shiga, Japan) for the spinal cord or a Transcriptor One-Step RT-PCR Kit (Roche, Mannheim, Germany) for the midbrain and pons. First-strand cDNA was synthesized using Oligo (dT) primer at 50°C for 30 min. PCR was performed using the Program Temp Control System PC-320 (ASTEC, Fukuoka, Japan), with 35 cycles of denaturation at 94°C for 10 s, annealing at 58°C for 30 s, and extension at 68°C for 30 s. GAPDH was used as an internal control. The sequence of the oligonucleotide primers used in this study, their

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positions on the corresponding mRNA sequences, and the expected sizes for the PCR products are shown in Table 1.

Cystometrogram. Rats (n = 24) were anesthetized with sevoflurane. After a lower abdominal incision, a polyethylene catheter (PE-50) was inserted into the urinary bladder. The bladder catheter was connected to a pressure transducer and an infusion pump using a T connector. Rats were then placed in a Ballman restraining cage (Natsume, Tokyo, Japan). After recovery from anesthesia, saline was infused into the bladder at a rate of 2.4 ml/h. Vesical pressure was recorded on an AP-601 polygraph (Nihon Kohden, Tokyo, Japan) and digitized with a converter for recording on a PowerLab system, version 5.0 (ADInstruments, Castle Hill, Australia). Three reproducible voiding cycles were recorded before drug injection and used as control values. Multiple doses of drugs starting with the smallest amounts were injected at intervals of at least 60 min in each rat. The following cystometric parameters were investigated: intercontraction interval (ICI) and maximum voiding pressure (MVP).

Intracerebroventricular injection. Anesthetized rats (pentobarbital sodium 50 mg/kg ip) were placed in a Ballman restraining cage (Natsume, Tokyo, Japan). After recovery from anesthesia, saline was infused into the bladder at a rate of 2.4 ml/h. Vesical pressure was recorded on an AP-601 polygraph (Nihon Kohden, Tokyo, Japan) and digitized with a converter for recording on a PowerLab system, version 5.0 (ADInstruments, Castle Hill, Australia). Three reproducible voiding cycles were recorded before drug injection and used as control values. Multiple doses of drugs starting with the smallest amounts were injected at intervals of at least 60 min in each rat. The following cystometric parameters were investigated: intercontraction interval (ICI) and maximum voiding pressure (MVP).

Intrathecal catheter in the spinal cord after each experiment. Statistical analysis. Data are expressed as means ± SE. The statistical significance of differences between means was analyzed by one-way ANOVA followed by a Bonferroni test. P < 0.05 was considered to be statistically significant.

RESULTS

mRNA localization of \( \alpha_1 \)-adrenergic receptor subtypes. To confirm whether injected antagonists could act on the afferent pathway to the spinal cord, the efferent pathway from the spinal cord, or the mesencephalic or pontine region associated with micturition, we examined the mRNA expression of three \( \alpha_1 \)-adrenergic receptor subtypes on the dorsal and ventral parts of the spinal cord, midbrain, and pons by RT-PCR. These sites involved inserting a length of polyethylene tubing after a laminectomy between L1 and L2 and carefully placing the catheter tip in the subarachnoid space of L5. Rats were allowed to recover over 4–5 days following implantation of the catheter filled with artificial CSF. The drugs were injected in a volume of 10 \( \mu \)l, followed by flushing with 10 \( \mu \)l of artificial CSF to ensure that each compound reached the spinal cord. Methylene blue was injected to confirm the position of the intrathecal catheter in the spinal cord after each experiment.

<table>
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<th>Table 1. Oligonucleotide primers used in the study</th>
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<tr>
<td>Gene</td>
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<td>( \alpha_1A )-Adrenergic receptor (( \alpha_1A ))</td>
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<td>GAPDH</td>
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Oligonucleotide sequence orientations are indicated as sense (S) and antisense (AS).
manner (0.0001–10 μg) on continuous-infusion cystometrograms in conscious rats (Fig. 2, A–C). Tamsulosin (1–10 μg), silodosin (1–10 μg), and BMY 7378 (1–10 μg) all induced significantly longer ICI (178.8 ± 17.6–203.9 ± 24.2, 175.3 ± 14.7–181.9 ± 25.2, and 166.5 ± 10.5–191.1 ± 17.9%, respectively) compared with controls (Fig. 3A). However, an intracerebroventricular injection of tamsulosin, silodosin, and BMY 7378 did not affect MVP (Fig. 3B). After the intracerebroventricular injection, methylene blue was found in the lateral ventricles, in the third ventricle, and in the cerebral aqueduct. ICI was also found to be prolonged by intrathecal injection of tamsulosin or silodosin in a dose-dependent manner (Fig. 4, A–C). ICI was significantly prolonged for tamsulosin (1–10 μg) and silodosin (1–10 μg; 162.9 ± 6.7–166.1 ± 9.5% and 146.4 ± 9.6–152.7 ± 11.1%, respectively), but not BMY 7378 (Fig. 5A). MVP was significantly reduced by intrathecal injection of tamsulosin (10 μg; 70.3 ± 2.0%), but not by silodosin or BMY 7378 (Fig. 5B). BMY 7378 (0.0001–10 μg) did not significantly affect ICI or MVP after an intrathecal injection. After the intrathecal injection, methylene blue was found in the L5–S1 spinal cord. All drugs did not significantly alter threshold pressure and baseline pressure. As a control, we performed the same time course of cystometry using intracerebroventricular or intrathecal injection of vehicle, CSF at pH 7.48 or 7.05 (same as 10-μg tamsulosin solution). The treatment with the vehicle at both pH levels during the same time course did not alter cystometric parameters at any volume.

**DISCUSSION**

The present study clearly demonstrated that both supraspinal and spinal α₁-adrenergic receptor subtypes are involved in the modulatory mechanisms of the micturition reflex in conscious rats. In conscious rats, intracerebroventricular injection of the α₁-adrenergic receptor antagonist tamsulosin, the selective α₁A-adrenergic receptor antagonist silodosin, and the selective

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**Fig. 2.** Effect of intracerebroventricular (i.c.v.) injection of tamsulosin (A), silodosin (B), and BMY 7378 (C) on continuous-infusion cystometrogram in conscious rats. All drugs prolonged the intercontraction interval (ICI), but not alter maximum voiding pressure (MVP) in a dose-dependent manner. Arrows indicate the injection of tamsulosin, silodosin, or BMY 7378.
α1D-adrenergic receptor antagonist BMY 7378 significantly prolonged ICI in a dose-dependent manner. These results suggest that supraspinal α1A- and α1D-adrenergic receptors are closely involved in micturition function and that activation of these receptors facilitates the micturition reflex. Support for this view comes from the finding that intracerebroventricular injection of the α1A-adrenergic receptor antagonist KMD-3213 (silodosin) markedly prolongs voiding (8). However, it was also shown that intracerebroventricular injection of the α1D-adrenergic receptor antagonist BMY 7378 was ineffective. These results might have been due to differences in the timing between the cannulation and the experiment, and in the cystometric parameters measured (e.g., interval of bladder contraction and bladder capacity).

The present results using RT-PCR assays demonstrated that α1A-, α1B-, and α1D-adrenergic receptor mRNAs were present in the rat brain (midbrain and pons) and spinal cord (dorsal and ventral parts of spinal cord). Our result is consistent with the previous studies that have used in situ hybridization with specific ribonucleotide probes to determine the distribution patterns of mRNA encoding the α1A-, α1B-, and α1D-adrenergic receptors in rat brain and spinal cord (3), and the expression of α1A-adrenergic receptor mRNA was found to be widespread throughout the rat central nervous system. Distribution of α1D-adrenergic receptor mRNA was the most discrete of the three receptors. The expression of α1D was strong in the olfactory bulb, cerebral cortex, hippocampus, reticular thalamic nucleus, regions of the amygdala, motor nuclei of the brain stem, inferior olivary complex, and spinal cord (3).

The micturition center in rats has been referred to as Barrington’s nucleus, comprising a group of cells between the locus coeruleus and dorsolateral segmental nucleus within the pontine micturition center (14, 15, 17, 18, 20). However, there are no noradrenaline neurons in Barrington’s nucleus. Numerous studies examining electric stimulation using cats have reported that areas above the pontine micturition center impact the micturition reflex (6, 7, 10), and in such areas micturition promotion and suppression sites are adjacent. It is also possible that the increase in frequency was associated with an increase in the firing rate of noradrenergic neurons in the locus coeruleus (5). Therefore, as clarified by the present study, the activation of supraspinal α1A- and α1D-adrenergic receptors enhances the micturition reflex, and the action site may exist in various regions, including the locus coeruleus.

As synaptic inputs to the micturition center in the lumbosacral spinal cord, descending pathways from various nuclei, such as the pontine micturition center, locus coeruleus, hypothalamus, and raphe nucleus, are known. In the present study, intrathecal injection of tamsulosin and silodosin in conscious rats prolonged ICI. In addition, we have not detected any significant change in residual urine volume by intrathecal or intracerebroventricular injections of these antagonists in our recent similar research (Yoshizumi M, Matsumoto-Miya K, Kawatani M, unpublished observations). These results indicate that both drugs inhibited the spinal cord level at the afferent pathway from the bladder. However, intrathecal injection of BMY 7378 had no significant effect on rat micturition function. Taken together with these results, the projection of noradrenergic nerves from the brain stem into the lumbosacral spinal cord excites α1A-adrenergic receptors in the micturition reflex afferent pathway, thereby enhancing the micturition reflex. The involvement of spinal α1D-adrenergic receptors in diuretic function is supported by previous studies (24, 25); bladder contraction is reduced by injection of 6-hydroxydopamine into the locus coeruleus, but recovers after intrathecal injection of an α1-adrenergic receptor agonist. In addition, bladder contraction induced by electric stimulation of the locus coeruleus is blocked by an α1-adrenergic receptor antagonist.

In anesthetized rats, Yoshiyama and de Groat (26) reported that intrathecal injection of a selective α1A-adrenergic receptor antagonist, RS-100329, increased the frequency of bladder contraction, indicating that α1A-adrenergic receptors inhibit the spinal processing of afferent input from the urinary bladder. However, Jeong and Lee (9) found that intrathecal injection of tamsulosin decreased the frequency of bladder contraction in anesthetized rats. Thus apparent contradictory actions have been documented, but these differences may have been due to the depth of anesthesia. In addition, previous studies have reported that
anesthesia affects glutamatergic signaling in the control of the micturition reflex (27, 28).

Yoshiyama and de Groat (26) reported that noradrenergic nerves from the brain stem regulate excitatory descending pathways from the pontine micturition center via \( \alpha_{1A} \)-adrenergic receptors at the sacral spinal cord level. However, in the present study, intrathecal injection of tamsulosin significantly lowered the MVP, while intrathecal injection of silodosin or BMY 7378 had no significant effect on MVP, indicating that \( \alpha_{1A} \) and \( \alpha_{1D} \)-adrenergic receptors did not appear to have an important role at the descending efferent limb of the micturition reflex pathway. Tamsulosin may affect some other receptor type to produce this inhibition, as it has an affinity almost equivalent to its \( \alpha_{1A} \) affinity for \( \alpha_{1D} \), dopamine D3, and 5-HT\( _{1A} \) receptors, with \( K_i \) values of 0.14, 0.28, and 0.74 nM, respectively (1, 11). Therefore, the effects of tamsulosin on MVP may include its antagonistic activity at the 5-HT\( _{1A} \) and/or dopamine D3 receptors. When the above-mentioned results are combined, the micturition reflex pathway at the lumbosacral spinal cord level is modified in an excitatory manner via the \( \alpha_{1A} \)-adrenergic receptor in the afferent pathway rather than the efferent pathway. In the present study, intrathecal injection of BMY 7378 had no significant effect on ICI and MVP. The results of a radioligand binding experiment (21) showed that the expression ratio of \( \alpha_{1A} \) and \( \alpha_{1B} \)-adrenergic receptor populations in the rat spinal cord comprise 70 and 30% of the total, respectively, while few \( \alpha_{1D} \)-adrenergic receptors were detected. Therefore, \( \alpha_{1A} \)-adrenergic receptors appear to be closely involved in the regulatory mechanisms of micturition function in the spinal cord.

Sugaya et al. (16) reported that intrathecal injection of the \( \alpha_{1D} \)-adrenergic receptor antagonist naftopidil abolished isovolumetric rhythmic bladder contraction in anesthetized rats. Naftopidil has a threefold greater affinity for \( \alpha_{1A} \)-adrenergic
receptors than does BMY 7378 (19). Furthermore, Yoshiyama and de Groat (26) indicated that intrathecal injection of BMY 7378 did not significantly alter the frequency or the amplitude of bladder contraction.

Clinically, $\alpha_1$-adrenergic receptor antagonists have been used for the treatment of both voiding and storage symptoms in benign prostatic hyperplasia patients. $\alpha_1$-Adrenergic receptor antagonists improve voiding symptoms by the relaxation of prostatic and urethral smooth muscle. A rat model of bladder outlet obstruction (BOO) related to storage symptoms seems to enhance the importance of supraspinal $\alpha_1$-adrenergic receptors than in normal rats (8). A recent study showed the potential for significant neurobehavioral consequences of BOO as a result of central noradrenergic hyperactivity (13). Therefore, supraspinal and/or spinal $\alpha_1$-adrenergic receptors as well as in smooth muscle may play an important role in the micturition reflex.

In conclusion, the results suggest that activation of supraspinal $\alpha_1A$- and $\alpha_1D$-adrenergic receptors enhances the micturition reflex. Furthermore, the projection of noradrenergic nerves from the brain stem works to enhance the micturition reflex via $\alpha_1A$-adrenergic receptors at the lumbosacral spinal cord level in the afferent pathway from the bladder rather than the excitatory descending pathway from the pontine micturition center.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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