Role of vasopressin $V_{1A}$ receptor in the urethral closure reflex in rats

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Submitted 10 November 2010; accepted in final form 5 January 2011

Role of vasopressin $V_{1A}$ receptor in the urethral closure reflex in rats. Am J Physiol Renal Physiol 300: F976–F982, 2011. First published January 12, 2011; doi:10.1152/ajprenal.00658.2010.—An enhanced urethral closure reflex via the spinal cord is related to urethral resistance elevation during increased abdominal pressure. However, with the exception of monoamines, neurotransmitters modulating this reflex are not understood. We investigated whether the vasopressin $V_{1A}$ receptor ($V_{1AR}$) is involved in the urethral closure reflex in urethane-anesthetized female rats. $V_{1AR}$ mRNA was highly expressed among the vasopressin receptor family in the total RNA purified from lamina IX in the spinal cord L6–S1 segment. In situ hybridization analysis of the spinal L6–S1 segment confirmed that these positive signals from the $V_{1AR}$s were only detected in lamina IX. Intrathecally injected Arg8-vasopressin (AVP), an endogenous ligand, significantly increased urethral resistance during an intravesical pressure rise, and its effect was blocked by the $V_{1AR}$ antagonist. AVP did not increase urethral resistance during an intravesical pressure rise, and its effect was blocked by the $V_{1AR}$ antagonist. AVP did not increase urethral resistance in rats in which the pelvic nerves were transected bilaterally. Urethral closure reflex responses to the intravesical pressure rise increased by up to threefold compared with the baseline response after AVP administration in contrast to no increase by vehicle. In addition, intravenously and intrathecally injected $V_{1AR}$ antagonists decreased urethral resistance. These results suggest that $V_{1AR}$ stimulation in the spinal cord enhances the urethral closure reflex response, thereby increasing urethral resistance during an abdominal pressure rise and that $V_{1AR}$ plays a physiological role in preventing urine leakage.

THE PUDENDAL NERVE, THE NUCLEI origin of which is located in Onuf’s nucleus in lamina IX of the caudal lumbar (L)-sacral (S) spinal cord, innervates the external urethral sphincter (22, 26). Recently, the pelvic-to-pudendal nerve-mediated reflex urethral closure mechanisms, which contribute to urethral resistance during events that cause the elevation of intravesical pressure, have been identified (9, 10, 13). Activation of the urethral closure increases urethral resistance during intravesical pressure increases (9–12). In fact, duloxetine, which is used clinically to treat stress urinary incontinence patients in Europe, enhances the urethral closure response by the potentiation of the urethral closure reflex (12, 19, 23). The mechanism is considered to act via the inhibition of the reuptake of serotonin and norepinephrine in the presynaptic terminals in Onuf’s nucleus to activate 5-hydroxtryptamine (5-HT) receptors and $\alpha_{1A}$-adrenoceptors, leading to stimulation of the pudendal nerves (2–5, 8, 12, 18, 19, 22, 23). Thus Onuf’s nucleus seems to play an important role in the control of the urethral closure reflex via the serotonin and norepinephrine pathways. However, few reports indicate that neurotransmitter pathways other

than serotonin and norepinephrine pathways are involved in the urethral closure reflex.

Arg8-vasopressin (AVP) is a neuromodulator in the central nervous system although it is well known as an antidiuretic hormone. The vasopressin receptor family consists of four subtypes, vasopressin $V_{1A}$, $V_{1B}$, $V_{2}$, and the oxytocin receptor ($V_{1AR}$, $V_{2R}$, $V_{1BR}$, and oxytocinR, respectively). $V_{1AR}$ is identified in rat Onuf’s nucleus by autoradiographic binding analysis (24, 25). Additionally, AVP potentiates the neuronal activity of cultured motor neurons from Onuf’s nucleus in neonatal rat via $V_{1AR}$, but not via the other vasopressin receptors, based on the findings of an electrophysiological study (16, 20).

We conducted a quantitative RT-PCR with total RNA from rat lamina IX containing Onuf’s nucleus after identification of area-innervating urethra by using Fast Blue and identified $V_{1AR}$ as the most highly expressed receptor among the vasopressin receptor family. We wondered whether $V_{1AR}$ stimulation would enhance the urethral closure reflex like 5-HT$_{2}$ receptor and $\alpha_{1A}$-adrenoceptor activation. In this study, we elucidated the distribution of $V_{1AR}$ mRNA in the spinal cord of female adult rats by in situ hybridization analysis. We pharmacologically evaluated the effect of intrathecally injected AVP on urethral resistance and the urethral closure reflex response in the rats. The $V_{1AR}$ antagonist was intrathecally used to identify the receptor responsible for the AVP-induced effects on urethral resistance. In addition, the effects of $V_{1AR}$ antagonists were investigated and the physiological role of $V_{1AR}$ in urethral resistance was discussed.

MATERIALS AND METHODS

Animals. Adult female Sprague-Dawley rats, weighing 200–300 g, were studied using experimental protocols approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Co., Ltd.

TaqMan RT-PCR analysis. To identify Onuf’s nucleus, a retrograde tracer experiment was performed as described by Kane et al. (14) with modification. Five rats were anesthetized by isoflurane inhalation (Abbott, Abbott Park, IL). The urethra was exposed through an abdominal incision, and 20 μl of 2% Fast Blue (Polysciences) in saline was injected. At 7 days after injection, a saline solution was perfused from the pulmonary artery after cutting of the inferior vena cava. After the perfusion, the spinal cord L4–S2 regions including the L6–S1 regions were removed. The ventral surface of the spinal cord was sectioned horizontally at 20 μm. Onuf’s nucleus was identified in several sections under a fluorescent microscope. The lamina IX area (20–200 μm in diameter) that contained labeled motor neurons was directly dissected by laser-capture microdissection (Takara Bio, Shiga, Japan). Total RNAs purified from five rats were mixed into one tube. TaqMan RT-PCR analysis was performed using a sequence detection system (Prism 7900HT; Applied Biosystems, Carlsbad, CA). The primers and probes listed in Table 1 were used with cDNA synthesized from 0.5 ng total RNA in each reaction against $V_{1AR}$, $V_{2R}$, $V_{1BR}$, and oxytocinR. Results indicate the average of two experiments.
Measurement of urethral resistance using the intravesical pressure clamp method. Urethral resistance was measured using the intravesical pressure clamp method as described previously (10) with slight modification. Briefly, after rats were anesthetized by isoflurane inhalation, the spinal cord was transected at the thoracic spine (Th8-Th9).

To fill the bladder, the urinary bladder was exposed through an abdominal incision, and a polyethylene catheter (PE-100, Clay Adams, Parsippany, NJ) was inserted into the dome and secured with a ligature. The bladder was connected to a saline reservoir via a bladder catheter and three-way stopcocks. The saline reservoir was mounted on a metered vertical pole, and intravesical pressure was increased in 2.5-cmH2O steps from 20 cmH2O upward. The intravesical pressure was released after 60 s by opening the three-way stopcocks, and the bladder was allowed to rest for 2 min. The pressure at which fluid leakage occurred was regarded as urethral resistance.

Urethral closure reflex responses induced by an increase in intravesical pressure. Urethral closure reflex responses were induced according to previously described methods with slight modification (10, 12). Briefly, under isoflurane anesthesia, spinal cord transection and bladder catheter insertion were performed as described for the intravesical pressure clamp method. The bladder neck was ligated with a suture to prevent fluid leakage from the bladder into the urethra. After surgery, isoflurane anesthesia was replaced with urethane anesthesia (1.2 g/kg ip). A 3.5-Fr-size nylon catheter with a side-mounted microtip transducer catheter located 1 mm from the catheter tip (SPR-524; Millar Instruments, Houston, TX) was inserted in a retrograde fashion into the middle urethral 12.5–15 mm from the urethral orifice. The bladder was connected with a saline reservoir and pressure transducer via a bladder catheter and three-way stopcocks. The intravesical pressure was abruptly changed from 0 cmH2O and maintained for 30 s at 50 cmH2O. To record the urethral contractile functions, a microtip transducer catheter was connected to an amplifier and an analog-to-digital converter (PowerLab; AD Instruments, NSW, Australia), and the urethral responses were digitally recorded at a sampling rate of 1,000 Hz using data-acquisition software (Chart version 5, AD Instruments) on a computer system equipped with an analog-to-digital converter. Because the first urethral response measured tended to be smaller than the second response, but the responses seemed to stabilize thereafter, measurements were repeated three times, and the last two measurements were averaged to calculate the urethral response. The urethral pressures were obtained from the average for 30 s before and after an increase in the intravesical pressure, and the urethral closure responses induced by an increase in the intravesical pressure were determined by the comparison between these urethral pressures.

In situ hybridization for V1AR. For immunohistochemical analysis, three animals were used. After cutting of the inferior vena cava, a saline solution was perfused from the pulmonary artery. The spinal cord containing the L6 segment was dissected out, frozen in liquid nitrogen, cut into 10-μm fresh frozen section that were attached to MAS-coated slides (Matsunami Glass Industries, Osaka, Japan), and fixed in 4% paraformaldehyde.

Table 1. TaqMan probe and primers for RT-PCR

<table>
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<tr>
<th>Gene</th>
<th>Probe</th>
<th>Primer 1</th>
<th>Primer 2</th>
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<tr>
<td>V1aR</td>
<td>TGCGTGCAAAGTTTCCCATGCTGC</td>
<td>GTGGCCATCTCTGGAAGA</td>
<td>GAATTGCTGGCGCATGCTGT</td>
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<tr>
<td>V2R</td>
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<td>CTTGCGGCTTTGCACTCTTT</td>
<td>CACTGCCAATTGGCCAGATCA</td>
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<tr>
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<td>TGGCTGCGGCTGCTGACCTGC</td>
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<tr>
<td>OxytocinR</td>
<td>AGGCTGCGCGCTCTAGTAGTACCGGT</td>
<td>CAAAGGCTACTGACATGGAT</td>
<td>CCGCTGCGTGGGATACCT</td>
</tr>
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V1aR, vasopressin V1a receptor.

Fig. 1. A: expression of vasopressin family receptor mRNA levels in total RNA purified from Onuf’s nucleus region in rats. B–D: in situ hybridization analysis for vasopressin V1a receptor (V1aR) mRNA in the spinal cord L6 segment. Shown is staining with antisense (B and D) and sense (C) RNA probes for V1aR. Scale bars: B and C = 300 μm; D = 50 μm.
Digoxigenin (DIG)-labeled RNA probes were transcribed from the PCR products amplified from the full length of vasopressin V1αR cDNA using a DIG labeling kit (Roche Diagnostics, Basel, Switzerland). The following primer pairs were used for the V1αR probe: 5'-ATGATTTCCCGCGAGGCTCCC-3' for the sense probe and 5'-TCAAGTGGAGACAGAATGAATCTG-3' for the antisense probe. In situ hybridization was performed on the dissected spinal cord. Probes were detected by alkaline phosphatase-conjugated anti-DIG antibodies (Roche Diagnostics) and nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Roche Diagnostics) was used as the substrate.

Drug and administration. AVP and HO-Phaa-d-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH2 (VPA), a selective V1αR antagonist, were purchased from Peptide Institute (Osaka, Japan). The polyethylene catheter (PE-10, Clay Adams) was inserted into the spinal cord from the Th12–Th13 vertebra, and the end of the catheter was positioned at the level of the L6–S1 spinal cord after a laminectomy. The intrathecally administered volume in saline was 20 µl in each experiment. SR-49059 (Tocris, Ellisville, MO) was dissolved in 50% N,N-dimethylacetamide (Wako, Osaka, Japan)-50% polyethylene glycol 400 (Wako).

Data analysis. All data are expressed as means ± SE for five rats in each experiment. Data were analyzed with a one-tailed Williams’ test (see Figs. 2A and 5, A and B), Student’s t-test (see Figs. 2B, 3A, and 4B), and Welch’s test (see Fig. 3B). For Student’s t-test and Welch’s test, a difference of P ≤ 0.05 was considered statistically significant, and for Williams’ test, a difference of P ≤ 0.025 was considered statistically significant.

RESULTS

Expression of V1αR mRNA in the spinal cord. The vasopressin receptor family consists of four subtypes, V1αR, V2R, V1βR, and oxytocinR. To examine whether the vasopressin receptor family is expressed in lamina IX containing Onuf’s nucleus (L6–S1), RT-PCR analysis was performed after identification of Onuf’s nucleus by using Fast Blue injection. V1αR mRNA was highly expressed in the total RNA compared with V2R, V1βR, and oxytocinR (Fig. 1A). To investigate the distribution of V1αR mRNA in female rats, in situ hybridization analysis was performed by using an L6 segment (Fig. 1, B–D). Positive signals (purple) were detected with the antisense V1αR RNA probe only in the cells in lamina IX, which contains rat Onuf’s nucleus, while no signal was detected in the sense RNA probe.

Spinal vasopressin induces increase in urethral resistance. We investigated whether intrathecal administration of AVP increases urethral resistance (Fig. 2A). Urethral resistance was compared before and 10 min after intrathecal administration of AVP in the spinal cord-transected rats. AVP increased urethral resistance in a dose-dependent manner, whereas the vehicle did not. The increment induced by AVP was statistically significant compared with that in the vehicle-treated groups. To examine whether V1αR is involved in the AVP-induced increase in urethral resistance, the effects of a V1αR antagonist, VPA, were tested. In the preliminary study, VPA at 6.0 nmol administered intrathecally decreased urethral resistance, while VPA at 3.0 nmol did not affect urethral resistance. Either VPA at 3.0 nmol or saline was intrathecally injected with AVP. An AVP-induced increase in urethral resistance was abolished in the VPA-injected rats compared with the saline-treated rats (Fig. 2B). The inhibitory effect of VPA on the AVP-induced increase in urethral resistance was statistically significant compared with saline treatment.

Next, we investigated whether selective agonists for vasopressin receptors, excluding V1αR, increase urethral resistance. Urethral resistances were compared before and 10 min after intrathecal administration of selective agonists in the spinal cord-transected rats. Increases in urethral resistance by intrathecally injected selective agonists for V1βR ([dCha4]-AVP, 2 nmol), V2R ([dCys1,dVal4,d-Arg8]-vasopressin, 2 nmol), and oxytocinR ([Thr4,Gly7]-oxytocin, 2 nmol) were −1.9 ± 0.6, −1.3 ± 0.6, and −0.6 ± 0.6 cmH2O, respectively. These agonists did not show any increment of urethral resistance.

Fig. 2. A: effect of intrathecal administration of AVP on urethral resistance in rats. Urethral resistance values in each group before drug administration were 31.9 ± 3.6, 33.8 ± 4.7, 41.9 ± 2.4, and 34.4 ± 3.4 cmH2O at 0.0, 0.2, 0.6, and 2.0 nmol in the AVP-treated group, respectively. ***P ≤ 0.025 to vehicle. B: intrathecal administration of HO-Phaa-d-Tyr(Me)-Phe-Gln-Asn-Arg-NH2 (VPA) blocked the AVP-induced increase in urethral resistance. Urethral resistance values in each group before drug administration were 44.4 ± 3.4 and 38.0 ± 5.0 cmH2O at 0.0 and 3.0 nmol in the VPA-treated group, respectively. ***P ≤ 0.001 to saline.
Urethral closure reflex mediation. The urethral closure reflex is involved in urethral closure responses to increase and maintain urethral resistance (8, 10). Hence, we investigated whether the AVP-induced increase in urethral resistance depends on the urethral closure reflex by the following two methods.

First, the effect of AVP was investigated in rats whose pelvic nerves had been bilaterally transected to abolish the urethral closure reflex. The baseline of urethral resistance in rats whose pelvic nerves had been bilaterally transected was significantly lower than that in intact rats (Fig. 3A). AVP was intrathecally administered to rats with and without nerve transection. No increment of urethral resistance was observed in the nerve-transected rats, whereas AVP increased urethral resistance in the intact rats (Fig. 3B). These differences were statistically significant.

Second, we directly examined whether AVP enhances the urethral closure reflex. In the spinal cord-transected rats, the elevation of intravesical pressure from 0 to 50 cmH2O induced the urethral closure reflex (Fig. 4A). Ten minutes after intrathecal administration of AVP, the increase in the urethral closure reflex was threefold higher than the baseline response before treatment (Fig. 4B). AVP and the vehicle used did not significantly change the baseline urethral pressure before elevation of intravesical pressure. Changes in the baseline urethral pressure values were 1.3 ± 0.8 and 3.9 ± 2.7 cmH2O in the vehicle- and AVP-treated groups, respectively.

Physiological role of V1AR on urethral resistance. To investigate the physiological role of V1AR on urethral closure responses, we examined effects of V1AR antagonists on urethral resistance in the spinal cord-transected rats. SR-49059, a nonpeptide V1AR antagonist, was intravenously injected and urethral resistance was measured. SR-49059 significantly decreased urethral resistance in a dose-dependent manner (Fig. 5A). Next, VPA was intrathecally injected and urethral resistance was measured. Intrathecally injected VPA decreased urethral resistance in a dose-dependent manner (Fig. 5B). The decrease caused by VPA was statistically significant compared with vehicle-treated groups.

DISCUSSION

Onuf’s nucleus in the spinal cord is innervated by serotonergic and noradrenergic terminals and contain 5-HT2 receptors and α1-adrenoceptors (22). Recent studies using pharmacological agents reveal that 5-HT2 receptors and α1A-adrenoceptors are involved in the urethral closure reflex (2, 17, 18). However, few findings have elucidated the enhancement of urethral resistance through the urethral closure reflex at the
spinal cord excluding serotonin or norepinephrine pathways. In this study, we indicated that V1AR mRNA in the spinal L6 is specifically distributed in the lamina IX, which contains Onuf’s nucleus in rats. Our findings are consistent with previous studies that used autoradiographic V1AR antagonists and indicated the localization of V1AR in Onuf’s nucleus in adult rats (24, 25). Therefore, it is likely that V1AR plays an important role in the regulation of the urethral closure response.

The intrathecal injection of AVP increased urethral resistance, and its effect was blocked by VPA. VPA has relatively weak selectivity over V1BR and oxytocinR (20). Because a highly selective V1AR antagonist was not available for the intrathecal injection in our study, selective agonists for other vasopressin receptors were applied to confirm the responsible receptor. The intrathecally administered selective agonists for V1BR, V2R, and oxytocinR did not affect urethral resistance. In addition, while V1AR was expressed in lamina IX confirmed by RT-PCR analysis, V1BR and oxytocinR were not. Taken together, these findings suggest that V1AR is responsible for the AVP-induced increase in urethral resistance. Ogier et al. (20) indicated that the cultured motor neurons from Onuf’s nucleus in neonatal rats can be potently excited by AVP. They showed that the excitation was mediated by V1AR but not by V1BR, V2R, or oxytocinR. Our in vivo pharmacological results were in good agreement with their findings. The results of Ogier et al., which indicate that Onuf’s nucleus would potentiate through V1AR stimulation, suggest the appropriate mechanism in the spinal cord for the AVP-induced increase in urethral resistance. That is, the stimulation of V1AR in Onuf’s nucleus would cause the increase in urethral resistance.

Recently, it has been shown that an increase in urethral resistance is induced by the enhancement of the urethral closure reflex via the spinal cord (9–11, 22). We postulated that the mode of action for the AVP-induced increase in urethral resistance depends on the enhancement of the urethral closure reflex (spinal reflex potentiation) as the peptide drug was injected into the spinal cord. After transection of the pelvic nerve containing the bladder afferent nerve, the urethral closure reflex responses to bladder pressure increase disappear (10). AVP did not show any effect on the urethral resistance in the rats without the bladder afferent nerves. Intrathecally injected AVP enhanced the urethral closure reflex responses observed when intravesical pressure increased. In addition, AVP induced no increase in baseline before the intravesical pressure rise, suggesting that AVP did not induce urethral contraction itself. These results indicate that the AVP-induced increase in urethral resistance apparently depends on spinal reflex potentiation.

The modulation of glutamate receptors, the NMDA receptor and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor, plays a crucial role in the excitation necessary for the urethral closure response (1, 5, 6, 22, 27). This Fig. 5. A: intravenous administration of SR-49059, a V1AR antagonist, decreased urethral resistance. Urethral resistances in each group before drug administration were 33.1 ± 2.8, 34.2 ± 1.7, 33.8 ± 5.8, 32.5 ± 2.7, and 33.8 ± 3.8 cmH2O at vehicle at 0.1, 0.3, 1.0, and 3.0 mg/kg in the SR-49059-injected group, respectively. †P ≤ 0.025 to vehicle. B: intrathecal administration of VPA also decreased urethral resistance. Urethral resistances in each group before drug administration were 33.1 ± 3.3, 39.4 ± 2.6, 33.1 ± 2.6, and 36.9 ± 3.6 cmH2O at 3, 6, and 12 nmol vehicle in the VPA-injected group, respectively. †P ≤ 0.025 to vehicle.

spinal cord excluding serotonin or norepinephrine pathways. In this study, we indicated that V1AR mRNA in the spinal L6 is specifically distributed in the lamina IX, which contains Onuf’s nucleus in rats. Our findings are consistent with previous studies that used autoradiographic V1AR antagonists and indicated the localization of V1AR in Onuf’s nucleus in adult rats (24, 25). Therefore, it is likely that V1AR plays an important role in the regulation of the urethral closure response.

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Fig. 6. Hypothetical schema shows the role of V1AR in the urethral closure reflex. The activation of Onuf’s nucleus enhances urethral closure responses. V1AR, 5-HT2, and α1A-adrenoceptors increase the excitation of glutamate receptors through a calcium influx, thereby enhancing the urethral closure responses.
modulation is caused by monoamines such as serotonin, norepinephrine, and dopamine (22, 27). Duloxetine has been shown to increase the urethral closure reflex via serotonin and norepinephrine pathways (12, 19, 22, 23, 26). These studies suggest that increases in serotonin and norepinephrine at the presynaptic gaps of Onuf’s nucleus would enhance the sensitivity of glutamate receptors. Ogier et al. (16, 20) indicated that AVP application induces both membrane depolarization and action potential discharge in motor neurons prepared in vitro from newborn rats. We consider that AVP would induce membrane depolarization but would not evoke action potentials enough to cause urethral closure responses in vivo because the V1AR-mediated increase in urethral resistance was not observed without the urethral closure reflex. Interestingly, the expression of V1AR in adults is significantly lower than in newborns (16). The low level of V1AR in adult motor neurons could cause a small depolarization, which modulates glutamatergic response, but is not sufficient to generate action potentials by itself. Together, it is likely that spinal AVP modulates the sensitivity of glutamate receptors in a manner similar to monoamines. The stimulation of the V1AR, 5-HT2 receptor and α1A-adrenoceptor at the spinal cord enhances the urethral closure reflex. These receptors are coupled with Gq protein, which increases intracellular levels of calcium. The calcium influx through the Gq protein-coupled receptor would have an important role in the increase of the urethral closure reflex (Fig. 6). On the other hand, the stimulation of the receptor-coupled Gi protein (e.g., dopaminergic D2-like receptors and 5-HT1A receptors), which decreases intracellular levels of cAMP, would decrease the urethral closure response at the spinal cord (18, 27). Gq and Gi protein-coupled receptors would control the urethral closure reflex in a coordinated manner. They would modulate the sensitivity of the glutamate receptors.

Systemic injection of SR-49059 and intrathecally injected VPA significantly decreased urethral resistance, suggesting that vasopressin and oxytocin, endogenous ligands for V1AR, play physiological roles via at least two possible pathways. First, the spinal-projecting vasopressergic and/or oxotremorine neuron from the supra-spinal cord would control the urethral closure response via V1AR on Onuf’s nucleus as well as serotonergic and noradrenergic neuron (Fig. 6) (22). Second, the AVP in plasma might be involved in the mechanism of urethral closure responses via V1AR on the smooth muscle of the urethra because the central nervous system penetration of SR-49059 is weak (25). SR-49059 might directly relax the internal urethral sphincter, but not the urethral closure reflex via Onuf’s nucleus. Further studies will be required to clarify two points for a physiological role of V1AR in the urethral closure response: 1) the possibility of a peripheral V1AR pathway, and 2) the contribution of spinal and peripheral V1AR pathways. However, our data were the first evidence that the blockade of the V1AR pathway causes urethral resistance reduction.

Stress urinary incontinence is the most common type of urinary incontinence in women (7). Urinary incontinence could be caused by the impairment of the closure mechanisms of the urethra. Studies have revealed that duloxetine enhances the urethral closure reflex via the spinal cord during increased abdominal pressure. We showed that AVP increased urethral resistance via the spinal cord during an intravesical pressure rise. This is the first evidence that the V1AR pathway would be involved in the urethral closure reflex and be necessary to maintain urethral resistance physiologically. Spinal V1AR could be a therapeutic target to increase urethral resistance. Moreover, the physiological role of V1AR suggests that the deficiency of the vasopressin pathway would be involved in lower urinary tract symptoms.

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

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