The involvement of urothelial $\alpha_{1A}$ adrenergic receptor in controlling the micturition reflex

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(Received 30 June 2008; and accepted 31 July 2008)

ABSTRACT
The current study was undertaken in an attempt to characterize the functional properties of urothelial $\alpha_{1A}$ adrenergic receptors, especially in modulating the micturition reflex. The expression of $\alpha_{1A}$ receptors in rat bladder was analyzed by immunohistochemistry and Western blotting. As a functional study, we obtained continuous infusion cystometrograms in conscious rats using noradrenaline (NA) and subtype selective $\alpha_{1}$ adrenergic receptor antagonists, tamsulosin ($\alpha_{1A}/\alpha_{1D}$ selective) and silodosin ($\alpha_{1A}$ superselective). $\alpha_{1A}$ receptors were immunohistochemically detected in rat urothelium. Intravesical infusion of NA (60 μM) significantly shortened the intercontraction interval (ICI). Pretreatment with tamsulosin at a dose of 0.4 μg/kg i.v. abolished intravesical NA infusion-induced reduction of ICI. Neither intravesical infusion of tamsulosin (20 μM) nor that of silodosin (0.2 μM) significantly altered ICI. After intravesical infusion of silodosin, intravesical NA infusion did not affect ICI. Urothelial $\alpha_{1A}$ receptors might modulate bladder afferent activity under pathophysiological conditions with augmented concentrations of NA in blood or urine.

$\alpha_{1}$ adrenergic receptor antagonists have been the predominant form of medical therapy for the treatment of lower urinary tract symptoms in benign prostatic hyperplasia (BPH) (17). $\alpha_{1}$ blockers were initially designed to improve voiding symptoms in BPH such as hesitancy and slow urinary flow, since $\alpha_{1A}$ adrenergic receptors enhance prostatic and urethral smooth muscle contraction to increase the resistance of urinary outflow. It turned out later that this therapy is also effective for storage symptoms such as frequency and urgency (8), suggesting the existence of another target mechanism of these drugs different from releasing bladder outlet obstruction (18).

$\alpha_{1}$ adrenergic receptors have been found at various sites in the nervous system controlling the bladder function. Intrathecal injection of $\alpha_{1}$ antagonist increased the bladder capacity and decreased the vesical pressure during bladder contraction in rats (15, 16). In the parasympathetic ganglia of cats, $\alpha_{1}$ antagonist prazosin inhibited the hypogastric nerve-induced facilitation of cholinergic transmission (19). In addition to their presence in the nervous system, all three subtypes of $\alpha_{1}$ adrenergic receptors ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$) have been identified in human and rat bladder (13, 21, 24). The functions of the three different subtypes in nerves and the urinary bladder were not studied until recently due to a lack of specific antagonists for each receptor subtype.

The urothelium has been appreciated to be a potential modulator of bladder sensory systems because of its neuron-like properties capable of detecting mechanical or chemical stimuli and releasing a number of signaling molecules (7). The urothelial cells express diverse sensor molecules including transient receptor potential (TRP) channels (5, 6), amiloride/mechanosensitive Na$^+$ channels (ENaC) (12), receptors for purines (20, 25) and acetylcho-
lines (3, 10), and receptors for inflammatory mediators, such as bradykinin (11) and neurotrophins (27). The urothelial cells release adenosine triphosphate (ATP) in response to stretch, or nitric oxide (NO) by capsaicin or α1 agonist stimulation (4, 6), which subsequently affects the activity of afferent sensory nerves located beneath the urothelium. Therefore, the urothelium seems to play an important role in controlling the micturition reflex, especially during storage phase.

In rat urothelium, mRNAs for all three subtypes of α1 adrenergic receptors were detected. Previously, we showed that α1a receptors facilitate the mechanosensitive bladder afferent nerve activity partly through distention-induced ATP release from the urothelium (14). In the present study, we used subtype selective α1 antagonists, tamsulosin (α1a/α1b) and silodosin (α1a superselective) to investigate the involvement of urothelial α1a receptors in modulating the micturition reflex.

MATERIALS AND METHODS

Animals. Wistar rats of either sex weighing 150–300 g (n = 30) were used for this study. Protocols for the experiments were approved by the Animal Research Committee at Akita University.

Immunohistochemistry. Rats were perfused with 4% paraformaldehyde under deep anesthesia. Urinary bladders were dissected out and post-fixed in the same fixative for an additional 4 h. After cryoprotection with 30% sucrose in 0.1 M phosphate buffer, frozen sections, 5 μm in thickness, were cut and mounted on poly-L-lysine-coated slides. They were pretreated with 0.3% Triton X-100 containing 0.03% H2O2 in methanol to inhibit endogenous peroxidase activity. After treatment with 4% Blockace (Dainippon Sumitomo Pharma, Japan), the sections were incubated with goat anti-α1a adrenergic receptor polyclonal antibody (1:200, Santa Cruz Biotech., USA), and then with amino acid polymer conjugated with peroxidase and anti-goat IgG (Histofine Simple Stain Rat MAX-PO(G); Nichirei, Japan). The antigen-antibody reaction was visualized by incubation in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.01% 3,3′-diaminobenzidine (DAB) and 0.001% H2O2. Nuclear counterstaining was done with hematoxylin. Negative control tests conducted without primary antibody showed elimination of the staining.

**Western Blotting.** The animals were sacrificed by exsanguination under general anesthesia. Whole urinary bladders were dissected out, homogenized with ice-cold Tris-HCl buffer (50 mM, pH 7.5) containing 10 mM EDTA and protease inhibitor cocktail (COMPLETE; Roche Diagnostics, Germany). A pellet obtained by centrifugation at 30,000 × g for 1 h at 4°C was resuspended in the same buffer and solubilized with 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride for 30 min on ice. Protein samples were reduced with 0.7 M 2-mercaptoethanol, separated by 7.5% SDS-polyacrylamide gel electrophoresis, and electroblotted onto nitrocellulose membranes. The membranes were blocked with 4% Blockace, and then α1a adrenergic receptors were detected using the same primary antibodies used in immunohistochemistry, and peroxidase-conjugated anti-goat IgG (Pierce, USA) as secondary antibody. Antibodies were diluted with 0.01 M PBS containing 0.05% Tween 20. Immunopositive reactions were visualized using an enhanced chemiluminescence detection reagent (SuperSignal West Pico; Pierce).

Cystometrograms. Under halothane anesthesia, an intravesical catheter (PE-50) was inserted through the bladder dome and connected with a T-stopcock to a syringe pump for infusion and a pressure transducer for recording. After the surgery, the rats were placed in a Ballman restraining cage (Natsume, Japan) and allowed to recover from anesthesia for a few hours. Intravesical pressure was recorded using data acquisition software (Chart 4; AD Instruments, Australia) on a computer system equipped with an analog-digital converter (PowerLab; AD Instruments) in response to continuous infusion at a constant speed (0.04 mL/min) of saline as a control. The infusion solution was subsequently changed to noradrenaline (NA, 60 μM). In seven rats, tamsulosin (0.4 μg/kg) was administered into the external jugular vein during saline infusion 1 h before NA infusion. In another seven rats, the vesical pressure was recorded during intravesical tamsulosin infusion (20 μM). Five rats were intravesically preinfused with silodosin (0.2 μM) before intravesical NA infusion. Statistical analyses were performed using Mann-Whitney and paired t tests, with P < 0.05 considered significant. All data are presented as the mean ± SEM.
RESULTS

Immunohistochemistry
Immunopositive reactions for $\alpha_{1A}$ adrenergic receptors were found in rat urothelium. $\alpha_{1A}$ immunoreactivity was localized mainly in the umbrella cells (Fig. 1A).

Western blotting
Western blotting showed an immunoreactive band of approximately 50–55 kDa, corresponding to the deduced molecular weight of $\alpha_{1A}$ adrenergic receptor (51.6 kDa) in rat whole bladder. We also detected another band of approximately 120 kDa, which was almost twice the size of the smaller one (Fig. 1B).

Cystometrograms: intravesical NA infusion enhanced the micturition reflex
When physiological saline was continuously infused into the bladder, the average ICI and MVP (maximum voiding pressure) were $510.6 \pm 52.1$ s and $32.3 \pm 2.7$ cmH$_2$O, respectively. Intravesical NA infusion (60 μM) significantly shortened ICI to $279.3 \pm 57.9$ s (55% of control, $P < 0.05$, n = 7), whereas MVP was not altered by NA infusion (Fig. 2).

The effect of tamsulosin on NA-induced ICI reduction
Pretreatment with tamsulosin at a dose of 0.4 μg/kg i.v. abolished the effect of intravesical NA infusion. The average ICI was $496.6 \pm 83.2$, $557.7 \pm 97.8$, and $546.6 \pm 94.3$ s in the control, after intravenous tamsulosin injection, and after intravesical NA infusion, respectively. There were no significant differences of ICI among the groups (n = 7) (Fig. 3). Intravesical application of tamsulosin (20 μM) did not affect ICI (n = 7) (Fig. 4).

The effect of silodosin on NA-induced ICI reduction
Intravesical administration of silodosin (0.2 μM) did not alter ICI. After intravesical pretreatment with silodosin, intravesical NA infusion did not affect ICI. The average ICI was $566.0 \pm 27.9$, $600.3 \pm 46.4$, and $646.0 \pm 60.2$ s in control, after intravesical silodosin administration, and after intravesical NA infusion, respectively. There were no significant differences of ICI among the groups (n = 5) (Fig. 5).

DISCUSSION

The present experiments showed that intravesical NA infusion significantly shortened ICI during continuous infusion cystometrograms in conscious rats. It was also demonstrated that intravenous administration of $\alpha_{1A}/\alpha_{1D}$ adrenergic receptor selective antagonist tamsulosin and intravesical infusion of $\alpha_{1A}$ superselective antagonist silodosin inhibited the intravesical NA infusion-induced reduction of voiding intervals. Immunohistochemical and Western blot analyses revealed the expression of $\alpha_{1A}$ receptors in the rat urothelium. Previously we showed that $\alpha_{1D}$ receptors were expressed in rat urothelium, and intravenous administration of an $\alpha_{1D}$ selective antagonist, naftopidil, inhibited the bladder primary after-
Man bladder (21, 24), OAB in the elderly population might partly be related to the increase in the plasma concentration of catecholamine with aging, and urothelial α1 receptors might play some roles in the pathophysiological process of OAB. How urothelial α1A acts on the afferent pathway remains unknown. Regarding the α1D receptor, we previously demonstrated that intravenous administration of naftopidil inhibited the release of ATP into the bladder lumen induced by bladder distention, suggesting that urothelial α1D receptors enhance afferent nerve activity through increasing ATP release from urothelial cells during the storage phase (14).

In the present study, neither intravenous administration of tamsulosin (0.4 μg/kg) nor intravesical infusion of tamsulosin (20 μM) or silodosin (0.2 μM) significantly affected ICI during continuous infusion cystometrograms, while in our previous study intravenous administration of naftopidil (1.0 mg/kg) significantly prolonged ICI. One explanation for this

It is said that the concentration of catecholamine in blood rises with aging, which may induce an increase in the frequency of α1 adrenergic receptor-related diseases, such as hypertension, cardiac hypertrophy, and prostatic hyperplasia, during aging (23). Overactive bladder (OAB) is a new entity defined as a symptomatic syndrome of urinary frequency and urgency with or without incontinence (1), whose prevalence also increases during aging (26). In most cases, the cause of OAB is unclear, although a significant proportion of male OAB patients also have BPH (2, 9). Since all three subtypes of α1 adrenergic receptors are expressed in the human bladder (21, 24), OAB in the elderly population might partly be related to the increase in the plasma concentration of catecholamine with aging, and urothelial α1 receptors might play some roles in the pathophysiological process of OAB.

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discrepancy of the effect on ICI is that there might be a facilitatory mechanism that normally and constitutively functions by intrinsic NA stimulation of $\alpha_1$ receptors, and the fact that the mechanism was affected only by naftopidil and not by the other two agents, may have been due to the different drug delivery systems used for the three drugs or to some unknown reasons. Alternatively, it may be accounted for by the difference in affinity for $\alpha_1$ receptor subtypes among the drugs, since the two antagonists used for the present experiments did not act on $\alpha_{1D}$ receptors for which naftopidil has quite high affinity. In contrast, intravesical infusion of tamsulosin and silodosin did antagonize the ICI reduction induced by intravesical NA infusion. Since intravesical NA infusion must have activated both $\alpha_{1A}$ and $\alpha_{1D}$ receptors in the urothelium to decrease voiding intervals, if the effects of tamsulosin and silodosin were mostly mediated by $\alpha_{1A}$ receptors, this result would be somewhat puzzling, and contradictory to the previous report of urothelial $\alpha_{1D}$ function in an afferent facilitatory system. It is too difficult to explain all these findings simply, although our findings, taken altogether, indicated that the $\alpha_{1D}$ receptors in the urothelium might be able to act as a part of the mechanism regulating the normal micturition reflex, whereas urothelial $\alpha_{1A}$ receptors might function only when an excess of NA exists in the blood or urine, as in the intravesical NA infusion in the present experiments. Future studies will be required to clarify the functional difference between urothelial $\alpha_{1A}$ and $\alpha_{1D}$ receptors.

Recently we reported that $\alpha_{1A}$ receptors might be involved in modulating the cell motility in the primary culture from mouse urothelium (22), suggesting that $\alpha_{1A}$ receptors might be a regulator to maintain the urothelium as the functional transitional epithelium. We need further investigation for fully understanding of functional properties of urothelial $\alpha_{1A}$ receptors.
REFERENCES