Male lower urinary tract symptoms and $\alpha_{1D}$-adrenoceptors

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ABBREVIATIONS AND ACRONYMS

AR = adrenoceptor

LUTS = lower urinary tract symptom

mRNA = messenger ribonucleic acid

BOO = bladder outlet obstruction

SHR = spontaneous hypertensive rat

KO = knock out

WT = wild type

ATP = adenosine 5’- triphosphate

BPO = benign prostatic obstruction

FDV = first desired volume

SDV = strong desired volume

FVC = frequency volume chart

PFS = pressure flow study

IPSS = international prostate symptom score

RT-PCR = reverse transcriptase polymerase chain reaction

OVX = ovariectomy

TRPM8 = transient receptor potential channel melastatin member 8
ABSTRACT

Historically, $\alpha_1$-adrenoceptors have been classified into 3 subtypes ($\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$) that are widely distributed in various organs. Research on the $\alpha_{1D}$-adrenoceptors in the bladder, urethra, and prostate has focused on the relationship between expression levels and symptoms of bladder outlet obstruction, and the implications and functional roles of $\alpha_{1D}$-adrenoceptors subtypes in these organs. The $\alpha_{1D}$-adrenoceptor mRNA and protein seem to be increased in obstructed bladders or small capacity bladders. On the other hand, $\alpha_{1D}$-adrenoceptor subtype knock-out mice have been found to have a prolonged voiding interval. Interestingly, an $\alpha_{1D}$-adrenoceptor antagonist was found to inhibit the facilitation of afferent nerve activity for the micturition reflex induced by intravesical infusion of acetic acid. Clinically, patients who felt urgency at low filling volumes and had a small bladder capacity were found to have more $\alpha_{1D}$-adrenoceptor mRNA in their bladder mucosa than patients, who felt urgency at high filling volumes and had a large bladder capacity. An $\alpha_{1D}$-adrenoceptor antagonist was found to increase the first desired volume and the maximum desired volume while decreasing detrusor overactivity in pressure flow studies. Thus, $\alpha_{1D}$-adrenoceptors in the lower urinary tract may play an important role in the pathophysiology of lower urinary tract disorders.

Keywords: storage symptoms, voiding symptoms, alpha1-adrenoceptor subtypes, voiding dysfunction, bladder
1. BASIC STUDIES

1.1. α1 adrenoceptor subtypes

Drugs targeting adrenoceptors (ARs) producing activation and/or inhibition are some of the most widely used therapeutic agents in clinical medicine. ARs bind and are activated by the endogenous catecholamines adrenaline and noradrenaline. α1-AR subtype cDNAs encoding three α1-AR subtypes (α1A, α1B, and α1D) have been cloned and characterized pharmacologically.1-6 The α1D-ARs have been shown to have 10 to 100-fold higher affinity for the endogenous neurotransmitters norepinephrine and epinephrine compared with the α1A- or α1B-AR subtypes.6 This finding provides a potentially important mechanistic rationale for targeting α1D-ARs when treating lower urinary tract symptoms (LUTS).

1.2. Expression of α1-AR subtypes in animal models and afferent nerve activity modulation through α1D-ARs in voiding function

In some animal models associated with bladder outflow obstruction (BOO), hypertension and aging have been found to complicate urinary bladder dysfunction.7-10 In each of these models an elevated expression level of α1D-AR mRNA or α1D-AR protein has been demonstrated (Fig. 1). To counteract these pathological changes, some of which are, at least partly, caused by α1D-AR stimulation, it is reasonable to assume that an α1D-AR antagonist could be useful.
1.2.1. Expression of α₁-AR subtype in animal models

In animal studies, Hampel et al. found that BOO produced a 6-fold increase in bladder weight vs. sham operation, and significantly increased voiding frequency.\textsuperscript{11} Although the bladder α₁-AR density did not increase overall, striking changes in α₁-AR subtype expression were demonstrated. In control animals, 70% of α₁-AR mRNA was the α₁A subtype, 5% was α₁B, and 25% was α₁D, whereas in obstructed animal bladders, α₁-AR expression changed to 23% α₁A, 2% α₁B, and 75% α₁D. Changes in α₁-AR mRNA expression were of similar magnitude throughout the bladder dome, mid body, and base. Parallel changes were also evident at the protein level, with 100% α₁A-AR expression in control animals changing to 36% (mean value) in animals with a 5-fold or greater increase in bladder weight. The data of Hampel et al. are suggestive, but whether a change in α₁D density could have functional consequences was not investigated. However, it has been shown pharmacologically that elevated urinary frequency in obstructed rats was decreased with tamsulosin (α₁A/1D antagonist) but not with 5-Me-urapidil (α₁A antagonist).\textsuperscript{12} Further, Barendrecht et al. reported that relaxation responses to the endogenous agonist noradrenaline through β-AR are turned into α₁-AR-mediated contraction responses in BOO, possibly due to up-regulation of α₁D-AR.\textsuperscript{10} These findings support the hypothesis that the α₁D-ARs are mechanistically involved in the development of storage symptoms, and they are plausible targets for therapeutic interventions to achieve a stable
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bladder condition and control storage symptoms. Recently, changes in the distribution of the α1-AR subtype in the urinary bladder have been demonstrated in a rat BOO model. The study was conducted with a sham group and a BOO group to evaluate the impact of BOO on α1-AR expression. While there was no major difference in weight between the sham and BOO groups, urinary bladder weight was higher in the BOO group (0.76 g) than in the sham group (0.11 g). The expression of α1D-AR subtype was higher in the urothelium in the BOO group than in the sham group. The expression of α1A-AR mRNA was markedly reduced in the BOO group (0.57) compared to the sham group (2.43). In contrast, the expression of α1D-AR mRNA was notably higher in the BOO group (1.79) than in the sham group (0.71). An actual difference in the α1-AR subtype between the smooth muscle layers and the urothelium could not be detected.

Persson et al. demonstrated that bladder function in spontaneously hypertensive rats (SHRs) differed from that in control rats and was characterized by a decrease in bladder capacity and micturition volume, as well as by an increase in nonvoiding bladder contractions. The results have furthermore suggested differences in smooth muscle and neuronal responsiveness to norepinephrine between SHR and control rats. The distribution of α1-AR subtype in the urinary bladder of SHRs has been reported; rats were assigned to 2 groups, and the reference group was fed a normal chow diet, while another group was fed experimental chow that contained 8% NaCl. Blood pressure increased slightly depending on the NaCl diet. While the expression of α1D-AR subtype was increased in the
NaCl diet group, the expression of $\alpha_{1A}$-AR subtype was dramatically decreased in both bladder urothelium and smooth muscle layers. The expression level of $\alpha_{1D}$-AR mRNA was similarly increased, and that of $\alpha_{1A}$ was decreased. These findings suggest that the dynamics of $\alpha_{1}$-AR expression could partly contribute to bladder function.

Using rat bladder, Dmitrieva et al. investigated whether aging affects expression of $\alpha_{1D}$-AR and whether $\alpha_{1D}$-AR mediates contraction. Immunofluorescent staining for $\alpha_{1D}$-AR was detected in sections of the urothelium. Furthermore, Western blotting confirmed more $\alpha_{1D}$-AR in the urothelium in aged rats than in young rats. Moreover, phenylephrine increased intravesical pressure in a concentration-dependent manner in both young and aged rats, and the effect of phenylephrine was significantly greater in aged than in young rats. It was also found that prazosin and BMY7378, an $\alpha_{1D}$-AR antagonist, decreased the contractile response induced by phenylephrine in a concentration-dependent manner in aged and young rats. BMY7378 produced significantly greater inhibition in aged rats, while prazosin did not. These findings suggest that $\alpha_{1D}$-AR was overexpressed in aged rat urothelium, resulting in enhancement of the contractile response.

1.2.2. Voiding function and afferent nerve activity modulation through $\alpha_{1D}$-AR

To examine whether a molecule is really important in some physiological responses, animal genetic models may have advantages. Two groups recently reported that the $\alpha_{1D}$-AR subtype plays a
unique role in voiding in $\alpha_{1D}$-AR knockout (KO) mice. Chen et al. demonstrated that the $\alpha_{1D}$-AR subtype has an important role in regulating bladder function.$^{15}$ Mean daily voiding frequency was significantly lower in $\alpha_{1D}$-AR KO mice (9 times) than in wildtype (WT) mice (16 times). Mean volume per void was significantly larger in $\alpha_{1D}$-AR KO mice than in WT mice. Similarly, cystometric analysis demonstrated larger bladder capacity (140%) and voided volume (146%) in $\alpha_{1D}$-AR KO mice than in WT mice. Moreover, Wang et al. suggested that locally-released noradrenaline activates urothelial $\alpha_{1D}$-AR and affects urinary bladder function.$^{16}$ Cystometry using wild-type mice demonstrated that intravesical infusion of noradrenaline into the urinary bladder shortened the intercontraction interval in a dose-dependent manner, without changing the maximum voiding pressure. In contrast, $\alpha_{1D}$-AR KO mice showed no change in the intercontraction interval in response to intravesical infusion of noradrenaline. These findings indicate a predominant involvement of $\alpha_{1D}$-AR in the facilitation of the micturition reflex by noradrenaline.

Ishihama et al. demonstrated that $\alpha_{1D}$-ARs were expressed in the urothelium of the rat bladder with Western blotting and immunohistochemistry, and that inhibition of these receptors affects reflex voiding through an afferent nerve decrease.$^{17}$ The rather selective $\alpha_{1D}$-AR antagonist naftopidil prolonged the intercontraction interval during continuous infusion cystometrograms in conscious rats (143% of the control value) and suppressed the excitatory effect of intravesical infusion of 0.1% acetic acid on the intercontraction interval (220%). Naftopidil inhibited the bladder afferent nerve
activity induced by bladder distension (32%) and acetic acid infusion (30%) and decreased ATP levels in the bladder perfusate during bladder distension (37%). Sugaya et al. also reported the effects of naftopidil on the urinary ATP level and bladder activity after bladder stimulation in rats using continuous cystometry with 0.1% acetic acid. The shortened interval between bladder contractions with the acetic acid solution was recovered with naftopidil treatment. The urinary ATP level increase caused by the infusion of acetic acid was less with naftopidil. Therefore, naftopidil’s inhibitory effect on bladder activity may be partly due to blocking ATP release from the bladder epithelium.

A relationship between sensory nerve activation and an increased voiding reflex response is well known. Cold stress was found to significantly decrease the voiding interval, micturition volume, and bladder capacity in conscious rats. TRPM8 expression on the skin partly mediated the micturition responses. But how does $\alpha_{1D}$-AR affect the above relationship? A novel and interesting finding associated with cold stress stimulation was recently reported using OVX rats, which have been shown to have a decreased sensory afferent nerve threshold. Cold stress stimulates the skin TRPM8. The TRPM8-positive area in the skin was significantly higher (2-times) in OVX rats than in sham rats. In the OVX rats, the voiding interval was shortened from 3.0 min to 2.0 min, and the bladder capacity was smaller, from 0.73 mL to 0.39 mL under the low temperature condition as compared to the sham rats. Furthermore, naftopidil, an $\alpha_{1D}$-AR antagonist, blocked the OVX-induced effects.
(voiding interval, 4.7 min; bladder capacity, 0.83 mL). Therefore, the increased TRPM8 in OVX rats may result in $\alpha_{1D}$-AR mediated detrusor overactivity induced by cold stress under afferent nerve activation.
2. CLINICAL STUDIES

2.1. $\alpha_1$-AR subtypes in the human lower urinary tract

Historically, Malloy et al. found species heterogeneity in $\alpha_1$-AR subtype expression (human vs. rat), with $\alpha_{1D}$ predominating in human detrusor. Total $\alpha_1$-AR expression in human bladder was 6 fmol/mg total protein. Although significantly less than the $\alpha_1$-AR density in human prostate, bladder $\alpha_1$-AR expression is 3 times higher than the density in the human coronary artery. At a subtype level, $\alpha_{1D}$-ARs are twice as abundant as $\alpha_{1A}$-ARs in human detrusor at both the mRNA and protein levels; no $\alpha_{1B}$-ARs were found in human detrusor. Overall $\alpha_{1D}$-AR expression in human tissue is more limited than that of other $\alpha_1$-AR subtypes.

2.2. Functional responses of $\alpha_1$-AR in obstructed bladder

Nomiya et al. found that $\alpha_1$-ARs were expressed at low levels in human bladder, and $\alpha_{1D}$-AR mRNA was increased 1.8 times in obstructed patients compared with the control group, but the difference was not significant. Their functional study showed that phenylephrine at concentrations up to $10^{-4}$ M produced no contractile response in obstructed or control bladders. However, Chapple et al. showed that an $\alpha_1$ agonist produced responses in 6 of 11 patients with overactive detrusor bladder specimens. Furthermore, Bouchelouche et al. showed significant contractile responses to phenylephrine in preparations from BOO bladders. In contrast, $\alpha_1$ agonist responses were slight in
all nonobstructed bladders, although potassium-induced contractions in these tissue strips were similar to those in BOO specimens. Another interesting finding was that phenylephrine induced contractile oscillations and tonic contractions in BOO preparations, while each type of contraction was dose dependently inhibited by tamsulosin. Such inhibition was also obtained with the α1D-AR antagonist BMY7378, by shifting the phenylephrine-induced dose-response curve. These results and previous animal investigations\(^{11}\) support the hypothesis that α1D-AR might be important in storage symptoms associated with male LUTS.

The correlation between bladder expression of α1-ARs and sensation in patients has been unclear. A recent investigation demonstrated expression of α1-AR mRNAs in the bladder mucosa of men with LUTS and BPO and the association of α1-AR mRNAs with urodynamic parameters during storage of experimentally infused contrast medium.\(^{28}\) Expressions of α1A- and α1B-AR mRNAs in bladder mucosa from two groups (group 1: FDV ≤ 200 mL and/or SDV ≤ 300 mL; group 2: FDV ≥ 201 mL and/or SDV ≥ 301 mL) revealed no significant differences between the groups with respect to α1A- and α1B-AR mRNA levels. However, mucosa from the first group of patients had significantly more α1D-AR mRNA than did that from the latter group of patients. There seem to be many molecular causes of storage symptoms,\(^{29,32}\) not all necessarily involving urothelial α1-ARs. Nevertheless, the finding of a relationship between urodynamic sensory parameters and the expression levels of urothelial α1-AR mRNAs suggests that α1D-ARs may play an important role in
storage symptoms in male LUTS patients.

2.3. What is the role of the $\alpha_{1D}$-ARs in the human bladder?

Since the nonselective $\alpha_1$-AR antagonist terazosin relieves nocturia, as assessed by frequency volume charts (FVCs), one (or more) $\alpha_1$-AR subtypes must be responsible for this effect. Both tamsulosin, which is an $\alpha_{1A/1D}$-AR selective antagonist (rather $\alpha_{1A}$-AR selective), and naftopidil, which is an $\alpha_{1D/1A}$-AR selective antagonist (rather $\alpha_{1D}$-AR selective), reduce the nocturnal frequency in FVC by decreasing nocturnal urine volume. By contrast, silodosin, which is an $\alpha_{1A}$-AR highly selective antagonist, does not decrease the number of nighttime voidings in FVC. Then, is there evidence for $\alpha_{1D}$-AR-related LUTS that was obtained by comparing the effects of a rather $\alpha_{1A}$-AR selective antagonist and a rather $\alpha_{1D}$-AR selective antagonist on bladder storage function? Nishino et al. reported interesting evidence from a PFS by comparing the two drugs in a randomized, cross-over design. This study was conducted with an average prostate size of 20 mL in patients with severe symptoms (total IPSS $\geq$ 20). Tamsulosin and naftopidil caused no significant difference in voiding symptoms or total IPSS, but relief of storage symptoms, especially nocturia, was significantly greater with naftopidil. In PFS, moreover, the increases in the maximum desired volume and the first desired volume were higher with naftopidil than with tamsulosin. In 7 subjects who showed disappearance of involuntary contractions, disappearances were found in 5 subjects in each
cross-over period. In one subject, however, involuntary contractions disappeared during the first naftopidil period, but they returned after the switch to tamsulosin. In another subject, the cross-over to naftopidil resulted in disappearance of involuntary contractions, although the contractions continued with tamsulosin. In addition, both drugs decreased BOO grade. Kakizaki et al. have also investigated detrusor overactivity using a filling cystometry procedure in BPH patients with total IPSS storage symptom scores (frequency, urgency, and nocturia) ≥ 7. In nine patients who had detrusor overactivity before naftopidil treatment, filling cystometry was repeated after treatment. Bladder volume at first desire to void increased significantly from 174 ± 92 mL to 259 ± 109 mL. These investigations support the suggestion that α₁D-AR may play an important role, at least in part, in sensory afferent nerve activity.

2.4. α₁A- or α₁D-AR subtype dominant in patients with BPH

Initial studies examining the α₁-AR subtype in human prostate using RNase protection assays and in situ hybridization approaches revealed that α₁A-AR predominates at the RNA level. However, recent evidence demonstrated that, in addition to α₁A-AR, the α₁D-AR subtype was also present to a significant extent in human prostate using the real time RT-PCR procedure. This study demonstrated that the median expression levels (interquartile range) of α₁A-AR and α₁D-AR were 1.25 (0.66-2.45) and 1.18 (0.71-2.27) × 1,000 copies/β-actin, respectively, with no significant
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difference. Furthermore, the ratio of the mean expression level of each subtype to total $\alpha_1$-AR was 41.2% and 49.1% for $\alpha_{1A}$- and $\alpha_{1D}$-AR mRNAs, respectively. The correlation between the expression of $\alpha_1$-AR subtype mRNA in the prostate and the clinical efficacy of subtype-selective $\alpha_1$-AR antagonists was also examined.\textsuperscript{41} Patients who did not have malignant tumors based on prostate biopsy results were divided into two groups and given either tamsulosin or naftopidil. The efficacy of tamsulosin and naftopidil differed depending on the dominant $\alpha_1$-AR subtype in the prostate. Tamsulosin was more effective in patients with dominant expression of the $\alpha_{1A}$-AR subtype, whereas naftopidil was more effective in those with dominant expression of the $\alpha_{1D}$-AR subtype. Although this theory may not be clinically applicable for all BPH patients because of the inconvenience of biopsy, the $\alpha_1$-AR subtype mRNA expression level in the prostate could be a predictor of the efficacy of subtype selective $\alpha_1$-AR antagonists. Genetic differences may be responsible for the diverse responses to these drugs.
3. SUMMARY

There is evidence that targeting only $\alpha_{1A}$-AR may not provide comprehensive therapy for LUTS associated with BPH/BPO. The presence of $\alpha_{1D}$-ARs in the lower urinary tract suggests that this subtype may play an important role in the pathophysiology of male LUTS.
Conflict of interest

None declared.
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Fig. 1

The expression level of $\alpha_{1D}$-adrenoceptor (AR) mRNA or $\alpha_{1D}$-AR protein increases by bladder outlet obstruction, hypertension and/or aging. This may be related to the male lower urinary symptoms, and $\alpha_{1D}$-AR may become the target of the therapy.
Fig. 1

Expression level

\( \alpha_{1d} \)-AR : ↑
\( \alpha_{1D} \)-AR : ↑

Bladder dysfunction

- Frequency : ↑
- Nonvoiding contraction : ↑
- Bladder capacity : ↓
- Contractile response : ↑

\( \alpha_{1D} \) adrenoceptor antagonist