IMPROVEMENT IN BLADDER STORAGE FUNCTION BY TAM SULOSIN DEPENDS ON SUPPRESSION OF C-FIBER URETHRAL AFFERENT ACTIVITY IN RATS

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Running head: $\alpha_1$-blocker and C-fiber urethral afferents

Key words: C-fiber, $\alpha_1$-blockers, detrusor overactivity, prostaglandin

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ABSTRACT

Purpose: α₁-blockers improve voiding symptoms by reducing prostatic and urethral smooth muscle tone. However, the mechanism underlying improvements in storage symptoms is not known. Topical application of prostaglandin (PG) E₂ to the rat lower urinary tract stimulates the micturition reflex (MR). Using an animal model, we investigated whether the α₁-blocker tamsulosin acts on C-fiber afferent activity, and if so the location of this effect.

Materials and Methods: To induce desensitization of C-fiber afferent activity resiniferatoxin (0.3 mg/kg, RTX) was subcutaneously injected in female Sprague-Dawley rats 2 days prior to experiments. Simultaneous recordings of urethral pressure and rhythmic bladder pressure were made under urethane anesthesia. PGE₂ (0.4 mg/ml) was continuously administered intravesically or intraurethrally to rats pretreated with RTX (RTX rats) or rats without pretreatment (non-RTX rats). We investigated the effects on MR of intravenous (2.2x10⁻¹ – 2.2x10³ nM/kg) or intrathecal (0.001 – 0.1 nmol) administration of tamsulosin.

Results: Bladder contraction interval (BCI) was markedly reduced after intravesical or intraurethral administration of PGE₂ in non-RTX rats, but was unchanged in RTX rats. This effect was antagonized by an EP1 receptor antagonist (ONO-8711). Intravenous administration of tamsulosin significantly increased BCI in non-RTX rats receiving intraurethral PGE₂, but had no effect on non-RTX rats receiving intravesical PGE₂. Intrathecal administration of tamsulosin produced a slight and insignificant increase in BCI in non-RTX rats receiving intraurethral PGE₂.
Conclusion: These results suggest that PGE\textsubscript{2} enhances MR through C-fiber afference and that tamsulosin had an inhibitory effect on the C-fiber urethral afferent nerves, thereby improving bladder storage function.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is an age-related increase in the volume of the prostate, which leads to voiding and storage dysfunction caused by bladder outlet obstruction (BOO). The functional importance of \(\alpha\text{-adrenoceptors (}\alpha\text{-ARs}\) in the sympathetic nerve terminals of the prostate has been indicated, and it has been shown that dynamic obstruction is mediated by \(\alpha\text{-AR stimulation}\). Medical treatment of voiding, storage or both symptoms suggestive of BOO is now the initial choice of therapy, and \(\alpha\text{-AR blockers}\) remain the most widely used pharmacological agents aimed at the dynamic component of prostatic obstruction.\(^1\)

Recent attention has focused on the classification of neural supply to the bladder and prostate. Presently, \(\alpha\text{-ARs}\) are generally subdivided into \(\alpha\text{\textsubscript{1A}}\text{-, }\alpha\text{\textsubscript{1B}}\text{-, and }\alpha\text{\textsubscript{1D}}\text{-AR subtypes.}\(^2\) The \(\alpha\text{\textsubscript{1A}}\text{-AR subtype}\) predominates in the prostatic stroma at the mRNA and protein level, and is responsible for the dynamic component of obstruction and related voiding symptoms.\(^3\) The \(\alpha\text{\textsubscript{1D}}\text{-AR subtype}\) is expressed in the detrusor, prostate, peripheral ganglia, and spinal cord in humans and rats.\(^4\text{-7}\) Recently, a number of experimental findings have indicated the involvement of the \(\alpha\text{\textsubscript{1D}}\text{-AR subtype}\) in the storage symptoms.\(^5\text{-8}\) Because \(\alpha\text{-AR blockers}\) act during the storage phase to allow an increase in bladder capacity and a decrease in urgency, it is thought that they exert an
inhibitory effect on afferent nerves. However, the mechanism by which these blockers improve storage symptoms remains unknown.

Prostanoids, in particular prostaglandin (PG) E\textsubscript{2}, have been implicated as endogenous modulators of bladder function under both physiological and pathophysiological conditions.\textsuperscript{9} PG synthesis occurs locally in the bladder muscle and mucosa, and is initiated by various physiological stimuli such as detrusor muscle stretch and nerve stimulation, as well as by injury and mediators of inflammation. Several investigators have shown that PGE\textsubscript{2} can contract isolated human as well as animal detrusor muscle.\textsuperscript{9} This effect is unlikely to contribute to voiding contraction even if PGE\textsubscript{2} does facilitate the action of the endogenous efferent neurotransmitter acetylcholine.\textsuperscript{10} \textit{In vivo}, endogenous prostanoids may enhance voiding efficiency through a direct or indirect effect on sensory nerves.\textsuperscript{11} Topical application of PGE\textsubscript{2} to the bladder stimulates the micturition reflex (MR) in humans and rats.\textsuperscript{12,13} Increases in COX-2 expression and PGs in the bladder wall have been shown to play an important role in the development of detrusor overactivity caused by BOO.\textsuperscript{14,15} To our knowledge, however, the influence of intraurethral PGE\textsubscript{2} on MR has not been investigated.

In the present study, we investigated whether tamsulosin,\textsuperscript{16} an $\alpha$\textsubscript{1A}-and $\alpha$\textsubscript{1D}-AR blocker, acts on C-fiber afferents by comparing its effect on induced detrusor overactivity in C-fiber-desensitized and C-fiber normal rats. We also studied the effect of intravenous (iv) and intrathecal (it) administration of tamsulosin on the detrusor overactivity induced by intravesicular or intraurethral PGE\textsubscript{2}. 

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MATERIALS AND METHODS

A total of fifty-two female Sprague-Dawley rats weighing 225-268 g (mean = 252 g) were used. They were housed at a constant temperature (23 ± 2°C) and humidity (50-60%) under a regular 12-h light/dark cycle (lights on 7:00 AM - 7:00 PM). Tap water and standard rat chow were freely available. All experiments were performed in strict accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Fukui.

Simultaneous recordings of urethral and rhythmic bladder pressure

To induce desensitization of C-fiber afferent activity, we subcutaneously injected resiniferatoxin (0.3 mg/kg, RTX) 2 days prior to experiments. All surgical and urodynamic procedures were performed under urethane anesthesia (1.0 g/kg). The bladder and proximal urethra were exposed through a midline abdominal incision. Urethral activity, measured as urethral perfusion pressure, was monitored using a polyethylene catheter (size 3; i.d. 0.5 mm, o.d. 1.0 mm; Kunii Co. Ltd., Tokyo, Japan) with the tip embedded in a cone-shaped plug that was introduced transvesically through an incision in the bladder dome and then seated securely in the bladder neck. The cone-shaped plug was fashioned from Eppendorf pipette tip. The catheter end was then exteriorized at the external urethral meatus. To monitor intravesicular pressure, the bladder end of a catheter (size 4; i.d. 0.8 mm, o.d. 1.3 mm; Kunii Co. Ltd., Tokyo, Japan) was heated to create a collar and passed through the same incision of the bladder dome. This arrangement permitted the functional separation of bladder and urethral activity without the risk of surgical damage to the vesicourethral innervation associated
with a urethral ligation or total urethrotomy. The bladder catheter was connected to a pump (TE-311; Terumo Co. Ltd., Tokyo, Japan) for infusion of physiological saline and to a pressure transducer (TP-200T; Nihon-Kohden Co., Ltd., Tokyo, Japan) by means of a polyethylene T-tube. The urethral catheter was connected to a pump for continuous saline infusion (0.075 ml/min) and to a pressure transducer by means of a polyethylene T-tube.

**Experimental protocol**

After a 30-min postsurgical stabilization period, pressure recordings from the bladder and urethra were started. The bladder was filled with saline at a rate of 0.1 ml/min to induce the micturition reflex, which was evident by rhythmic, large-amplitude bladder contractions. Bladder filling was then discontinued and isovolumetric pressure was recorded. The urethra was continuously infused with saline (0.075 ml/min). Thus, isovolumetric bladder and urethral perfusion pressure were recorded independently and simultaneously. The values of the three parameters (bladder contraction interval, BCI; bladder contraction pressure, BCP; and bladder contraction duration, BCD) were obtained from the micturition reflex measurements (Fig. 1).

**Drug administration**

PGE$_2$ (0.4 mg/ml) dissolved in 0.1 M phosphate buffer (pH 7.4) was continuously administered intravesically or intraurethrally to rats pretreated with RTX (RTX rats) or rats without pretreatment (non-RTX rats). To confirm whether the effect of PGE$_2$ on MR was mediated by the EP1 receptor, ONO-8711 {6-[(2S,3S)-3-(4-chloro-2-methylphenylsulfonylaminomethyl)-bicyclo[2.2.2]octan-2-yl
]-5Z-hexenoic acid}, a selective EP1 antagonist chemically synthesized at Ono Pharmaceutical Co., Ltd. (Osaka, Japan), was given by intravenous administration. The effect of tamsulosin (Astellas Pharma Inc., Tokyo, Japan) on intravesical or intraurethral PGE_{2}-stimulated MR was investigated at iv doses of 2.2 \times 10^{-1} – 2.2 \times 10^{3} \, nM/kg and iv doses of 0.001 – 0.1 nmol.

Data analysis

Data are expressed as the mean ± standard error of the mean (SEM). Statistical comparisons were performed using one- or two-way repeated measures analysis of variance (ANOVA), with subsequent individual comparisons conducted using Fisher’s PLSD test. The two groups were compared using Mann Whitney’s U-test or Wilcoxon signed-ranks test. A level of p < 0.05 was considered statistically significant.

RESULTS

Effects of PGE_{2} on micturition reflex

BCI was markedly reduced after intravesical or intraurethral administration of PGE_{2} in non-RTX rats (Figs. 1, 2, 3), but was unchanged in RTX rats. Intravesical and intraurethral administration of PGE_{2} decreased BCI by 40.9% and 23.1%, respectively, with these ratios expressed as 0% in Fig. 3 as baseline ratios for the next experiment. These effects were antagonized by the EP1 receptor antagonist ONO-8711 (1 mg/kg, iv). PGE_{2} by intravesical administration gradually increased voiding threshold pressure (Fig. 1), whereas that by intraurethral administration had no effect (Fig. 2).

Effects of tamsulosin on PGE_{2}-stimulated micturition reflex
Intravenous administration of tamsulosin significantly increased BCI in rats receiving intraurethral PGE$_2$ (p <0.05), but had no particular effect on those receiving intravesical PGE$_2$ (Figs. 3, 4, 5). The percentage increases in BCI in rats receiving intraurethral and intravesical PGE$_2$ at 2.2 x 10$^3$ nM/kg tamsulosin were 89.4% and 18.9%, respectively. The high dose of tamsulosin did not increase BCI in rats receiving intravesical PGE$_2$, whereas ONO-8711 (1 mg/kg iv) completely reversed the influence of PGE$_2$ on BCI (Fig. 5). Further, in rats not receiving PGE$_2$, tamsulosin showed no significant effect on BCI (Fig. 3), and had no effect on BCI in RTX rats. The high dose of tamsulosin (2.2 x 10$^3$ nM/kg) decreased BCP in rats receiving intraurethral or intravesical PGE$_2$ and in rats not receiving PGE$_2$ by 26.1%, 8.2%, and 47.5%, respectively (Fig. 6). Further, tamsulosin by intravenous administration had no effect on BCD in any treatment group (Fig. 7), although intrathecal administration produced a slight and insignificant increase in BCI in rats receiving intraurethral PGE$_2$.

**DISCUSSION**

Our findings indicate that PGE$_2$ produces an excitatory effect on MR by stimulation of C-fiber afferent nerves via the EP1 receptor. This effect of PGE$_2$ was seen on application to either the bladder or the urethra. Tamsulosin by intravenous administration had an inhibitory effect on this agonistic effect on MR of intraurethral but not intravesical PGE$_2$ and did not produce a decrease in BCP at low doses. Tamsulosin by intrathecal administration, in contrast, had only a slight and insignificant inhibitory effect on intraurethral PGE$_2$-stimulated MR. These results support the
hypothesis that this $\alpha_1$-AR blocker improves detrusor overactivity by inhibiting C-fiber afferent activity in the urethra rather than in the spine, and this effect does not depend on the inhibition of C-fiber afferent activity in the spine.

Studies in rats and humans have demonstrated that intravesical administration of PGE$_2$ results in detrusor overactivity.$^{12,13}$ PGE$_2$ produces its endogenous activity via the EP receptor family of G protein-coupled receptors, of which four subtypes have been identified to date. Using EP1 receptor knockout mice, Schröder demonstrated that the EP1 receptor was not essential for normal micturition but did play a role in the development of detrusor overactivity caused by PGE$_2$ and BOO.$^{15}$ In the present study, continuous intravesical administration of PGE$_2$ caused a significant decrease in BCI in non-RTX rats, whereas ONO-8711 antagonised this effect. These findings indicate that these effects of PGE$_2$ are attributable to the stimulation of EP1 receptors. Further, RTX rats did not respond to intravesical PGE$_2$, leading us to hypothesize that PGE$_2$ produces its excitatory influence on MR by stimulating C-fiber afferent nerves via the EP1 receptor.

In the present study, the excitatory effects on MR produced by intravesical administration of PGE$_2$ were also seen in non-RTX rats receiving intraurethral administration, but not in RTX-rats. The mechanism of this overactivity is unknown, but may be initiated by a PGE$_2$–mediated increase in urethral afferent activity. It has been suggested that detrusor overactivity caused by BOO may be initiated from the bladder outlet region rather than from the bladder itself.$^{18}$ In cats, urethral perfusion triggered spontaneous bladder contraction of such intensity and frequency that bladder filling was
not possible. Pharmacological activation of urethral afferent nerves by intraurethral capsaicin elicited a biphasic change in MR, initially decreasing BCI within minutes, followed 15 to 30 min later by complete MR blockage. Immunohistochemical data have indicated the presence of capsaicin-sensitive primary afferent fibers in the rat proximal urethra. Considering these findings and the antagonistic effect of ONO-8711 on PGE$_2$-stimulated MR, urethral C-fiber afferent activity may be a trigger to the induction of detrusor overactivity via the EP1 receptor in rats.

A recent study reported that $\alpha_{1d}$-AR mRNA is present in the human detrusor, that the ratio of $\alpha_{1d}$-AR mRNA is higher than that of $\alpha_{1a}$-AR mRNA, and that the $\alpha_{1D}$-AR subtype is closely related to the storage symptoms encountered in patients with BPH. The ratio of $\alpha_{1d}$-AR subtype to all $\alpha_1$-AR subtype mRNAs has been reported to be higher in the obstructed rat bladder. $\alpha_1$-AR blockers with significant affinity for the $\alpha_{1D}$-AR subtype are therefore thought able to improve storage symptoms related to BOO. Nomiya et al. found that mRNAs of the $\alpha_{1a}$-, $\alpha_{1b}$-, and $\alpha_{1d}$-AR subtypes are expressed at low levels in the obstructed human bladder, whereas $\beta_3$-AR mRNA is highly expressed. They suggested that bladder $\alpha_1$-ARs are not likely to be responsible for the detrusor overactivity and storage symptoms in patients with BPH.

In the present study, tamsulosin had an inhibitory effect on intraurethral PGE$_2$-stimulated MR, but not on intravesical PGE$_2$-stimulated MR. This result lead us to hypothesize that tamsulosin exerts an inhibitory effect on C-fiber afferent nerves in the urethra. Tamsulosin is a combined $\alpha_{1A}$ and $\alpha_{1D}$-AR antagonist which exerts a relaxant effect on urethral rather than bladder smooth muscle. The $\alpha_1$-AR subtype
predominates in prostatic stroma at the mRNA and protein level, and is responsible for the dynamic component of obstruction.\textsuperscript{3} Taken together, these findings suggest that tamsulosin exerts an inhibitory effect on C-fiber urethral afferent nerves by decreasing urethral tonus, and thereby improving of storage symptoms. However, using urethane treated animal increases the risk that effects of \(\alpha_1\)-AR blockers in the spinal cord will be masked by the urethane. In order to really confirm C-fiber urethral afferent nerves, it is ideally necessary to record the activity from identified C-fibers. Further research on the underlying mechanisms of the interaction between \(\alpha_1\)-AR and C-fiber afferent activity in the urethra may lead to new therapeutic modalities targeted at detrusor overactivity.

CONCLUSION

In vivo animal study reveals that exogenous PGE\(_2\) enhances MR through effects on C-fiber efferent nerves in both the bladder and urethra. Tamsulosin has an inhibitory effect on C-fiber urethral afferent nerves, thereby improving bladder storage function. These findings are likely to be applicable to the human subject and to explain the mechanism that \(\alpha_1\)-AR blockers improve storage symptoms.

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FIGURE LEGENDS

Figure 1. Simultaneous recordings of isovolumetric bladder and urethral perfusion pressure before (A) and 10 min after (B) intravesical administration of prostaglandin (PG)E₂. Intravesical PGE₂ facilitated rhythmic bladder contraction and gradually increased voiding threshold pressure. BCI: Bladder Contraction Interval, BCP: Bladder Contraction Pressure, BCD Bladder Contraction Duration.

Figure 2. Simultaneous recordings of isovolumetric bladder and urethral perfusion pressure before (A) and 10 min after (B) continuous intraurethral administration of PGE₂. Intraurethral PGE₂ facilitated rhythmic bladder contraction but did not increase voiding threshold pressure.

Figure 3. Effects of intravenous tamsulosin (2.2 x 10⁻¹ – 2.2 x 10³ nM/kg) on bladder
contraction interval (BCI) in urethane-anesthetized rats. Intraurethral and intravesical administration of PGE₂ significantly reduced BCI (circles and triangles, respectively). BCI values after intravesical or intraurethral administration of PGE₂ are expressed as 0%. Increases in BCI were recognized at increasing doses of tamsulosin in rats receiving intraurethral PGE₂, whereas no change was seen in rats receiving intravesical PGE₂. Single asterisk indicates p <0.05 vs rats receiving intravesical PGE₂. No change in BCI was seen with increasing doses of tamsulosin in rats not receiving PGE₂ (squares).

Figure 4. Effect of intravenous tamsulosin (2.2 nM/kg) on simultaneous recordings of isovolumetric bladder and urethral perfusion pressure bladder contraction interval in urethane-anesthetized rats receiving intraurethral administration of PGE₂. Note that tamsulosin increased bladder contraction interval.

Figure 5. Effect of a high dose (2.2 x 10³ nM/kg) of intravenous tamsulosin on simultaneous recordings of isovolumetric bladder and urethral perfusion pressure in urethane-anesthetized rats receiving intravesical administration of PGE₂ (A). Note that tamsulosin slightly increased bladder contraction interval (BCI). Intravenous administration of ONO-8711 increased BCI at a dose of 1 mg/kg (B).

Figure 6. Effect of intravenous tamsulosin (2.2 x 10⁻¹ – 2.2 x 10³ nM/kg) on bladder contraction pressure (BCP) in urethane-anesthetized rats. Intraurethral and intravesical administration of PGE₂ significantly reduced BCP (circles and triangles, respectively). BCP values after intravesical or intraurethral administration of PGE₂ was expressed as 0%. In rats not receiving PGE₂ (squares) BCP values before administration of
tamsulosin are expressed as 0%. Decreases in BCP were seen with a high dose (2.2 x 10^3 nM/kg) of tamsulosin in rats receiving intraurethral PGE\textsubscript{2} and in rats not receiving PGE\textsubscript{2}. Single asterisk indicates p <0.05 vs before administration of tamsulosin.

Figure 7. Effect of intravenous tamsulosin (2.2 x 10^{-1} – 2.2 x 10^3 nM/kg) on bladder contraction duration (BCD) in urethane-anesthetized rats. Intraurethral and intravesical administration of PGE\textsubscript{2} significantly reduced BCD (circles and triangles, respectively). BCD values after intravesical or intraurethral administration of PGE\textsubscript{2} are expressed as 0%. BCD values before administration of tamsulosin are expressed as 0% in rats not receiving PGE\textsubscript{2} (squares). No changes were found in the three treatment groups.
Fig. 1

A

Urethra

Bladder

10 cmH$_2$O

1 min

B

BCI

BCP

BCD
Fig. 2

A
Urethra

B
Bladder

10 cmH₂O
1 min
Fig. 3

change in bladder contraction interval (% of control)

Dose of tamsulosin (nM/kg)

Control PGE₂ 2.2 × 10⁻¹ 2.2 2.2 × 10⁻² 2.2 × 10⁻³

-40 -20 0 20 40 60 80 100 120 140
Fig. 4

Urethra

Tamsulosin 2.2 nM/kg

Bladder

10 cmH₂O

1 min
Fig. 5

A
Tamsulosin $2.2 \times 10^3$ nM/kg
Urethra
Bladder

B
ONO-8711 1 mg/kg
10 cmH$_2$O
1 min
Fig. 6

Change in bladder contraction pressure (% of control) vs. Dose of tamsulosin (nM/kg)
Fig. 7

Dose of tamsulosin (nM/kg)

Change in bladder contraction duration (% of control)