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Investigations into the Presence of Functional β1, β2 and β3-Adrenoceptors in Urothelium and Detrusor of Human Bladder

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ABSTRACT

Purpose: We investigated the presence of functional β1, β2 and β3-adrenoceptor in urothelium and detrusor muscle of human bladder through in vitro pharmacology of selective β3 adrenoceptor agonist solabegron.
Materials and Methods: Expression of these adrenoceptors in surgically separated human urothelium and detrusor muscle were investigated using RT-PCR. The effects of activating these receptors were studied by determining the relaxation produced by β-adrenoceptors agonist in pre-contracted human detrusor strips.
Results: The results confirmed the presence of mRNA for β1, β2 and β3-adrenoceptor in both human urothelium and detrusor. In an in vitro functional bladder assay, Solabegron and other agonists for β-adrenoceptors such as procaterol and isoproterenol evoked potent concentration-dependent relaxation of isolated human bladder strips with pD_2 values of 8.73 ± 0.19, 5.08 ± 0.48 and 6.28 ± 0.54, respectively.
Conclusions: Selective β3-adrenoceptor agonist may be a potential new treatment for the overactive bladder OAB syndrome. Existence of β3-adrenoceptor mRNA exists in the urothelium in addition to the detrusor muscle suggest multiple site of actions for the β3-adrenoceptor in the lower urinary tract.

Key words: *bladder; urothelium; detrusor; adrenoreceptors*
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INTRODUCTION

The overactive bladder (OAB) syndrome affects more than 17 million people in the United States. Muscarinic receptor antagonists are the most common form of pharmacologic treatment therapy prescribed for treating OAB, but are associated with mechanistic side effects related to effect of these agents on muscarinic receptors at other sites (1). Muscarinic receptor antagonists act to block nerve evoked bladder contraction and alternative approach may be to develop drugs acting on the storage phase of micturition (2). It is also commonly accepted that the muscarinic receptor antagonists act during the storage phase (3). Towards that goal, it seems logical that bladder relaxation mediated by β-adrenoceptors will be a viable target, because increased relaxation of the detrusor smooth muscle will lengthen the duration of storage phase in micturition cycle and thereby alleviate the symptoms of OAB (4).

Studies have shown that bladder relaxation evoked by β-adrenoceptor agonists is mainly mediated by β3-adrenoceptor in most species (5). β3-adrenoceptor agonist are said to stimulate the G protein (Gs) and activate adenyle cyclase (AC) to increase the intracellular level of adenosine 3',5'-cyclic mo-

nophosphate and causes relaxation of smooth muscle in the bladder (5). Other mechanisms involved in β-adrenoreceptor induced relaxation of bladder are also not ruled out (5). Studies have shown that at least 95% of the adrenoceptor transcripts in human bladder are represented by β3-adrenoceptor subtype (6). Selective β3-adrenoceptor agonists have been shown to cause significant relaxation of human bladder strips as compared with β1 and β2-adrenoceptor agonists, and this was observed in normal and neurogenic bladders (7). β3-adrenoceptor agonists are expected to be clinically useful agents in the treatment of the OAB syndrome.

Although expression of β-adrenoceptors in human bladder has been previously reported using receptor-binding studies, but the anatomical distribution of the expression and which subtype(s) of β-adrenoceptor are expressed in the urothelium has only recently been indicated (8). Expression of β-adrenoceptors in urothelium is favored by the mounting evidence in support of a important role played by bladder epithelial cells in modulating bladder activity in response to local chemical and mechanical stimuli (9). Thus, in this study, we aimed to investigate the presence of β1-, β2- and β3-adrenoceptor in separate tissue of urothelium and detrusor muscle from human bladder. Functional significance of β3-adrenoceptors was investigated by studying the effects of GW427353 or Solabegron®, a selective β3-adrenoceptor agonist in human detrusor muscle.

MATERIALS AND METHODS

Human bladders - The human bladders were obtained via Institutional Review Board approved informed consent from the next of kin of the six organ donors using an honest broker system from the Health Sciences Tissue Bank at University of Pittsburgh Medical Center. The organ donors were 4 males and 2 females aged between 18-69 years. The health and disease status of organ donors was not available to the study investigators.

Isolated bladder strips - The mucosa and adventitia was removed and longitudinal detrusor strips of approximately 10x5x3 mm were obtained. Fine silk sutures were tied to each end of the strips, and tissues were lowered into the myobath multi-channel tissue bath system (World Precision Instruments, Sarasota, Florida, USA). The changes in muscle tension were digitally recorded by PowerLab Software (Charts 5, ADInstruments, Colorado Springs, CO, USA). Each tissue sample was suspended in a 7 mL organ bath containing oxygenated Krebs solution (NaCl, 118 mmol/l; KCl, 5.6 mmol/l; NaHCO3, 25 mmol/l; KH2PO4, 1.2 mmol/l; CaCl2, 2.5 mmol/l; MgSO4, 1.2 mmol/l; glucose 6.1 mmol/l; pH 7.4; aerated with 95% O2 / 5% CO_2) and maintained at 37ºC and constantly aerated with 95% oxygen and 5% carbon dioxide. The tissues were subjected to a resting tension of 1-2 grams and allowed to equilibrate for 60 min, during this time the tissue was washed every 15 min and resting tension was adjusted. After obtaining basal tension of 1-2 grams, muscle contractions were induced using 30 mM KCl bath application. For electrical field stimulation (EFS) studies, the equilibrated strips were given EFS using following parameters (5-40 Hz, 0.1 ms pulses, 1s train duration, 80V SIU). Bladder strips were incubated for 15 min with β3-adrenoceptor agonist prior to testing EFS responses and with a 10 min wash after each stimulus.

Chemicals - KCl, procaterol, isoproternol and papaverine were obtained from Tocris bioscience Catalog (Ellisville, Missouri, USA). GW427353 or solabegron (a selective β3-adrenoceptor agonist) was provided by GlaxoSmithKline Pharmaceutical Company (King of Prussia, PA, USA). All solutions were freshly prepared daily. Drugs were dissolved in deionized distilled water. Further dilutions were carried out in Krebs solution. The concentrations are expressed as the final molar concentration in the tissue chamber.

RT-PCR studies - Total RNA extraction was performed with TRIzol® reagent using manufacturer instructions (Invitrogen™) from surgically separated urothelium and detrusor tissues. Reverse transcription with 3 µg of RNA was followed by PCR with primers specific for the cDNA coding for human ß1, ß2, and ß3 receptor subtypes (Table-1). Reactions were performed under certain conditions, including 95°C for 10 minutes, 40 cycles at 95°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute. Good separation of the bladder layers was evaluated by H&E staining.

Table 1 *– Primer sequences.*

Primer	Sequence	PCR Product
β1	Forward: TCGTGTGCACCGTGTGGGCC Reverse: AGGAAACGGCGCTCGCAGCTGTCG	265bp
β2	Forward: GCCTGCTGACCAAGAATAAGGCC Reverse: CCCATCCTGCTCCACCTTGG	329bp
β3	Forward: GCTCCGTGGGCCTCACGAGAACAGC Reverse: CCCAACGGCCAGTGGCCAGTCAGC	314bp

Immunostaining - Separated tissues of urothelium and detrusor were snap frozen in liquid nitrogen immediately after dissection and embedded in OCT freezing medium. Cryosections 10 μM thick were used for Hematoxylin & Eosin staining.

Statistical evaluation - Results are expressed as means ± SEM of measurements in strips obtained from at least 6 different human bladders, and a maximum of three strips per bladder were used. Contractile responses are expressed as absolute values (M) or as percentage of the relaxation induced by 10^{-3} M papaverine. Concentration-response data of KCl were evaluated by sigmoid curve fitting and -logEC_{50} values (pD_2) were calculated by non-linear regression analysis using GraphPad Prism. Differences between mean values were statistically analyzed using an unpaired student t-test. A probability value of $p < 0.05$ was regarded to be significant. All analyses were performed using GraphPad Prism software (version 4.0; Graphpad Software Inc., San Diego, CA).

RESULTS

Presence of transcripts for ß1-, ß2-, and ß3-adrenoceptors was determined by PCR amplification of cDNA obtained from surgically separated detrusor and urothelium tissue of human bladder. Specific primers for the ß1-, ß2-, and ß3-adrenoceptor gene produced a single PCR product of expected size in all sets when separated on ethidium bromide stained 1.5% agarose gels (Figure-1). Separation of urothelium and detrusor was demonstrated by lack of any smooth muscle tissue stain in H&E staining (Figure-2). The lack of contamination from detrusor tissue in the separated urothelium was further verified by lack of immunoreactivity for nonvascular smooth muscle specific protein desmin in urothelium (data not shown). The detrusor tissue showed strong stain for desmin compared to a faint stain in urothelium.

Functional significance of these receptors was tested by relaxation of human bladder strips by ß3-adrenoceptor agonist Solabegron. Responses of drugs were expressed as a percentage of the 10^{-3} M papaverine induced relaxation of tension produced

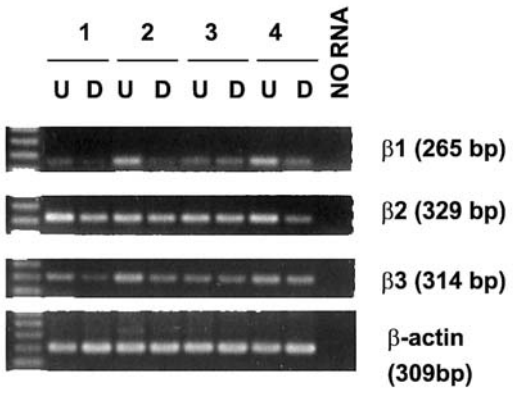

Figure 1 *– Expression ß-adrenoceptors in human urothelium and detrusor. The representative ethidium bromide stained agarose gel of RT-PCR products for β1, β2 and β3-adrenoceptors show their mRNA expression in separated detrusor and urothelium tissue from 4 bladder specimens marked 1-4 on top. Lane from urothelium tissue of each bladder specimen is marked by* **U** *and lane for detrusor tissue is marked by* **D**. *Left lane is 100 bp DNA ladder in all gels and products of expected size are shown by bands for β1, β2 and β3-adrenoceptors in gels from top to bottom. Right most lane representative control with no template.*

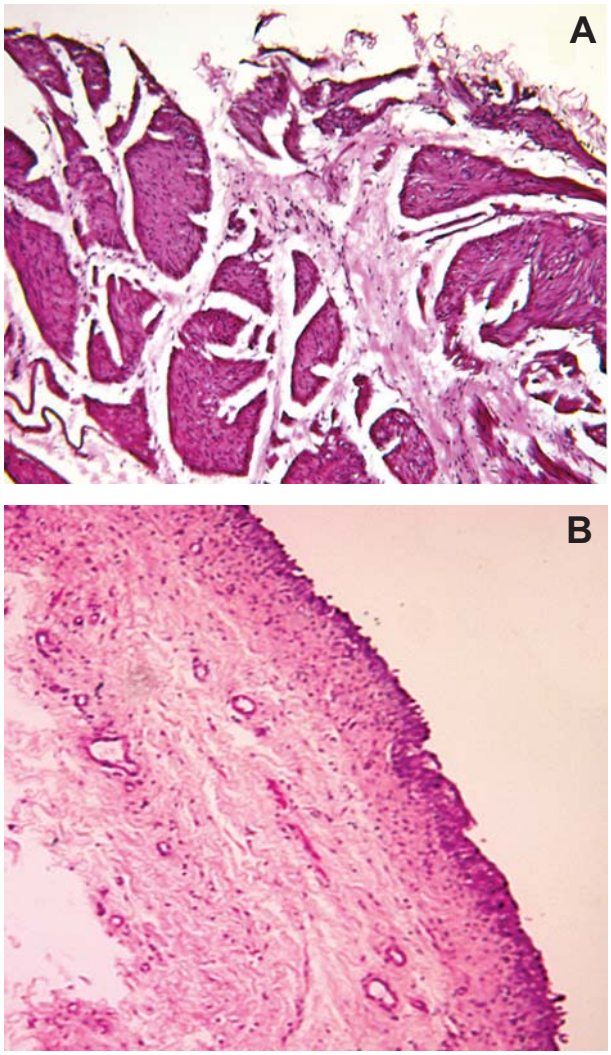

Figure 2 *– H & E staining of surgically separated tissue of detrusor (Panel A) and urothelium (Panel B). Absence of any smooth muscle cells in urothelium section verify lack of contamination from detrusor in the expression studies demonstrated in Figure-1.*

by KCl. Cumulative addition of Solabegron into the myobath evoked concentration-dependent relaxation of KCl precontracted human bladder strips and attained a significant effect at nanomolar concentration range. Solabegron relaxed the human bladder strips that were pre-contracted with 30 mM KCl with a pD_2 value of 8.73 ± 0.19 (Figure-3).

Isoproterenol (a non-selective ß-adrenoceptor agonist) produced significant relaxation of pre-contracted muscle strips at $>10^{-6}$ M, whereas, solabegron produced significant relaxation at $> 10^{-9}$ M (Figure-3). When compared with procaterol (a selective ß2 agonist), solabegron produced a significant ($p < 0.05$) relaxation (Figure-3). The pD_2 values were calculated from the concentration-relaxation curve were 5.08 ± 0.48, 6.28 ± 0.54 and 8.73 ± 0.19 for procaterol, isoproterenol and solabegron, respectively.

In other experiments, efficacy of solabegron to relax human bladder contraction in response to electrical field stimulation EFS at the lower frequencies (5, 10 and 15 Hz) was compared against isoproterenol. Drugs were incubated for 20 min. in the myobath prior to testing their effect on EFS (Figure-4). There was a significant inhibition of detrusor contraction at 10 Hz by solabegron and isoproterenol at 10^{-4} M (Figure-4) and the detrusor response was reduced at all frequencies. However, maximum suppression was observed at EFS induced contractions of lower frequencies as 5 Hz. The force-frequency curve was shifted to the right by both solabegron and isoproterenol, at 10^{-4} M (the percentage effect become similar for frequencies of 10-15 Hz). Efficacy of solabegron was slightly better than isoproternol in suppressing EFS evoked contraction, but not statistically significant.

COMMENTS

A previous study by Yamaguchi has determined the relative abundance of ß1-, ß2-, and ß3-adrenoceptor in the human bladder, but the anatomical distribution of expression in bladder in terms of urothelium and detrusor has not been determined (6). In our study, we demonstrated expression of all three ß-adrenoceptors mRNAs urotheium as well as in detrusor muscles of the human bladder. The functional importance of ß-adrenoceptors expressed in urothelium remains to be completely investigated. It is interesting to note that effect of ß-adrenoceptors agonists on micturition is mediated principally by these receptors (10).

The effect of Solabegron on human bladder strips was recently reported (11). Our results on the effect of same drug Solabegron on human bladder generally agree with the earlier study showing relaxation of human detrusor strip pre-contracted with carbachol (11). However, the few notable

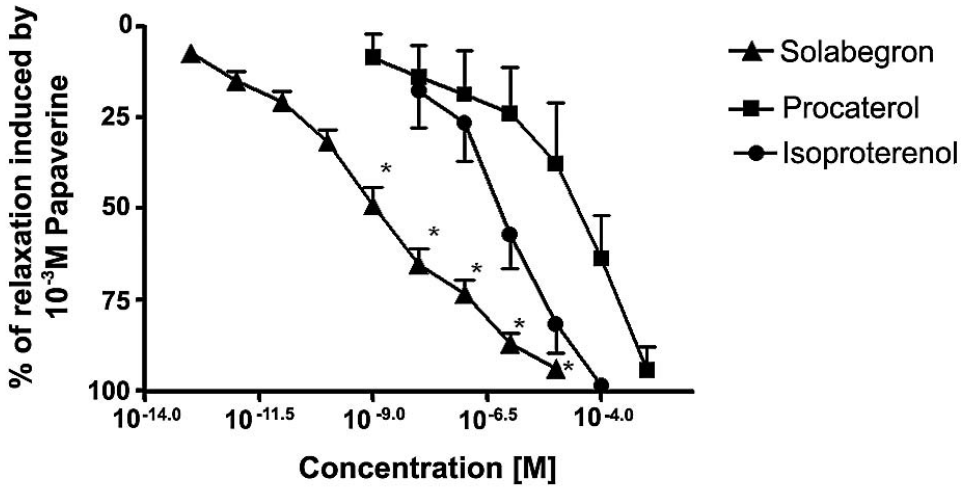

Figure 3 – *Concentration dependent relaxation of the KCl-induced tone of human detrusor strips by Solabegron, Procaterol and Isoproterenol. Relaxation evoked by drugs in muscle strips pre-contracted with 30nM KCl is expressed as percentage of the relaxation induced by 10-3 M papaverine. Values are expressed as the mean (SEM) and significant difference from control was considered at *p < 0.05. Dose response curve were evaluated by sigmoid curve fitting and -logEC50 values (pD_2) were calculated by non-linear regression analysis using Graph Pad Prism.*

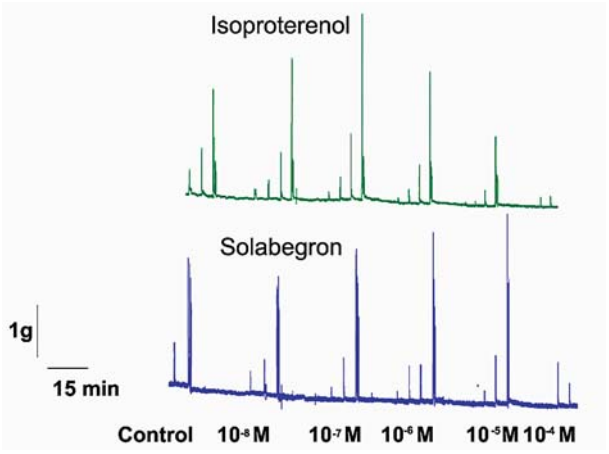

Figure 4 – *A representative trace for the response of a human detrusor strip to EFS (5, 10, 15 Hz) in the presence of oxygenated Krebs' solution, and after 20 min incubation in Solabegron and* ***Isoproterenol*** *(10^{-8}-10^{-4} M). Both the drugs suppressed EFS evoked detrusor contraction at 10^{-4} M concentration. The maximum suppression was observed at EFS induced contractions of lower frequencies as 5 Hz.*

differences are the use of different stimuli of KCl to evoke contraction prior to the addition of solabegron in this study. The significant relaxation of KCl pre-contracted muscle strips were produced at much lower concentration of 10^{-9} M, as against 10^{-7} M reported for relaxation of carbachol tone (11). Another major difference between the two studies on human bladder was the lack of difference observed in this study with respect to the suppression of EFS induced detrusor contraction. Biers et al. reported that only solabegron was able to suppress EFS contraction at 10^{-4} M, whereas we observed that isoproternol was equipotent in suppressing the EFS induced contraction of human detrusor (Figure-4). It is possible that different experimental methodology followed in the two studies will explain the differences reported here. On other hand, the EFS response on tissue strips was measured prior to testing of drugs and no drugs were added to myobath before EFS was done. Whereas the previous study (11), the tissue strips were subjected to carbachol 10^{-5} M contraction prior to EFS response. In addition to different experimental methodology to explain different results, there could be differences in the sensitivities of bladder specimens used in the two studies to drugs and electrical stimulation.

Evidence suggests that ß-adrenoceptor activation by isoproterenol in rat urothelial cells can trigger production and release of NO due to an increase

in intracellular Ca^{2+} following activation of the adenylate cyclase pathway in the urothelial cells (12). It has been reported that intravesical administration of NO scavenger decreased bladder capacity inducing bladder contractions (13) and that an intravesically applied NO donor decreased bladder overactivity induced by a chemical irritant, cyclophosphamide in rats (14). Considering the minimal relaxing effects of NO on rat bladder smooth muscles (15); it is believed that effect of NO on reflex bladder activity is much better explained by suppression of excitability of and/or the release of transmitters from bladder afferent nerves (16). Thus, it seems reasonable to assume that information about β-adrenoceptors expressed in the human urothelium might be involved in the regulation of bladder sensory functions.

Studies have shown that stimulation of β2- and β3-adrenergic receptors existing in the human detrusor can produce direct relaxation of the detrusor smooth muscle without blocking voiding induced bladder contraction (2,6). This β-adrenergic-stimulated relaxation is mediated through the stimulation of adenyl cyclase and the accumulation of cyclic AMP (17). The involvement of β3-adrenoceptor activation in mediating the relaxation of human detrusor by β3-adrenoceptor agonists was demonstrated by lack of suppression of its effect by selective β1 and/or β2-adrenoceptor antagonists such as dobutamine and procaterol (6). The role of β3-adrenoceptor is further verified by the blockade of Solabegron evoked relaxation by selective β3-adrenoceptor antagonists.

Considering the role of β3-adrenoceptors in modulating the control of bladder smooth muscle tone in humans; these results support the hypothesis that β3-AR agonists represent a useful clinical strategy for treating detrusor overactivity (18). β-adrenoceptor antagonists blockers have also been advocated for stress urinary incontinence owing to inappropriate reflex urethral relaxation, because propranolol prevents the reduction in urethral pressure after sacral root stimulation (19). However, β-adrenoceptor antagonists are not particularly useful in treating bladder or urethral disorders (20). Currently therapy for the OAB syndrome, such as with antimuscarinic agents or direct acting smooth muscle relaxants, can produce the result of urinary retention and other mechanistic side-effects (21).

CONCLUSIONS

The present study demonstrates that stimulation of β3-receptor with Solabegron evokes human bladder relaxation, suggesting that selective β3-adrenoceptor agonist may be a valuable new treatment for the OAB syndrome. The expression of β1-, β2- and β3-adrenoceptors in urothelium apart from detrusor may suggest additional mechanism of action for the β3-adrenoceptor in the lower urinary tract.

CONFLICT OF INTEREST

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REFERENCES

1. Andersson KE: LUTS treatment: future treatment options. Neurourol Urodyn. 2007; 26: 934-47.
2. Takeda H, Yamazaki Y, Akahane M, Igawa Y, Ajisawa Y, Nishizawa O: Role of the beta(3)-adrenoceptor in urine storage in the rat: comparison between the selective beta(3)-adrenoceptor agonist, CL316, 243, and various smooth muscle relaxants. J Pharmacol Exp Ther. 2000; 293: 939-45.
3. Finney SM, Andersson KE, Gillespie JI, Stewart LH: Antimuscarinic drugs in detrusor overactivity and the overactive bladder syndrome: motor or sensory actions? BJU Int. 2006; 98: 503-7.
4. Yoshimura N, Kaiho Y, Miyazato M, Yunoki T, Tai C, Chancellor MB, et al.: Therapeutic receptor targets for lower urinary tract dysfunction. Naunyn Schmiedebergs Arch Pharmacol. 2008; 377: 437-48.
5. Frazier EP, Peters SL, Braverman AS, Ruggieri MR Sr, Michel MC: Signal transduction underlying the control of urinary bladder smooth muscle tone by muscarinic receptors and beta-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol. 2008; 377: 449-62.
6. Yamaguchi O: Beta3-adrenoceptors in human detrusor muscle. Urology. 2002; 59: 25-9.
7. Igawa Y, Yamazaki Y, Takeda H, Kaidoh K, Akahane M, Ajisawa Y, et al.: Relaxant effects of isoproterenol and selective beta3-adrenoceptor agonists on normal, low compliant and hyperreflexic human bladders. J Urol. 2001; 165: 240-4.
8. Otsuka A, Shinbo H, Matsumoto R, Kurita Y, Ozono S: Expression and functional role of beta-adrenocep-

tors in the human urinary bladder urothelium. Naunyn Schmiedebergs Arch Pharmacol. 2008; 377: 473-81.
9. Murakami S, Chapple CR, Akino H, Sellers DJ, Chess-Williams R: The role of the urothelium in mediating bladder responses to isoprenaline. BJU Int. 2007; 99: 669-73.
10. Yamaguchi O, Chapple CR: Beta3-adrenoceptors in urinary bladder. Neurourol Urodyn. 2007; 26: 752-6.
11. Biers SM, Reynard JM, Brading AF: The effects of a new selective beta3-adrenoceptor agonist (GW427353) on spontaneous activity and detrusor relaxation in human bladder. BJU Int. 2006; 98: 1310-4.
12. Birder LA, Nealen ML, Kiss S, de Groat WC, Caterina MJ, Wang E, et al.: Beta-adrenoceptor agonists stimulate endothelial nitric oxide synthase in rat urinary bladder urothelial cells. J Neurosci. 2002; 22: 8063-70.
13. Pandita RK, Rønn LC, Jensen BS, Andersson KE: Urodynamic effects of intravesical administration of the new small/intermediate conductance calcium activated potassium channel activator NS309 in freely moving, conscious rats. J Urol. 2006; 176: 1220-4.
14. Ozawa H, Chancellor MB, Jung SY, Yokoyama T, Fraser MO, Yu Y, et al.: Effect of intravesical nitric oxide therapy on cyclophosphamide-induced cystitis. J Urol. 1999; 162: 2211-6.
15. Andersson KE, Persson K: Nitric oxide synthase and the lower urinary tract: possible implications for physiology and pathophysiology. Scand J Urol Nephrol Suppl. 1995; 175: 43-53.
16. Masuda H, Kim JH, Kihara K, Chancellor MB, de Groat WC, Yoshimura N: Inhibitory roles of peripheral nitrergic mechanisms in capsaicin-induced detrusor overactivity in the rat. BJU Int. 2007; 100: 912-8.
17. Harmon EB, Porter JM, Porter JE: Beta-adrenergic receptor activation in immortalized human urothelial cells stimulates inflammatory responses by PKA-independent mechanisms. Cell Commun Signal. 2005; 3: 10.
18. Furuta A, Thomas CA, Higaki M, Chancellor MB, Yoshimura N, Yamaguchi O: The promise of beta3-adrenoceptor agonists to treat the overactive bladder. Urol Clin North Am. 2006; 33: 539-43.
19. Kaisary AV: Beta adrenoceptor blockade in the treatment of female urinary stress incontinence. J Urol (Paris). 1984; 90: 351-3.
20. Zinner NR, Koke SC, Viktrup L: Pharmacotherapy for stress urinary incontinence : present and future options. Drugs. 2004; 64: 1503-16.
21. Reynard JM: Does anticholinergic medication have a role for men with lower urinary tract symptoms/benign prostatic hyperplasia either alone or in combination with other agents? Curr Opin Urol. 2004; 14: 13-6.

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EDITORIAL COMMENT

Recent interest of the bladder physiology has focused on urothelial/suburothelial cells of the urinary bladder. The present study demonstrates the presence of three subtypes of β-adrenoceptors on the urothelium and the detrusor of human bladder, and would support recent findings that β-adrenoceptor subtypes are functionally expressed in the urinary bladder urothelium (1,2). Although many findings about the urothelial muscarinic receptor subtypes have been reported, little remains known as regards

the distribution and functional roles of urothelial β-adrenoceptor subtypes.

Multiple lines of evidence suggest that β_3-adrenoceptors are predominantly abundant in the human detrusor muscle of the urinary bladder, and play important roles in detrusor relaxation during urinary storage in humans. Besides, it is postulated that β_3-adrenoceptor agonists could be highly promising agents to treat overactive bladder by a clinical trial (3).

In the near future, I hope novel findings of mechanism of the urothelial/suburothelial β-adrenoceptor subtypes in the urinary bladder may shed light on the pathological conditions such as overactive bladder and interstitial cystitis.

REFERENCES

1. Murakami S, Chapple CR, Akino H, Sellers DJ, Chess-Williams R: The role of the urothelium in mediating bladder responses to isoprenaline. BJU Int. 2007; 99: 669-73.
2. Otsuka A, Shinbo H, Matsumoto R, Kurita Y, Ozono S: Expression and functional role of beta-adrenoceptors in the human urinary bladder urothelium. Naunyn Schmiedebergs Arch Pharmacol. 2008; 377: 473-81.
3. Chapple CR, Yamaguchi O, Ridder A, Liehne J, Carl S, Mattiasson A, et al: Clinical proof of concept study (Blossom) shows novel β3 adrenoceptor agonist YM178 is effective and well tolerated in the treatment of symptoms of overactive bladder. Eur Urol (Suppl). 2008; 7: 239.

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