Changes in Awake Cystometry and Expression of Bladder $\beta$-adrenoceptors after Partial Bladder Outlet Obstruction in Male Rats

Min Gu Park, Hong Seok Park¹, Jeong Gu Lee¹, Hyung Jee Kim²

Department of Urology, Inje University College of Medicine, Busan, ¹Korea University College of Medicine, Seoul, ²Dankook University College of Medicine, Cheonan, Korea

Purpose: To explore possible changes in awake cystometry and expression of beta-adrenoceptors (ARs) as a cause for bladder dysfunction in a male rat model of partial bladder outlet obstruction (pBOO).

Materials and Methods: Awake cystometry was performed in rats with pBOO (16) and sham-operated rats (16), 8 weeks after the operation. The expression of mRNA and protein of $\beta$-ARs was assessed by real-time PCR and western blot.

Results: The bladders with pBOO (1030mg) were increased compared to those in control rats (230mg). In the cystometric studies, the maximum intravesical pressure significantly increased in the pBOO group compared to control group rats (p=0.001). The time to reach maximal intravesical pressure during micturition in the pBOO group was significantly longer than the sham group (p=0.003). The frequency of non-void contraction in the pBOO group was significantly more than the sham group (p=0.006). The mRNA expressions of $\beta$2- and 3-ARs were increased insignificantly in pBOO group compared to sham group. The data of pBOO group expressed as folds of corresponding expression in sham group were 1.28 and 1.46 respectively in $\beta$2- and 3-ARs. Compared to the sham groups, the density of 60Kda protein band recognized by $\beta$2-AR antibodies and the density of 45Kda protein band recognized by $\beta$3-AR antibodies were higher in the bladder from pBOO group rats.

Conclusion: PBOO of male rats increase the maximal intravesical pressure and contraction time during micturition and the frequency of non-void contraction as well as weight of bladder. The expression of $\beta$2- and 3-ARs subtypes was increased insignificantly compared to sham operated group. This study demonstrates that the changes of cystometric or non-void contraction parameters in pBOO is one of the pathophysiolc processes potentially associated with the alterations of bladder $\beta$-ARs. Int Neurourol J 2010;14:157-63.

Key words: Bladder outlet obstruction, Beta-adrenoceptor, Rat, Bladder

Introduction

Symptoms of benign prostatic hyperplasia (BPH) are called lower urinary tract symptoms (LUTS) and are categorized as voiding and storage symptoms. Storage symptoms such as frequency and urgency have been associated with bladder dysfunction caused by bladder outlet obstruction (BOO) [1]. Evidence of a link between BPH and
voiding symptoms is well established, but there is no direct evidence of a link between BPH and storage symptoms. Although obstructions can be relieved by prostatectomy or administration of alpha-adrenergic blockers, up to 38% of men with BPH continue to suffer from storage symptoms [2]. Obstructed bladder dysfunction caused by BPH is characterized by alterations in bladder mass, tissue composition, capacity, compliance and the response to pharmacological agents. Storage symptoms are displayed in the compensatory stage subsequent to these changes [3]. Many pathophysiological mechanisms have been proposed, including changes in detrusor morphology and innervation, intercellular communication and electrical properties, detrusor receptors, ischemic/reperfusion injury, increased synthesis and deposition of connective tissue, urothelial mechanoafferent signaling, and central nervous system regulation [4,5]. Storage symptoms due to BPH have been treated with anticholinergics, but anticholinergics have side-effects including acute urinary retention. So other materials have been investigated for treating storage symptoms.

A recent study has suggested that there is a predominant expression of β3-adrenoceptor (AR) messenger RNA (mRNA) in human bladder, with 97% of total β-AR mRNA being represented by the β3-AR subtype and only 1.5 and 1.4% by the β1-AR and β2-AR subtypes, respectively [6]. Recently β3 agonist was investigated for application in the treatment of storage symptoms. But many studies have been investigated primarily in animals with overactive bladder without bladder outlet obstruction.

In this study, we investigated the changes in an awake cystometry after induction of partial bladder outlet obstruction in male rats and confirmed the development of urgency, and the expression of β2 and 3-ARs subtype in the rat bladder.

Materials and Methods

Procedures of partial bladder outlet obstruction

Animals

Male Sprague-Dawley rats at age 8 weeks (243-280 g) were housed in a temperature and light controlled room (illumination according to a 12 hour light/dark cycle, temperature of 22±1°C and a relative humidity of 50±20%) and were allowed free access to food and water. The experimental protocol of this study was approved by the Institutional Animal Care and Use Committee of Korea University, Seoul, Korea.

Surgical procedure

After a quarantine period of 1 week, animals were randomized into two groups: a sham operation group (16 rats) and a partial bladder outlet obstruction (pBOO) group (16 rats).

1. Procedures for partial bladder outlet obstruction

Partial outflow obstruction was done using sterile surgical technique under an intraperitoneal ketamine (100 mg/kg body weight) anesthesia. The bladder was exposed by a lower abdominal incision followed by bilateral retraction of the prostate in order to expose the bladder neck and urethra. Special care was taken to avoid damaging the bladder and its nerve innervation. The ureter and urethra were dissected bilaterally. A 22 gauge needle was placed anterior to the urethra. A 2-zero silk suture was placed around the needle and urethra. The suture was tied and the needle was removed. The prostate was returned to its original position. The abdominal wall was closed with a running 3-zero sterile silk sutures. In sham operated animals the suture was passed around the urethra and removed.

2. Intravesical catheter implantation for cystometry

Eight weeks after partial bladder outlet obstruction, 2nd surgical procedure was done for cystometry. After establishing intraperitoneal anesthesia with ketamine (100 mg/kg body weight), the bladder was exposed through an incision in the lower abdomen. Polyethylene catheter (PE-50; Clay-Adams, Parsippany, NJ) with a cuff was inserted through an incision into the dome of the bladder and held in place with a purse-string suture. The catheter was tunneled subcutaneously and anchored to the skin of the back with a silk ligature. The free end of the catheter was sealed. The abdominal wound was closed and the ani-
animals received 150 mg/kg body weight ampicillin intramuscularly.

**Cystometric investigations**

Cystometric investigations were performed without any anesthesia 3 days after bladder catheter implantation. The conscious rat were placed without any restraint in a metabolic cage (16 cm high, 16.5 cm wide and 20 cm deep an equipped with feces trapping mesh), which also enabled measurement of micturition volumes by means of a fluid collector connected to a force displacement transducer integrated into a data acquisition system (Grass model 7 polygraph, Grass instrument Co, Quincy, Mass, USA). The bladder catheter was connected via a T-tube to a pressure transducer (Harvard Apparatus, Holliston, USA) and a micro injection pump [PHD22/2000 pump; Harvard Apparatus]. Room temperature saline was infused into the bladder at a rate of 10 ml/hr. All rats were given a period of 60 to 80 minutes for the voiding pattern to get stabilized, Thereafter, reproducible micturition cycles, recorded for a 1 hour period, were used for evaluation.

The following cystometric parameters were investigated: micturition volume, micturition pressure [the maximal bladder pressure during micturition], micturition interval, time to reach maximal pressure during micturition, frequency of non-void contractions (spontaneously occurring changes in intravesical pressure).

**Bladder harvest**

Urinary bladders of two groups were excised under anesthesia induced by intraperitoneal injection following the cystometry. The bladders only with increased bladder weight and intravesical pressure and decreased micturition interval in UDS were chosen and mRNA expression of $\beta$-AR subtypes was assessed by quantitative real-time PCR. Following this, all rats were exsanguinated after cervical dislocation. The bladders were placed in liquid nitrogen after weighing.

**Real time PCR**

1. **Preparation of total RNA**

Total RNA was extracted from rat bladder samples. Bladder samples were homogenized using homogenized in 1ml of TRizol reagent per 50-100mg of tissue. The total RNA was isolated according to the manufacture’s protocol. The RNA concentration was determined by spectrometry using the Nanodrop (Nanodrop technologies, ND-1000 spectrophotometer, USA).

2. **Synthesis of cDNA**

cDNA was synthesized by reverse transcription according to the manufacturer’s protocol using the Reverse transcription system (Promega Corporation, Madison, WI, USA). The reaction mixture were 1ug total RNA, 5mM MgCl$_2$, 10Xbuffer, 1mM each dNTP, 1u/ul recombinant RNasin ribonuclease inhibitor, 0.5ug random primers and 15u/ug AMV Reverse transcriptase in a total volume of 20uL. After brief spin down, the reactions were incubated at room temperature for 10 minutes, 42ºC for 15minutes and then heating at 95ºC for 5minutes.

3. **Real-time reverse transcription polymerase chain reaction (RT-PCR)**

Expression level of $\beta_2$- and $\beta_3$-AR mRNA were

| Gene       | Accession numbers        | Sequence               | Amplicon size |
|------------|--------------------------|------------------------|---------------|
| $\beta_2$-adrenoceptor | Uniprot/SWISSPROT; P10608 | tgctatcacatcgcccttc   | 60nt          |
| $\beta_3$-adrenoceptor | Uniprot/SWISSPROT; P26255 | accactcgggctttctt     |               |
|            |                          | gaacctcaccgctcaacagtc  |               |
|            |                          | tggatgtcctacagctgta    | 64nt          |
Table 2. Comparison of the results of cystometry and weight of bladder between sham and PBOO groups.

|                       | Sham [n=16] | PBOO [n=16] | p value |
|-----------------------|-------------|-------------|---------|
| Intravesical pr. [mmHg] | 16.0±7.0    | 54.8±17.5   | 0.001*  |
| Micturation vol. [ml]  | 0.27±0.10   | 0.30±0.13   | 0.635   |
| Micturation interval [sec] | 164.3±92.3 | 244.7±84.8  | 0.059   |
| T 1/2 of contraction [sec] | 6.7±2.6     | 29.6±13.9   | 0.003*  |
| Non voided contraction [n] | 0.17±0.36   | 2.2±1.4     | 0.006*  |
| Weight of bladder [g]  | 0.23±0.08   | 1.03±0.25   | <0.001* |

Sham: sham-operation, PBOO: partial bladder outlet obstruction, pr.: pressure, vol: volume, sec: second

quantified by RT-PCR using LightCycler FastStart DNA Master SYBR Green I (Roche, Mannheim, Germany) according to the manufacture’s protocol. The reaction mixture (20µl) were Light Cycler FastStart DNA Master SYBR Green I, 4mM MgCl₂, 0.5µM of primer pairs and 2µl of the cDNA as a template. The sequences of primer pairs are summarized in table 1. The PCR reaction was performed at 95°C for 10minutes, followed by 45cycles at 95°C for 10seconds, at annealing temperature for 10seconds and at 72°C for (amplicon [bp]/25)s. After 45cycle, melting step was performed at 65°C 15seconds and at 40°C for 30seconds. The RT-PCR was performed in Light cycler 1.5 (Roche Diagnostics, Germany). To control for variations, all PCRs were normalized against glyceraldehydes-3-phosphate dehydrogenase (GAPDH) expression.

Western blotting
The bladder bodies from urethane-anesthetized

![Image](beta2.png)

**Figure 2.** Changes in the expression of β2 adrenoceptor. sham: sham operation, po: partial obstruction.

![Image](beta3.png)

**Figure 3.** Changes in the expression of β3 adrenoceptor. sham: sham operation, po: partial obstruction.

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rats were snap-frozen on dry ice and stored separately. Tissues were homogenized in lysis buffer [RIPA buffer (Sigma, USA)]. The following proteinase inhibitors were added to the buffer before use: pepstaitin, leupeptin, aprotinin (Roche, Mannheim, Germany). Samples were sonicated and centrifuged (14,000 rpm, 30 min, 4°C). Supernatant was collected, aliquoted and stored at 80°C. Protein concentrations were measured using BCA protein assay kit (Pierce, USA). The whole bladder samples were processed individually. A total of 30 μg of protein was loaded into a 10% sodium dodecyl sulfate-polyacrylamide gel for electrophoresis. The proteins were transferred onto a nitrocellulose membrane. Blocking was done with 5% non fat dried milk dissolved in 0.1% Tween 20 in TBS. The membranes were incubated overnight at 4°C with anti-rat β2- and β3-AR antibodies diluted in 2.5% Tween 20 in TBS (β2: rabbit, 1:500, Abcam Inc, USA, β3: chicken, 1:2000, Abcam Inc, USA) and then for 1 h at room temperature with horse-radish peroxidase-linked anti-rabbit antibodies [β2: 1:5000, Cell Signaling Inc, USA] and anti-chicken antibody (β3: 1:3000, Santa Cruz Biotechnology Inc, USA). The immunoreactive bands were visualized using ECL kit [Amersham Bioscience Inc, USA]. After stripping, the membranes were incubated with monoclonal anti-rat antibodies against actin (mouse 1:3000, Sigma Inc, USA). The secondary antibodies were anti-mouse (1:5000, Amersham Chemicon Inc, USA).

Results

Cystometric investigation

Bladder weight and cystometric data in the experimental animals are shown in table 2. The urinary bladders of the obstructed rats were generally enlarged and thickened. Mean bladder masses were 0.23±0.08g in control bladder (sham operation group) and increased to 1.03±0.25g in pBOO bladder (p<0.001). The control group did not exhibit any pathological lesions of the urinary bladder. In the cystometric studies, the maximum intravesical pressure significantly increased in the pBOO group compared to sham group rats (54.8 and 16.0 mmHg, respectively, p=0.001)

One micturition volume in the pBOO group was larger than that in the sham group (0.30 and 0.27 ml, respectively) without statistical significance (p=0.635). The micturition interval in the pBOO group was longer than that in the sham group (244.7 and 164.3 sec, respectively) without statistical significance (p=0.059). The time to reach maximal intravesical pressure during micturition in the pBOO group was significantly longer than the sham group (29.6 and 6.7 sec, respectively, p=0.003). The frequency of non-void contraction in the pBOO group was significantly more than the sham group (2.2 and 0.17, respectively, p=0.006).

Real time PCR and Western blotting

The mRNA expressions of β2- and β3-ARs were increased insignificantly in pBOO group compared to sham group. The data of pBOO group expressed as folds of corresponding expression in sham group were 1.28 and 1.46 respectively in β2- and β3-ARs (fig. 1). The results of RT-PCR were confirmed by Western blot. Compared to the sham groups, the density of 60Kda protein band recognized by β2-AR antibodies and the density of 45Kda protein band recognized by β3-AR antibodies were higher in the bladder from pBOO group rats (fig. 2, 3).

Discussion

This study was designed to investigate the change of β-ARs after pBOO, therefore, the results of cystometric investigations were important. In this study, we used a rat model of pBOO. The results of cystometric investigation showed that the number of non-void contraction and maximal intravesical pressure, both significantly increased, indicating that the micturition reflex is influenced by pBOO. And another changes in pBOO group, such as significant increase of bladder weight and extension of the time to reach maximal intravesical pressure during micturition, were lined with the results of previous other pBOO studies [7,8]. Through it, we could confirm the well establishment of pBOO rat models in present study.

Although LUTS/BPH in men has traditionally
voiding symptoms and urinary storage symptoms, the pharmacological therapies have been primarily aimed at improving urinary flow rates and optimizing voiding efficiency. The two principal classes of drugs targeting the prostate are α-blockers and 5ARIs [9]. However, in recent reports, urinary storage symptoms are more bothersome than voiding symptoms in LUTS/BPH [10-12]. Also Irwin et al. suggested that men with OAB symptoms reported more LUTS and greater severity of symptoms than the general population and symptom bother was related to the number of LUTS and urgency severity. [13] But with the abundance of data supporting the prevalence and bother of OAB symptoms in LUTS/BPH, it is unusual that more men are not actively treated for OAB [9].

Because there are the concern regarding the development of acute urinary retention or voiding difficulty following treatment with antimuscarinic agents, many clinician may hesitate the usage of antimuscarinic agents for treatment of male OAB. Beside of the concern of AUR, antimuscarinic agents have many side effects such as dry mouth, constipation, cognitive malfunction, etc. The prevalence of OAB and BPH increases with age. Therefore, old age tend to receive the antimuscarinics more than young age. Along the increasing the intake of antimuscarinics, the side effects will be developed more. Side effects are dose-related and may be dangerous, especially in old age [14]. So other agents such as β3-AR agonist have been investigated for treating storage symptoms. Andersson reported that sympathetic nerves stimulation of urinary bladder contributes to urine storage by relaxing the detrusor muscle via activation of β-ARs [15].

Beta-ARs are currently classified into β1-, β2-, and β3-subtypes [16]. In rat, canine, rabbit and human, the β2-AR subtype have proposed to have an important role in relaxation of detrusor smooth muscle [17-19]. Yamazaki, et al. suggested that the relaxation induced by AR agonists in urinary bladder smooth muscle may be mediated mainly via β2-AR in rabbits, via both β2- and β3-AR in rats, but mainly via β3-AR in dogs [20]. However, there was a predominant expression of β3-AR subtypes in human detrusor muscle [21,22]. Yamaguchi and Chapple reported that β3-AR agonists increase bladder capacity without influencing bladder contraction and have only weak cardiovascular side effects in rat models [6]. Although pharmacological differences exist between rat and human β3-ARs, [6] both of β2- and β3-subtypes seems to be important to study the function of bladder relaxation in this investigation.

According to the recent experiment with isolated bladder strip, the relaxation responses to β agonists are not altered to a major extent in rat BOO model, and the mRNA expression for β2- and β3-ARs subtypes was not significantly altered by BOO [23].

In our preliminary animal study, although, the increase of mRNA expression of β2- and β3-ARs subtypes did not show statistical significance, we could confirm the tendency to increase of expression of β2- and β3-ARs through the results of western blot. Therefore, additional larger-scale studies are needed and if statistical significance is achieved in those studies, the selective β-AR agonists can be considered as new therapeutic agents for treatment of the OAB with BPH.

Conclusions

PBOO of male SD rats increase the maximal intravesical pressure and contraction time during micturition and the frequency of non-void contraction as well as weight of bladder. The expression of β2- and 3-ARs subtypes was increased insignificantly compared to sham operated group. Additional larger-scale studies are needed and especially in humans are required to fully elucidate the role of bladder β-ARs and their potential alterations in BOO.

Conflicts of Interest:
The authors have nothing to disclose.

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