Functional plasticity

of autonomic pelvic ganglia

in rat models of overactive bladder

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ABSTACT

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Peripheral autonomic major pelvic ganglion (MPG) neurons are essential for the generation of storage and voiding reflexes. Under certain pathophysiological conditions such as spinal cord injury and chronic cystitis, plastic alterations in MPG neurons may cause abnormal functions of the bladder. Benign prostatic hyperplasia (BPH), a common problem affecting middle-aged and elderly men, is characterized by the overgrowth of prostatic tissue surrounding the urethra which causes lower urinary tract symptoms (LUTS) such as urgency, frequency, nocturia, incomplete bladder emptying, and weak urine flow. Overactive bladder (OAB) is a syndrome characterized by urinary urgency with frequency and nocturia. To date, neurogenic mechanisms underlying OAB remain poorly understood. The hypothesis of the present study is that OAB is

associated with changes in functions of MPG and dorsal roots ganglion (DRG) neurons in bladder outlet obstruction (BOO) rat models. To prove the hypothesis it was examined whether partial BOO alters expression and activity of nicotinic acetylcholine receptors (nAChRs) involved in synaptic transmission within MPG, and excitability of MPG and DRG neurons innervating the urinary bladder. Toward this end, rat models of partial urethral obstruction (PUO) and BPH were produced by partial urethra ligation and sc injection of testosterone/17-β-estradiol, respectively. The bladder muscles were hypertrophied in PUO and BPH rats. Cystometry revealed that PUO and BPH increased bladder capacity, threshold pressure for voiding, micturition volume, residual volume after voiding, and intermicturition oscillatory frequency, indicating development of OAB. Real-time PCR analysis showed that the nAChRs α3 and β4 subunits were up-regulated in MPG neurons of BOO rats. Then, nAChR currents were measured in DiI-labeled sympathetic and parasympathetic MPG neurons innervating the bladder detrusor muscles. As results, nAChR current densities were significantly increased in parasympathetic MPG neurons, but not in sympathetic PG neurons. Taken together, these data suggest that BOO-induced OAB is associated with phenotype-specific up-regulation of nAchRs in the MPG neurons innervating the bladder. Under the current-clamp mode of the patch-clamp technique, action potentials were recorded in DRG and MPG neurons of control and BOO rats. BOO produced hyperexcitability of the bladder DRG and MPG neurons via reducing rheobase and AHP duration. One of the ionic mechanisms underlying the hyperexcitability is up-regulation of T-type Ca^{2+} channels expressed in DRG and sympathetic MPG neurons. The decreased AHP duration also suggest that expression of Ca^{2+}-activated Cl^{-} and/or K^{+} (i.e., SK channels) might be altered in the bladder DRG and MPG neurons of BOO rats. In conclusion, BOO-

induced OAB might be associated with enhanced ganglionic transmission and/or hyperexcitability of peripheral autonomic motor and sensory neurons innervating the bladder. Clinically, use of T-type Ca^{2+} channel blockers might be a potential option of pharmacologic management of OAB.

I. INTRODUCTION

1.1.   The neural control of the lower urinary tract

The functions of the lower urinary tract (LUT) are to store urine and to periodically eliminate it. The LUT is composed of the urinary bladder (reservoir) receiving urine from the ureter, and an outlet consisting of the bladder neck, urethra and urethra sphincter. The bladder wall is lined with bundles of intertwining smooth muscle fibers, called as the detrusor. The smooth muscles lining the outlet form the internal sphincter, which is surrounded by striate muscle, the rabdosphincter. The periurethral striate muscle and the rabdosphincter together form the external urethral sphincter (EUS). The bladder and outlet function reciprocally. During the storage phase, the bladder accommodates increasing volumes of urine without changes in intravesical pressure, achieved by relaxation of detrusor muscles. At the same time, the internal and external sphincters contract to increase outlet resistance. Moreover, neural pathways for stimulating detrusor contraction for micturition are also silent during this phase, while the inhibitory pathways are active (Andersson et al., 2004b; Yoshimura et al., 2008). The storage process occurs slowly and continues until the bladder volume reaches a critical threshold. When the bladder distension is sensed at a threshold, voluntary micturition occurs by a rapid increase in intravesical pressure and decrease in outlet resistance (Lincoln et al., 1993). Bladder contraction is sustained such that small volumes of urine are completely eliminated. As described above, the bladder has two principal modes of operation: storage and elimination which are required to occur in a coordinated manner. For the coordination, the neural circuits controlling the bladder show switch-like (phasic)

patterns of activity in contrast to tonic patterns exhibiting in autonomic pathways to other visceral organs such as gastro-intestinal and cardiovascular organs.

The bladder and outlet are controlled by three sets of peripheral nerves: sacral parasympathetic (pelvic nerve), thoracolumbar sympathetic nerves (hypogastric nerves and sympathetic chain) and sacral somatic nerves (pudental nerves) (Fig. 1). The sacral parasympathetic outflow provides the major excitatory innervation to the detrusor muscles of the bladder. Cholinergic preganglionic neurons located in the intermediolateral gray matter of the lumbosacral spinal cord send axons via the pelvic nerves to pelvic or vesical or intramural ganglion neurons. There is some species variation in the specific spinal segments from which parasympathetic nerves arise. For example, parasympathetic preganglionic nerves arise from S2-S4 in humans, S1-S3 in cats and monkeys, and L6-S1 in rats (Brindley 1988; Fletcher et al., 1978; Maggi et al., 1986; Thomas, 1982). The sacral parasympathetic nucleus receives inputs from neurons in the pons micturition center (Noto et al., 1989) and from sensory afferents supplying the LUT. When the bladder is empty, parasympathetic preganglionic neurons are quiescent and (Maggi et al., 1986). When the bladder is distended to reach a critical threshold, neurons in the sacral parasympathetic nucleus are activated by higher centers in the brain and sensory reflexes. This produce was contraction of the detrusor by the discharge of postganglionic neurons (Downie et al., 1981; Maggi et al., 1986; McMahon 1986; Hattori et al., 1983).

Sympathetic preganglionic pathways arise from the intermediolateral nuclei of the low thoracic and upper lumbar segments of the spinal cord (T11-L2) (de Groat et al., 2001; Yoshimura et al., 2008). They leave the spinal cord and travel to the lumbo-sacral sympathetic chain ganglia as well as the prevertebral inferior mesenteric ganglia (de

Groat 1993). Some postganglionic neurons from these ganglia innervates the LUT via hypogastric and pelvic nerves. In addition, sympathetic preganglionic axons are also found to contact with pelvic ganglion neurons which finally innervates the LUT (Janig 1986; Janig et al., 1987). Although the sympathetic pathways are not essential for performance of micturition, they play primary roles in providing an excitatory input to the bladder neck and urethra to maintain closure of the outlet (Lincoln et al., 1993). In addition, several studies have suggested that the sympathetic supply to the bladder inhibit parasympathetic pathways during the storage phase (Burnstock 1986). Surgical interruption or pharmacological blockade of the sympathetic innervation can reduce urethral outflow resistance and bladder capacity, and increase the frequency and amplitude of bladder contractions recorded under constant volume conditions (de Groat 1993; Morrison et al, 2005)

The somatic motor supply to the striated muscles of the external urethral sphincter originates from the anterior horn cells lying in the ventral horns of the sacral spinal segments S2-S4. The somatic efferent axons are mostly carried in the pundendal nerve (Juenemann et al., 1988).

Coordination of the bladder storage and elimination modes requires precise sensory infromation from periphery. The majority of the afferent supply of the bladder and urethra originates in the lumbosacral region and travels via the pelvic nerve, the supply being greater in the bladder than the urethra (Dixon et al., 1987; Nance et al., 1988). These afferent pathways consist of small myelinated (A) and unmyelinated (C) fibers that convey sensory information from tension receptors and nociceptors in the bladder wall (Morrison et al., 2005). Electrophysiological studies have shown that A   bladder afferent fibers respond to both passive distension and active contraction of cat bladder

in a graded manner (Torrens and Morrison, 1987; Janig et al., 1986). On the other hand, C bladder afferent fibers in cat have very high thresholds and commonly do not respond to even high levels of intravesical pressure (Habler et al., 1990). However, these afferents can be activated by chemical irritation of the bladder mucosa (Habler et al., 1993) or by cold temperatures (Fall 1990). Administration of capsaicin, a neurotoxin that desensitizes C-fibers, blocks the bladder overactivity induced by noxious stimuli, but does not block normal micturition reflexes in rats, indicating that C-fiber afferent pathways are no essential for normal voiding (de Groat 1993; Maggi et al., 1993). About 70% of bladder afferent neurons in the rat are the C-fiber type based on responsiveness to capsaicin (Yoshimura et al., 1997a; Yoshimura et al., 2003; Yoshimura et al., 1996). These C-fiber afferent neurons express high threshold tetrodotoxin(TTX)-resistant sodium channels and action potentials. In addition, they exhibit phasic firing in response to prolonged depolarizing current injection. In contrast, A   bladder afferent neurons are resistant to capsicin, have low-threshold TTX-sensitive sodium channels and action potentials, and tonic firing to depolarizing current injection.

   The organization of micturition reflex pathways are shown in Fig.1. Micturition is mediated by activation of the sacral parasympathetic efferent pathway to the bladder and the urethra as well as reciprocal inhibition of the somatic pathway to the urethral sphincter. In animals with intact sppinal cord, micturition is initiated by a supraspinal reflex pathway called "spinobulbo-spinal micturition reflex pathway passing through pontine micturion center. The pontine micturition center is the prime determinant of LUT function as it sets the volume at which the LUT switches from storage to voiding mode, thereby effectively determining maximum bladder capacity. The switch between these

two phases is triggered by myelinated A afferent fibers, which are connected to the tension receptors in the bladder wall (detrusor). However, during chronic thoracic spinal cord injury, micturition is induced by unmyelinated C-fibers by spinal micturition reflex pathway as described (Cheng et al., 1999). Voluntary control of micturition depends on connections between the prefrontal cortex, hypothalamus, and other forebrain structures such as anterior cingulate gyrus, amygdala, bed nucleus of the stria terminalis and septial nuclei where electrical stimulation elicits micturition (de Groat 2006).
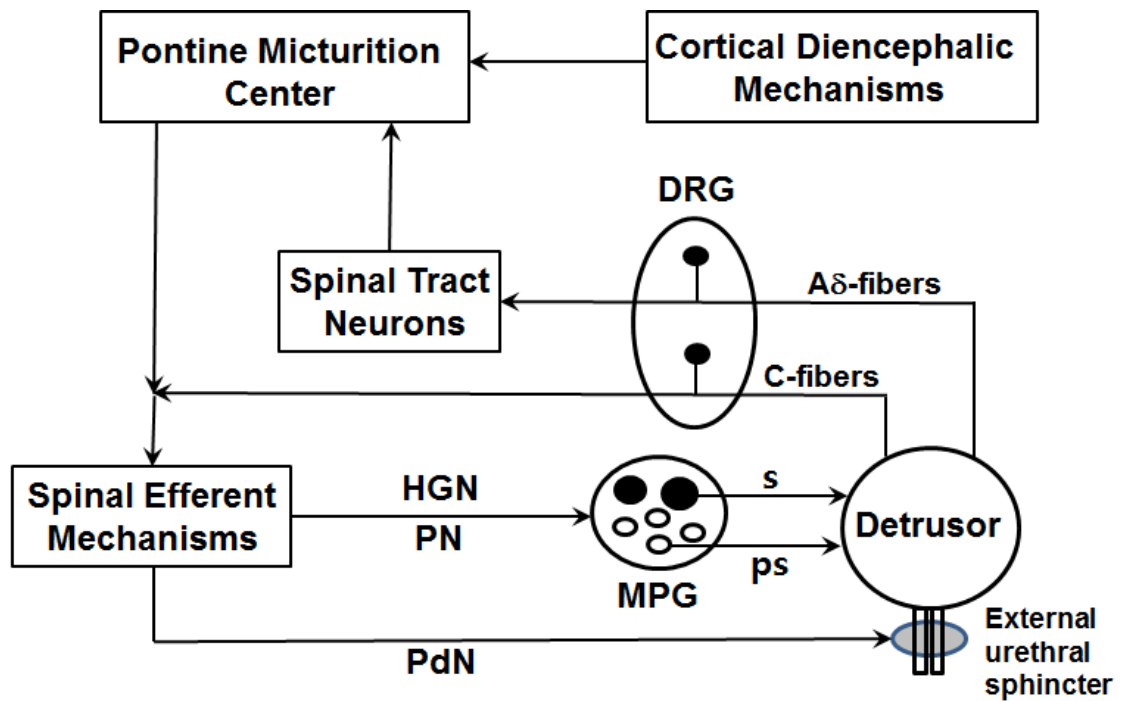
Figure 1. Schematic diagram showing the organization of micturition reflex pathways.

1.2. Characteristics of major pelvic ganglia

The pelvic plexus provide autonomic innervation to the urogenital system including the descending colon, the urinary bladder, and the external genitalia (Dail et al., 1983; Langworthy 1965). The pelvic plexus consists of autonomic ganglia as well as axonal pathways that convey afferent and efferent signals between the CNS and the peripheral target organs. Therefore, the pelvic plexus mediates autonomic visceral reflexes such as urination, evacuation, ejaculation, and erection (Dail et al., 1983). Accordingly, it is important to understand the precise neuronal circuit and neural regulation of the pelvic plexus for treatment of dysfunctions of pelvic organs such as urinary incontinence, constipation, and impotence. Pelvic ganglia in most animal species are widely dispersed among the pelvic viscera. Exceptionally, pelvic ganglion neurons in male rat are located in a pair of one large ganglion called the "major pelvic ganglion (MPG)" and "paracervical ganglion" in the female. The MPG are located on the lateral surfaces of the prostate gland and contain significantly larger number of ganglion cell (about 30,000) than paracervical ganglion (about 10,000) (Greenwood et al., 1985). Because the MPG are morphologically simple, it has been widely used in a variety of anatomical, pharmacological and physiological studies. Many previous studies have reported that anatomical and functional plasticity of the MPG are observed in pathophysiologically complex processes such as dysfunction of micturation and impotence (Keast et al., 1992). For examples, obstruction of bladder outlet produces marked hypertrophy of the MPG neurons innervating the bladder (Somogyi et al., 1993). Decreased testosterone levels by castration reduced expression of nicotinic acetylcholine receptors in the MPG neurons, producing neurogenic erectile dysfunction (Huang et al., 2011). Therefore, the

MPG neurons provide a useful model system for studying the autonomic control of pelvic organs during normal and pathological conditions.

1.3. General aspects and pharmacologic management of overactive bladder

Overactive bladder (OAB) is a symptom-based diagnosis by the International Continence Society(ICS) as urgency with without urgency incontinence usually with urinary frequency and nocturia (Abrams et al., 2002; Ouslander 2004). Paitients with OAB typically present with symptoms of a sudden and compelling need to urinate which is difficult to defer (urgency), involuntary leakage of urine with feelings of urgency (urgency incontinence), frequency (≥8 micturitions/day) and nocturia (≥one awakening per night to void) (Chu et al., 2006; Herbison et al., 2003; Ouslander 2004; Stewart et al., 2003). OAB affects approximately 17% of the population in the United States. Incidence of OAB is similar in men and women (Irwin et al., 2006) and increases with advanced age although individulas of all age groups are affected. OAB seems to be associated with various disease conditions such as stroke, Parkinson's disease, spinal cord injury, benign prostatic hyperplasia (BPH), acute bladder inflammation, metabolic syndrome, etc., Detrusor overactivity (DO) is an urodynamic observation characterized by involuntary contractions during the filling phase of cystometry (Abrams et al., 2002). The symptom of urgency described by OAB patients often correlates with DO demonstrable on cystometry. To date, the pathophysiology of the OAB syndrome and DO is still incompletely known, but most probably multifactorial. There are three hypotheses for explaining pathophysiological basis of DO: myogenic (Brading 1994;

1997), peripheral autonomy (integrative) (Drake et al., 2001), neurogenic (de Groat 1997). Myogenic hypothesis explain that an increased myogenic activity of the detrusor muscle may be an important mechanism inducing OAB and DO (Brading 1997). This mechanism seems to be more applicable to patients with bladder outlet obstruction (BOO). It has been observed that BOO increases intravesical pressure and induces bladder hypertrophy and partial denervation of the detrusor smooth muscle, leading to functional changes. These changes include denervation supersensitivity of cholinergic receptors (Speakman et al., 1987), increase in expression of purinergic receptors such as P2X1 and in purinergic receptor-mediated contractile responses (O'Reilly et al., 2001), and changes in the cell-to-cell communication in detrusor muscle due to up-regulation of gap-junction proteins, such as connexin 43 (Haferkamp et al., 2004). The peripheral autonomy (integrative) hypothesis arises from the observation that the bladder shows complex autonomous activity when descending inhibitory influences are removed (Coolsaet et al., 1993; Drake et al., 2003a; Drake et al., 2003b). This hypothesis proposes that other cell types in addition to muscle (interstitial cells, urothelium, and peripheral nerves) contribute to normal generation of localized spontaneous activity and thus low pressure sensing of filling state. For example, interstitial cells has been proposed for a pacemaking role in spontaneous acticvity of the bladder (Andersson et al., 2004a; Yoshimura and Chancellor, 2007). A study has shown that the number of interstitial cells is increased in a guinea pig model of BOO (Kubota et al., 2007). Furthermore, c-kit tyrosine kinase inhibitors, which inhibit interstitial cell activity, decreased the amplitude of spontaneous contractions in the guinea pig and human bladder (Biers et al., 2006; Kubota et al., 2006). Taken together, the interstitial cells may be involved in the emergence of DO through enhancing

autonomous detrusor activity. The neurogenic hypothesis propose that DO is caused by neural plasticity within the central nervous system controling the detrusor muscle (de Groat 1997). Neural plastic changes may occur in descending inhibitory pathways from higher brain centers including the pontine micturition center. However, little is known about functional plasticity of the peripheral nervous system including postganglionic efferent and sensory afferent neruons in animals and human with OAB and/or DO.

   Treatment of OAB is based on a clinical diagnosis following exclusion of other pathologies such as urinary tract infection, bladder stones, and diabetes. Combination of lifestyle interventions, pelvic floor muscle exercise (Kegel exercise), bladder training, and medical non-invesive pharmacotherapy represent the initial treatment options for OAB patients. The principals of treatment are to increase voiding volumes, thereby reducing frequency and nocturia, decrease urgency and reduce urgency incontinence episodes. Stimulation of M2/M3 receptors by acetylcholine causes bladder contractions that results in voiding urine (Chu et al., 2006; Erdem et al., 2006). Thus, antimuscarinic agents have been considered as an primary option for pharmcotherapy of OAB (Erdem et al., 2006). Unfortunately, however, these agents are limited by undesirable antocholinergic adverse effects such as dry mouth, constipation, sedation, impaired cognitive function, tarchycardia, and blurred vision (Erdem et al., 2006). Although many studies have suggested potential targets for treatment of OAB including ligand receptors (e.g. neurokininin, purinergic, or 3-adrenergic receptors), ion channels (e.g. K^+ and TRPV1 channels), and trk (NGF) receptors, few new therapies have emerged showing clinical benefits (Latini et al., 2011).

1.4. BOO models in OAB research

Several animal models have been developed in a variety of species in attempt to study the pathophysiology of OAB. However, OAB is a symptom-based diagnosis in which the conscious perception of urgency is key to the diagnosis. There is no way of knowing whether an animal is experiencing urgency. The inability to quantify urgency in animals necessitates the use of surrogate markers. That is why most experimental models have focused on DO because this can be objectively measured using cystometry. However, significant proportion of patients diagnosed with OAB has no evidence of DO on urodynamic evaluation (Hashim et al., 2006). Therefore, for validation of the OAB animal models, other surrogate markers including urinary frequency, voided volumes, and changes in micturition habit. It should be also used as clinically meaningful endpoints (Parsons et al., 2011).

BOO is a common problem for aging male as a consequence of benign prostatic enlargement (BPE). The mechanisms underlying the OAB seen in patients with BOO are not fully understood. Effects similar to BOO in humans are relatively straightforward to replicate in animals. This has been achieved in a variety of animal species including the pig, rat, guinea-pig, and rabbit by partial obstruction of the urethra using some form of ligature that either occludes the urethra immediately or does so gradually as the animal grows (Drake et al., 2006; Pampinella et al., 1997; Wolffenbuttel et al., 2001a; Wolffenbuttel et al., 2001b). These BOO models show many of the structural and physiological changes in bladder seen in human BOO, including muscle hypertrophy, altered responsiveness to stimuli, increased spontaneous myogenic activity with development of non-micturition contractions and enlarged sensory and parasympathetic ganglia (Parsons et al., 2011). Therefore, partial BOO appears to be a

good model to study lower urinary tract sympatoms as it can be reliably reproduced with etiological validity.

II. PURPOSES

The hypothesis of the present study is that OAB is associated with changes in functions of MPG and DRG neurons in bladder outlet obstruction (BOO) rat models. To prove the hypothesis I examined whether partial BOO alters expression and activity of nAchRs involved in synaptic transmission within MPG, and excitability of MPG and DRG neurons innervating the urinary bladder.

III. MATERIALS AND METHODS

3.1. Animals

Male Sprague-Dawley rats (8-week-old) were obtained from Orient Inc. (Orient Bio Inc., Seoul, Korea). All rats used in this study were housed in a light-dark (12 hr on/off; light on at 9:00 a.m.) and temperature-controlled environment with food and water available ad libitum in the Yonsei University Wonju College of Medicine Animal Facility. All animal maintenance and experiments were performed in accordance with the guidelines established by the Yonsei University Wonju College of Medicine Institutional Animal Research Committee (IACUC). For surgical study, rats were anesthestized with interaperitoneal (*i.p*) injection of ketamin/rompun mixture (ketamin 50 mg/kg; rompun 10 mg/kg) or pentobarbital sodium (50 mg/kg).

3.1.1. Partial bladder outlet obstruction model

A small 1 cm midline incision was made from the penoscrotal junction to the mid scrotum to gain access to the bulbous urethra. The urethra was then isolated from the cavernous bodies. A sterile steel rod with an outer diameter of 0.9 mm was placed on the prostatic urethral surface, and a 3-0 polypropylene suture was used to place a tie around both the prostatic urethra and the steel rod. After tying the knot, the steel rod was removed and a 4-0 silk suture was used to both muscle layer and skin. Sham-operated animals underwent similar surgery without obstruction (Melman et al., 2005).

3.1.2. Benign prostatic hyperplasia

Male rats (8-week-old) were treated with long acting sex hormones including testosterone enanthate (12.5 mg per rat) plus 17β-estradiol valerate (0.125 mg per rat) in 0.2 ml sesame oil given 4 times by weekly for 4 weeks intramuscular injection (Tatemichi et al., 2006). Age matched animals that received sesame oil served as the control. The experiments were performed after the initiation of hormone treatment (age 12 weeks).

3.2. Di-I labeling of MPG and DRG neurons innervating the bladder

Retrograde tracing of MPG and DRG neurons innervating the bladder using Di-I was performed. A small 1-2 cm midline incision was made along the lower abdominal ventral wall, and then exposed bladder. Di-I (1,1' dioctadecyl-3,3,3,'3'-tetramethylindocarbocyanine perchlorate; 50 mg/ml in DMSO; Molecular Probes, Eugene, OR) was injected into six to eight sites of the wall of the bladder base around the trigone using a 30-gauge needle (6 µl per site) (Chen et al., 2010). One week later, the rat MPG and DRG neurons were enzymatically dissociated and cultured for electrophysiology as described below (3.5). Di-I labeled MPG and DRG neurons were identified under fluorescent microscope (TE2000-U, ECLIPSE, Nikon)

3.3. Cystometry

3.3.1. Surgical procedure

The abdomen was opened through a midline incision, and the bladder was exposed.

A polyethylene catheter (PE-50; Clay Adams, Parsippany, NJ) was implanted in the bladder through the dome. Another PE-50 catheter connected 0.05 balloon on the tip cuff and exteriorized at the scapular level (Lee et al., 2008). Each rat was housed individually after surgery, and food and water were given ad libitum. Animals were allowed to recover for at least 48 h.

3.3.2. Cystometric recordings

Cystometric investigations were performed in conscious animals 2 days after the bladder catheter implantation. The rats were placed in metabolic cages without restraint and the bladder catheters were connected via a 3 way-valve to a strain gauge and an injection pump (KD200; KD Scientific, Holliston, MA) and a pressure transducer (Edwards Lifesciences, Irvine, CA). Micturition volumes were recorded with a fluid collector connected to a force displacement transducer (FT03D, Grass Instrument, Quincy, MA). Warmed saline (37°C) was infused into the bladder at a rate of 6 ml/h. Intravesical pressure was continuously recorded using a powerlab interface (AD Instruments, Oxford, UK) and Chart software (version 7). The following cystometric parameters were measured or calculated bladder capacity (BC, ml), basal pressure (BP, mmHg), peak micturition pressure (MP, mmHg), threshold pressure (TP, pressure at which micturition occurs, mmHg), micturition volume (MV, ml), residual volume (RV, volume infused minus MV, ml), IOF, (intermicturition oscillatory frequency, Hz), and MIOP (mean intermicturition oscillatory pressure, mmHg)

3.4. Histological analysis

For routine histological analysis, bladders from each of the groups were fixed with 4% paraformaldehye at 4°C overnight. Thereafter, they were frozen by dry ice/ethanol for cryosectioning or dehydration and embedded in paraffin wax for sectioning. Hematoxylin and eosin staining was performed on paraffin-embedded sections and evaluated independently by a veterinary pathologist.

3.5. Isolation of MPG and DRG neurons

Single neurons of the MPG and DRG from male rats were enzymatically isolated using a modification of the method described previously (Lee et al., 2002; Won et al., 2006). Briefly, adult male rats (200–300 g) were anesthetized with pentobarbital sodium (50 mg/kg, i.p). The MPG which are located on the lateral surfaces of the prostate gland and DRG (L6 and S1) were dissected out and placed in cold Hanks' balanced salt solution (HBSS, pH 7.4). The ganglia were desheathed, cut into small pieces, and incubated with 10 mg/ml collagenase type D (Roche, Diagnostics, Germany), 1.5 mg/ml trypsin (Worthington. Corporation, Harrison, New Jersey), and 1 mg/ml DNase type I (Sigma, St Louis, MO) in 10 ml of modified Earle's balanced salt solution (EBSS, pH 7.4) in a 25-cm^2 tissue culture flask. The EBSS was modified by adding 3.6 g/L glucose (Sigma) and 10 mM HEPES (Sigma). The flask was then placed in a shaking water bath 100rpm at 35.5°C for 55 min. After incubation, the ganglia were dispersed into single neurons by vigorous shaking of the flask containing ganglia. After centrifugation at 50g for 5min, the dissociated neurons were resuspended in minimum essential medium (MEM) (Invitrogen, CA, USA) containing 10% fetal bovine serum (Invitrogen), L-glutamine (Invitrogen) and 1% penicillin-streptomycin (Hyclone, Logan UT). Neurons were then plated onto culture

dishes (35-mm) coated with poly-L-lysine (Sigma) and maintained in a humidified 95% air-5% CO_2 incubator at 37°C until use.

3.6. RNA isolation and real-time PCR

Total RNA from the dissociated MPG neurons were extracted using RNeasy plus Kit (Qiagen, Valencia, CA). RNA samples used for RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) were treated with DNase mixture (Qiagen) and purified further using RNeasy easy column. The quantity and quality of RNA were assessed by spectrophotometry at 260 nm. The first strand cDNA was synthesized from 1 μg of total RNA using 50 U M-MLV (Money Murine Leukemia Virus) reverse transcriptase (Roche, Mannheim, Germany) by incubating at 42°C for 1 hr. Parallel reactions without reverse transcriptase were performed to confirm no amplification of genomic DNA. PCR reaction was initiated by a first denaturation at 95°C for 5 min, followed by 35 cycles consist of 94°C for 30s, 60°C for 40s, and 72°C for 30s. The PCR reaction was terminated by maintaining temperature at 72°C for 7 min. As an internal reference, β-actin or GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) gene was amplified. The resultant PCR products were separated on a 1.5% agarose gel and visualized by ethidium bromide staining.

Expression of nAchR subunits were quantified using real-time PCR as previously described (Huang et al., 2004). One μg of total RNA was converted into cDNA and used as a template for real-time PCR. PCR amplification was conducted using a Rotor-Gene (RG-3000) thermal cycler (Corbett Research, Sydney, Australia) detection system with the QuantiTest SYBR Green PCR kit (Qiagen). The sequences of real-time PCR primers

are shown in Table 1. As an internal reference, the β-actin gene was amplified. The specificity of each reaction was confirmed by analysis of the melting curve generated at the end of the PCR. The fold changes in gene expression were analyzed by the comparative $2^{-\Delta\Delta CT}$ method (Livak et al., 2001).

The quantitative process used by real-time PCR makes use of a defined threshold value (CT) by the accumulated PCR product. To measure the quantitative difference of mRNA in between control and overactive bladder group, the difference between the respective CT values (ΔCT) can be used and analyzed by subtracting the CT value of the amplification of overactive bladder group from those of control. The defined threshold was set at level of given ΔCT of heterozygous a-thallassemia-1 SEA control DNA was equal to zero.

3.7. Electrophysiological recordings

Ligand-gated ion channel (nicotinic acetylcholine (nAch), 5-HT$_3$ and GABA$_A$ receptor) and voltage-gated Ca^{2+} channel currents were recorded using the whole cell-ruptured configuration of the patch-clamp technique (Hamill et al., 1981). Patch electrodes were fabricated from a boroslicate glass capillary (Corning 8250, Garner Glass Co. Claremont, CA, USA). The electrodes were coated with sylgard 184 (Dow corning, Midland, MI, USA), fire polished on a microforge, and had resistance of 2~3 MΩ when filled with the internal solution described below. An Ag/AgCl pellet connected via a 0.15 M NaCl/agar bridge was used to ground the bath. The cell membrane capacitance and series resistance were compensated over 80% electronically using an EPC-10 amplifier and pulse/pulsefit (Ver 8.50) software (HEKA Electronik, Lambrecht, Germany). Current traces were generally

low-pass filtered at 5 Hz using the 4-pole Bessel filter in the clamp amplifier, digitized at 2 kHz, and stored on the computer hard drive for later analysis. The resistance of the filled pipettes ranged from 3 to 5 MΩ. Electrophysiology recording began 5~10 min after formation of a giga ohm seal when the series resistance was stabilized to below a 10 MΩ. Current-clamp recordings were performed under the gramicidin-perforated whole-cell configuration of the patch-clamp technique using EPC-10 amplifier and Plus/Pulsefit software.   All experiments were performed at room temperature (20~24°C).

3.8. Solutions and drugs for electrophysiology

To measure ligand-gated ion channel currents including nAch receptor, patch pipettes were filled with an internal solution containing (in mM); 30 KCl, 100 K^+-gluconate, 11 ethylene glycol bis (2-aminoethyl ether) -*N,N,N',N'*-tetraacetic acid (EGTA), 10 HEPES, 1 $CaCl_2$, 4 MgATP, 0.3 Na_2GTP, and 14 Creatine-PO_4 (pH 7.2, 298 mOsm/kg H_2O). Normal physiological salt solution (PSS) was used as bath solution contained 135 NaCl, 5.4 KCl, 10 HEPES, 10 Glucose, 1.8 $CaCl_2$, and 1 $MgCl_2$. $6H_2O$ (pH 7.4, 318 mOsm/Kg H_2O). To isolate Ca^{2+} currents, patch pipettes were filled with an internal solution containing 120 N-methyl-D-glucamine (NMG)-methanesulfonate (MS), 20 tetraethylammonium (TEA)-MS, 20 HCl, 11 EGTA, 1 $CaCl_2$, 10 HEPES, 4 Mg-ATP, 0.3 Na_2-GTP, and 14 creatine phosphate (pH 7.2, 290 mOsm/kg H_2O). External recording solution contained 145 TEA-MS, 10 HEPES, 10 $CaCl_2$, 15 glucose, and 0.0003 tetrodotoxin (TTX) (pH 7.4, 325 mOsm/kg H_2O). For perforated whole-cell patch clamp recordings, a stock solution of gramicidin was prepared at 50 mg/ml (in dimethyl sulfoxide) and diluted (50 μg/ml final concentration) in a pipette solution containing 135 NaCl, 5.4 KCl, 10 HEPES, 10 Glucose,

1.8 CaCl$_2$, and 1 MgCl$_2$ (pH 7.4, 318 mOsm/Kg H$_2$O) before recording. Normal PSS was used for bath solution for perforated recordings. Drugs were applied to single neurons via a gravity-fed fused silica capillary tube connected to an array of seven polyethylene tubes.

3.9. Data analysis

Statistical comparisons between two groups of data were made using two-tailed unpaired Student's *t*- test. Multiple comparisons were determined using one-way analysis of variance followed by post-hoc test (Tukey's multiple comparison test). *p* values less than 0.05 and 0.01 were considered significant for single and multiple comparisons, respectively. Data were presented as means ± SEM.

Table 1. List of primers used for real-time PCR.

Use	Target	Accession	Sequence	Size
Real-Time PCR	nAChR-3	AY574254	Forward ACTCCAAAAGCTGCAAGGAA Reverse CTTGTGAGGTTGGCACTGAA	103 bp
	nAChR-4	AY574260	Forward CTCCTGAACAAAACCCGGTA Reverse CTGATGAGCTGGGACAGTGA	101 bp
	α1H	NM153814.1	Forward TCTGTCCAACGACAGCACTC Reverse AGGCTACGGCGTTTAACCTT	108 bp
	β-actin	NM031144.2	Forward ATGGTGGGTATGGGTCAGAA Reverse ACCAACTGGGACGATATGGA	119 bp

IV. Results

4.1. Evaluation of BPH rat model

4.1.1. General characteristics of BPH rat model

A BPH rat model was used to study the effect of bladder outlet obstruction on bladder function. Body weight has been shown to decrease in BPH group (468.2 ± 5.2 and 344.82 ± 3.2 g, control and BPH groups, respectively, $p<0.001$, Fig. 2A) while bladder weight was increased (0.03 ± 0.002 and 0.044 ± 0.002 g, control and BPH groups, respectively, Fig. 2B). The increase of bladder weight in BPH groups compare with the control group was significant. Moreover, normalized dorsal and ventral prostate weights were also significantly increased compared with that in the control group (Fig. 2C and D). Partially outlet obstruction in BPH causes enlargement of ventral prostates (Choo et al., 2000). The increase of ventral prostate weight was significantly greater than that of dorsal prostate (ventral prostate/body weight in the control and BPH group was 0.05 ± 0.002 and 0.09 ± 0.01 %, respectively, $p<0.001$; dorsal prostate/body weight, 0.15 ± 0.002 and 0.35 ± 0.02 %, respectively, $p<0.001$).

4.1.2. Histological analysis of bladder of BPH rat

To analyze bladder defects by BPH, bladders were sectioned and its histology was examined (Zhang et al., 2004). Compared with control bladder, BPH bladder exhibited hypertrophy of the bladder detrusor muscle (Fig 3). Both bladder wall thickness and

smooth muscle bundles of BPH rats were hypertrophic compared with control bladder (Fig 3).
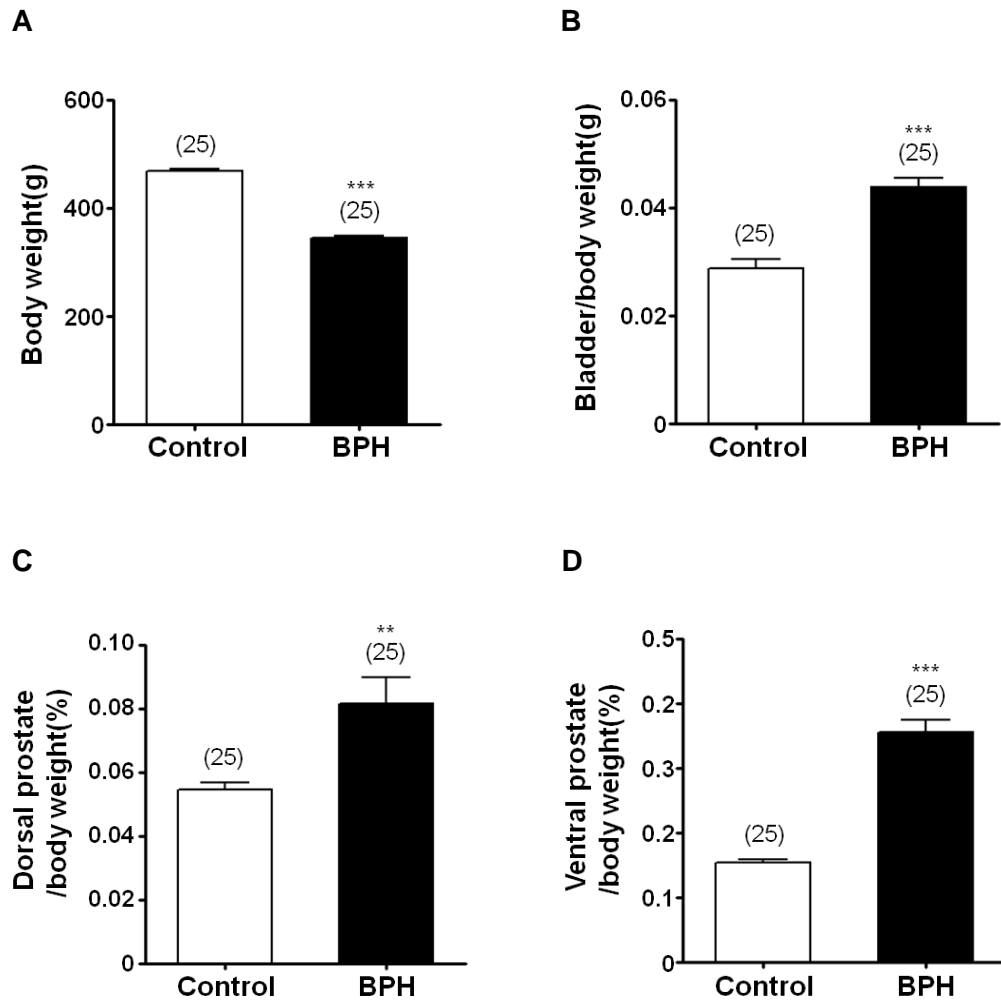
Figure 2. Change of body, bladder, and prostate weight in BPH rats.

Mean body (A), bladder (B), dorsal prostate (C), and ventral prostate (D) weight for experimental groups after 4 weeks under co-injected testosterone and 17-β-estradiol. Bladder and prostate weight was normalized to body weight. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. ** P<0.01; * P<0.05; compared with control.
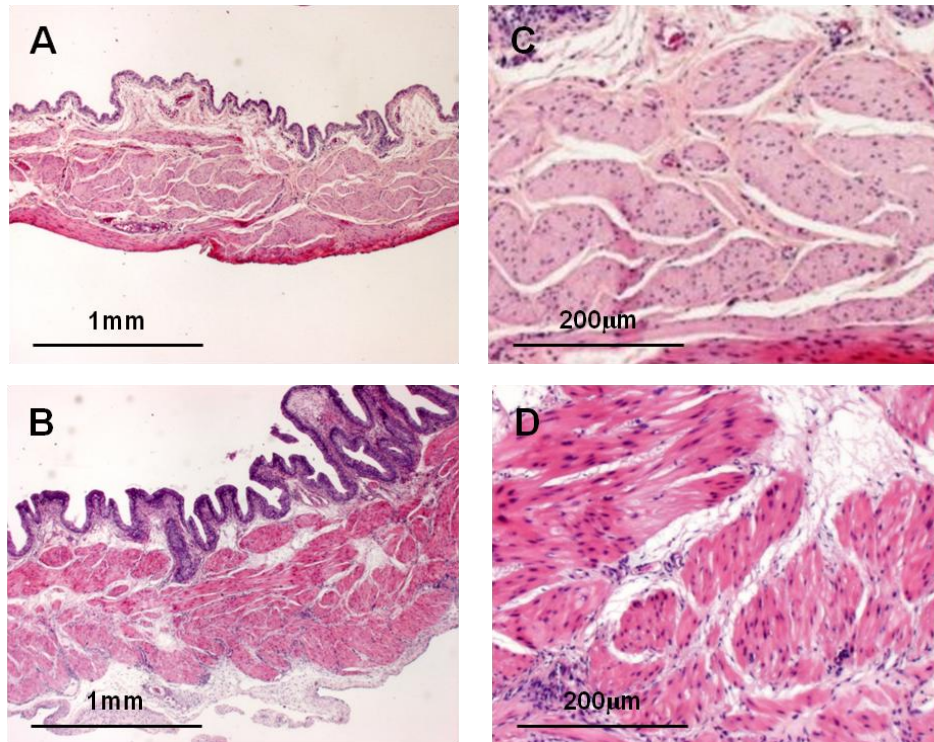
Figure 3. H & E staining in bladder of sham and BPH rat.

H & E staining of sections of bladder from control (sham; A, C) and BPH (B, D) rats. Bladder of BPH rat showed hypertrophic change in smooth muscle layer. H & E staining of control and BPH bladder shown at X 20 (A, B) or X 100 (C, D) magnification.

4.1.3. Cystometry recordings in BPH rat

It is known that the BPH rat model exhibits abnormal micturition patterns. Fig. 4 shows the typical cystometrical recordings of micturition cycles obtained in control and BPH rats (Fig. 4, table 2). Cystometrical recordings were performed under an awaken condition and cystometric parameters were monitored over 50 min recording periods. The voiding pattern of testosterone-treated BPH rats was significantly altered (Fig 4). For control rats, the bladder capacity, micturition volume, and residual volume were 0.93±0.07 ml, 0.89±0.05 ml, and 0.013±0.004, respectively. The bladder capacity and both micturition and residual volumes were significantly increased to 1.59±0.13 ml, 1.6±0.12 ml, and 0.184±0.04 ml in BPH rat, respectively. Threshold pressure in BPH rats was increased to 14.8±1.3 mmHg compared with control rats (10.5±0.6 mmHg), while both basal and micturition pressures had no difference between groups (Table 2). Intermicurition oscillatory frequency and pressure were also increased in BPH rats compared with control rats (Table 2).

A
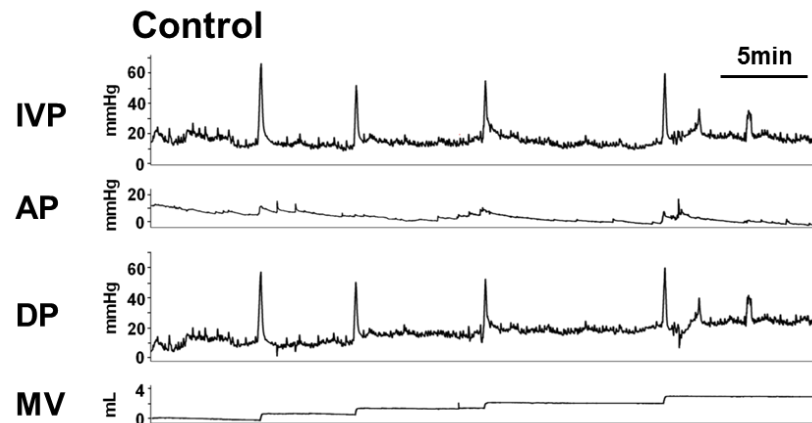
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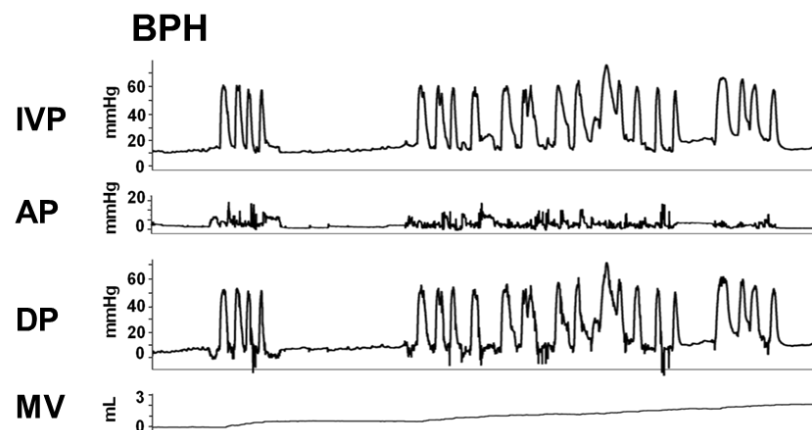
Figure 4. Cystometrical recordings of micturition cycles obtained in sham control and BPH rats. Cystometry recordings in sham control (A) and BPH (B) rats. Warmed saline was infused continuously into the bladder at rate of 6 ml/hr. The following parameters including intravesical pressure (IVP), intra-abdominal pressure (IAP), detrusor pressure (DP), and micturition volume (MV) were measured. Additional cystometric values and *n* numbers are provided in table 2.

Table 2. Cystometrical parameters in BPH rats.

Parameters	Sham (n=3)	BPH (n=3)
BC (ml)	0.93±0.07	1.59±0.13**
BP (mmHg)	9.3±1.3	9.8±0.9
TP (mmHg)	10.5±0.6	14.8±1.3*
MP (mmHg)	39.7±3.5	47.3±1.3
MV (ml)	0.89±0.05	1.6±0.12**
RV (ml)	0.013±0.004	0.184±0.04*
IOF (Hz)	4.83±0.83	9.32±1.59*
MIOP (mmHg)	2.9±0.3	30.2±4.5**

Cystometric parameters; BC, bladder capacity; BP, basal pressure; TP, threshold pressure, MP, micturition pressure; MV, micturition volume; RV, residual volume; IOF, intermicturition oscillatory frequency; MIOP, mean intermicturition oscillatory pressure. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. * $p<0.05$; ** $p<0.01$; ***; compared with control.

4. 2. Electrophysiological properties of MPG neurons innervating bladder

Major pelvic ganglia (MPG) have a unique feature that is the colocalization of both sympathetic and parasympathetic neurons within the same ganglionic capsule (de Groat and Booth, 1993; Keast 1999). Cell type of MPG neurons could be characterized in according to the previously established criteria such as cell size as assessed by electrical capacitance and expression of T-type Ca^{2+} channels (Lee et al., 2002; Zhu et al., 1997). In addition, sympathetic MPG neurons expressing presence of T-type Ca^{2+} current also express $GABA_A$ receptor currents. In contrast, most of parasympathetic neurons have a small membrane capacitance and $5\text{-}HT_3$ current.

MPG neurons innervating bladder were identified by retrograde tracing using Di-I and electrophysiological properties of Di-I-positive neurons were examined. MPG neurons innervating bladder also were also classified both sympathetic and parasympathetic neurons. Compared to the parasympathetic neurons (24.9±1.4 pF), the sympathetic neurons (61.8±8.4 pF) have a relatively larger cell size as measured in electrical capacitance. In addition, $GABA_A$ and $5\text{-}HT_3$ receptor current were recorded to confirm the cell types of MPG. GABA evoked a large inward Cl^- current while 5-HT had no effect in sympathetic MPG neurons expressing T-type Ca^{2+} channel. Conversely, 5-HT elicited a large inward current but GABA currents were negligible in parasympathetic MPG neurons lacking T-type Ca^{2+} current (Fig 5).

It has been reported that both sympathetic and parasympathetic MPG neurons show different properties of membrane potential and action potential firing (Lee et al., 2002; Park et al., 2006). In fig. 6, sympathetic MPG neurons showed tonic firing in response to injection of depolarizing supra-threshold current for 400 ms while the parasympathetic

MPG neurons showed phasic firing in response to that. The firing frequencies in sympathetic and parasympathetic MPG neurons were 18.7±1.8 and 5.1±2 Hz, respectively. To identify mechanism underlying the difference in spike firing between the sympathetic and parasympathetic MPG neurons, afterhyperpolarization (AHP) following a single action potential was compared. Sympathetic MPG neurons generated AHP with high amplitude and short duration in response to a depolarization current injection for 10 ms, while parasympathetic neurons showed AHP with low amplitude and long duration. On average, AHP amplitudes were -14.9±3 and -10.4±3 mV, and AHP durations were 119.8±3 and 223.7±3.3 ms in sympathetic and parasympathetic MPG neurons, respectively.
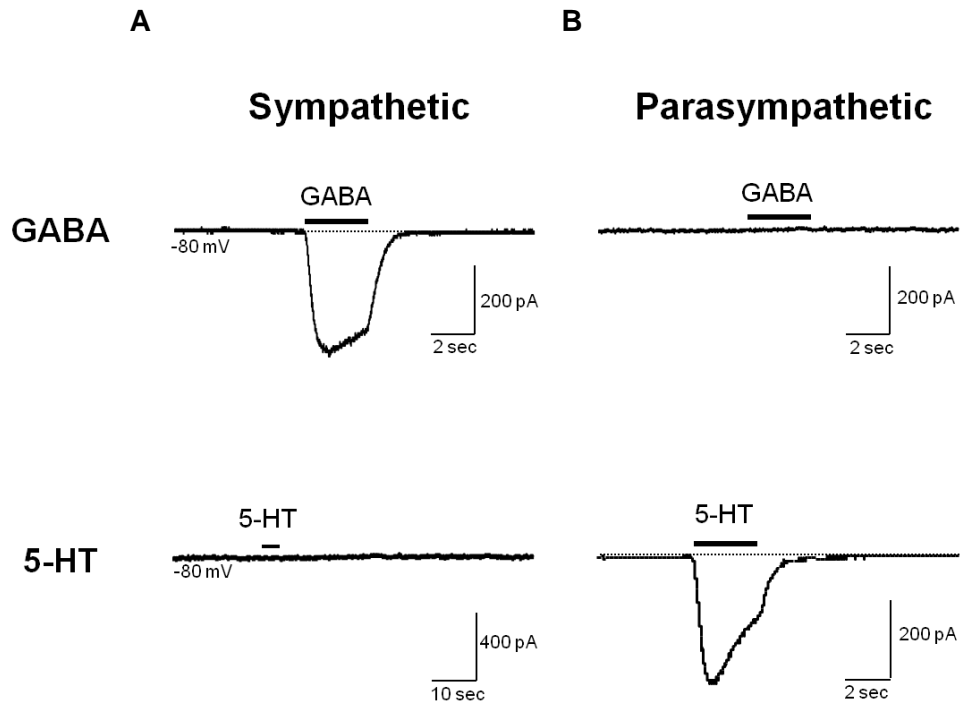
Figure 5. Cell-specific expression of GABA$_A$ and 5-HT$_3$ receptor in MPG neurons innervating bladder.

Representative traces of inward currents evoked by application of GABA (100 µM) or 5-HT (10 µM) in (A) the sympathetic and (B) parasympathetic MPG neurons measured using whole-cell patch-clamp techniques. Note that the GABA and 5-HT currents are hallmarks of sympathetic and parasympathetic MPG neurons, respectively. Cells were held at -80 mV.
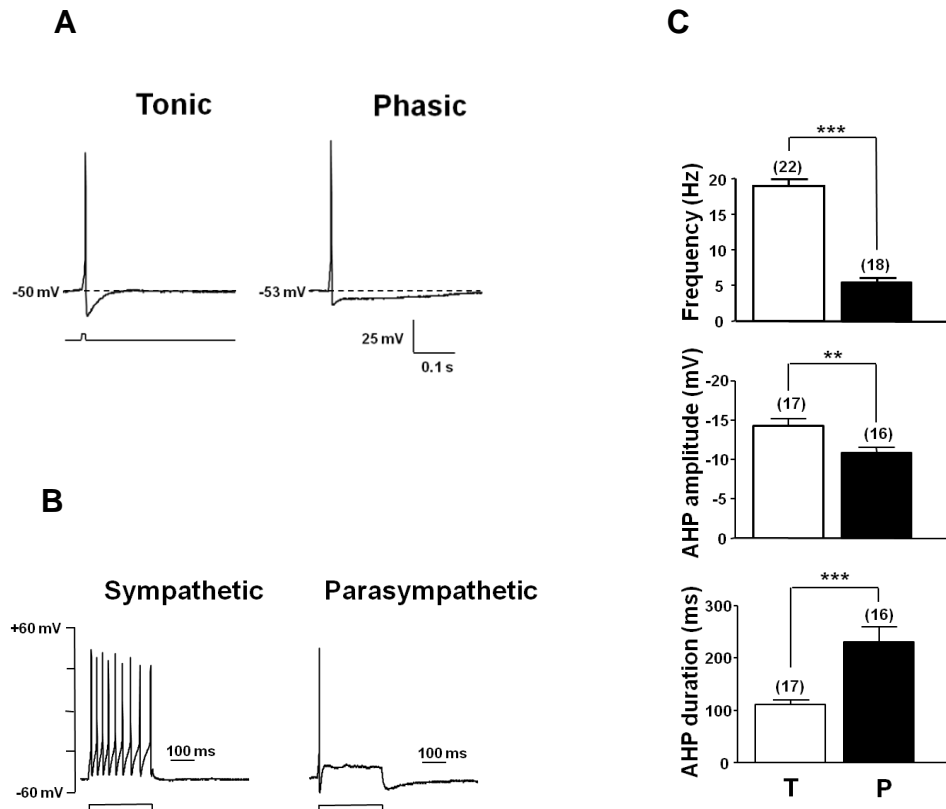
Figure 6. Electrophysiological properties of sympathetic and parasympathetic MPG neurons innervating bladder.

Membrane potentials were measured under current-clamp mode using gramicidin-perforated patch-clamp techniques. A, a AHP (afterhyperpolarization) is followed by single action potential that differs from the one found tonic firing MPG neurons showed a larger amplitude and a shorter duration than that of the phasic and parasympathetic ones. B, Representative traces of tonic and phasic spike firing in the sympathetic and parasympathetic MPG neurons was induced by injection of supra-threshold current for 400 ms, respectively. C, Bar graph showing differences in the firing frequency (top), AHP amplitude (middle), and AHP duration (bottom) between the sympathetic and

parasympathetic MPG neurons. Numbers of animals tested are indicated in parentheses.

Data are presented as mean±SEM. *** P < 0.001; ** P < 0.01; compared with control.

4.3. Nintotinic acetylcholine receptor in the MPG neurons of BPH rat.

Nicotinic acetylcholine receptors (nAChR) involve in excitatory postsynaptic potential and intracellular Ca^{2+} increase in the MPG neurons. An $\alpha3\beta4$ subunit combination acts as a major functional nAchR in male rat MPG neurons (Park et al., 2006). Thus, it was tested whether overactive bladder by BPH alters channel properties and/or expression of nAchR subunits in MPG neurons. The expression of $\alpha3$ and $\beta4$ subunit of nAchR was examined using quantitative real-time PCR. As shown in Fig. 7, both $\alpha3$ and $\beta4$ subunits were all upregulated in the MPG of BPH rats. These data suggest that increased expression of major subunits of nAchR, at least partly, may contribute augmentation of ganglionic transmission and excitability in MPG neurons of BPH rat.

Expression of major nAchR subunits was increased in bladder outlet obstruction by BPH. Thereafter, functional analysis of nAchR was performed using whole cell-ruptured patch-clamp techniques. Ach-induced inward current was evoked by 100 μM ACh in MPG neurons held at -60 mV. No significant difference was found in Ach-induced current density of sympathetic and parasympathetic MPG neurons from both control and BPH rats. However, the increase in Ach-induced current density of sympathetic neurons of BPH rats compared with control rats was significant (372.38±26.15 and 503.23±37.24 pA/pF, control and BPH groups, respectively, $p<0.05$, Fig. 8A). Similarly, Ach-induced current density in parasympathetic MPG neurons of BPH rats were also significantly increased compared with control rats (345.75 ± 18.12 and 471.76 ± 37.18pA/pF, control and BPH groups, respectively, $p<0.005$, Fig. 8 B). These data suggest that augmented nAchR current may, at least partly, contribute increased ganglionic transmission and excitability of MPG neurons in BPH rat model.
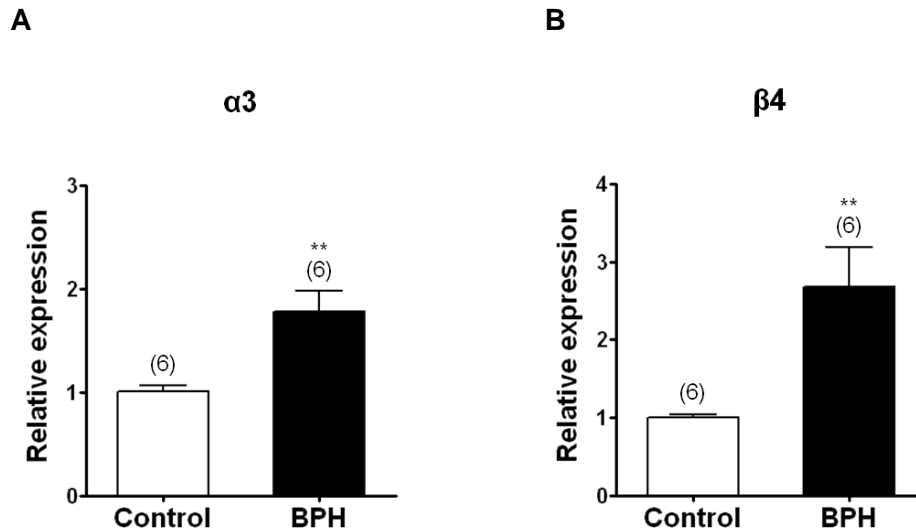
Figure 7.   Expression levels of nAChR subunits in BPH rat model.

mRNA levels of α3 (A) and β4 (B) nAChR subunits were examined in MPG of control and BPH rats using real-time PCR. The graph shows an average percentage of change in mRNA level relative to control. β-actin mRNA was used as an endogenous control. All values are form triplicate measurements. Similar results were found in three independent experiments. Numbers of cells tested is indicated in parentheses. Data are presented as mean±SEM. ** $P < 0.01$; compared with control

A
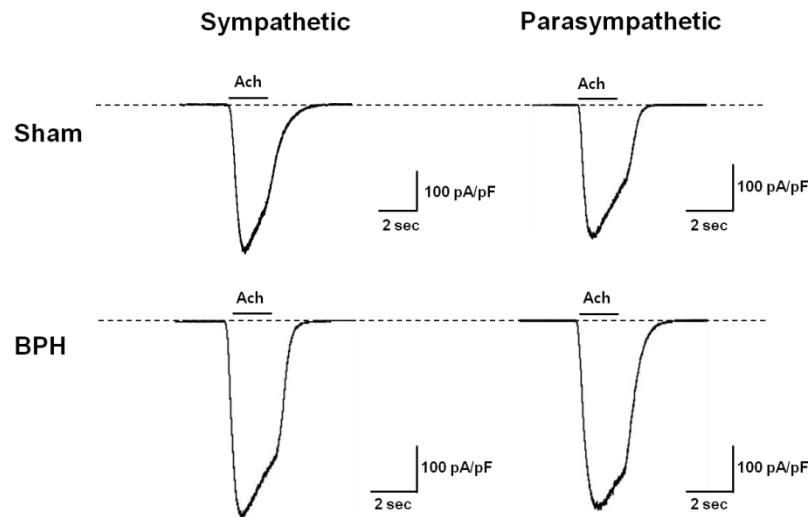
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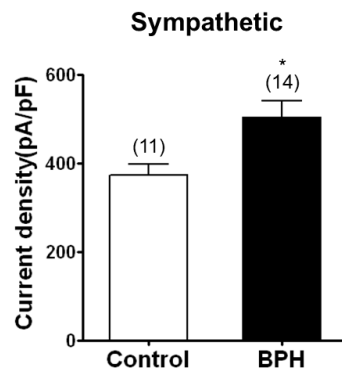
C
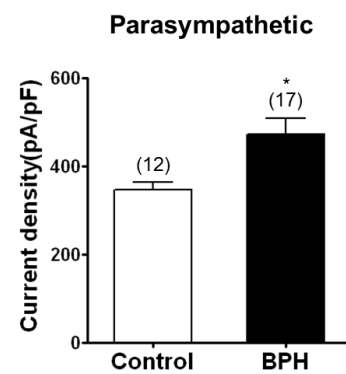
Figure 8. Cell-type specific changes of Ach-induced current in MPG neurons of BPH rats.

Ach-induced currents were measured using whole-cell ruptured patch-clamp techniques.

A, representative trace of inward currents evoked by application of 100 μM Ach in both

sympathetic and parasympathetic MPG neurons held at -60 mV. Upper and lower trace was from shame control and BPH rats, respectively. B and C, Ach-induced current density of MPG neurons innervating bladder was summarized. Ach-induced current density was significantly increased in both sympathetic and parasympathetic MPG neurons from BPH rats compared with control group (B, C). Data are presented as mean±SEM. * P < 0.05; compared with control.

4.4. Neuronal excitability of MPG and DRG neurons innervating bladder in BPH rats.

Neuronal excitability of both MPG and DRG neurons were examined using gramicidin perforated patch-clamp techniques. MPG neurons show tonic or phasic firing patterns in response to prolonged current injections (Fig. 6B, Fig 9). However, DRG neurons show only phasic firing pattern (Fig 10). Neurons isolated from BPH rats showed increased excitability.

In MPG neurons (Fig. 9, table 3), control group showed tonic firing of 12.5 Hz, 18 Hz, and 30 Hz in response to 1 X, 2 X, and 3 X threshold stimulation, respectively. However, firing rates of BPH group were increased to 15 Hz, 26 Hz, 37.5 Hz when the same sequence of stimulation was applied. In BPH group, rheobase (pA) was significantly reduced to compared with control group (126.6±18.7 and 56.4±7.1 pA, control and BPH group, respectively, p<0.01). In the some way, AHP duration (ms) significantly reduced (121.5±17.7 and 89.6±10.7, control and BPH group, respectively, p<0.05) while amplitude had no change (table 3).

In DRG neurons (Fig. 10, table 4), firing frequency in BPH groups was also significantly increased compared with control group. Firing frequencies of control group were 3.7±0.7, 4.2±0.6, and 6.7±0.6 Hz in response to 1 X, 2 X, and 3 X threshold stimulation, respectively. Interestingly, firing pattern was changed from phasic to tonic in DRG neurons in BPH rat. Firing frequencies of BPH group were 7.5±1, 14.2±0.6, and 20±1 Hz, respectively. Rheobase of DRG neurons in BPH group was significantly reduced to 56.7±6.7 pA, compared with control group (100.7±16.7 pA). Furthermore, both amplitude and duration of AHP were significantly reduced compared with control group. Additional

- 39 -

electrophysiological values are provided in table 4. Taken together, these data suggest that neuronal excitability of MPG and DRG neurons innervating bladder was increased in BPH rat model.
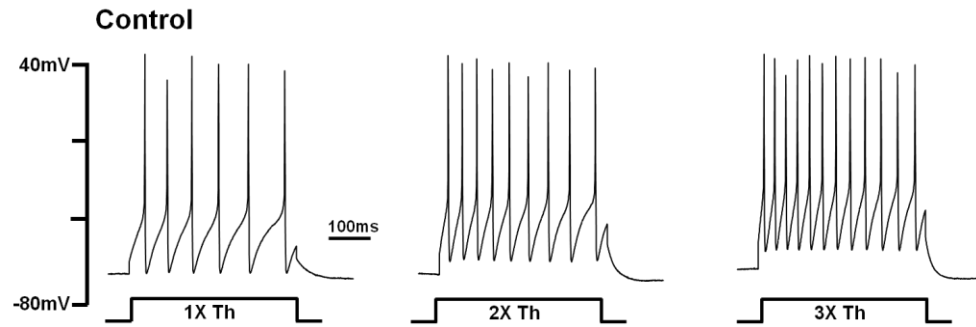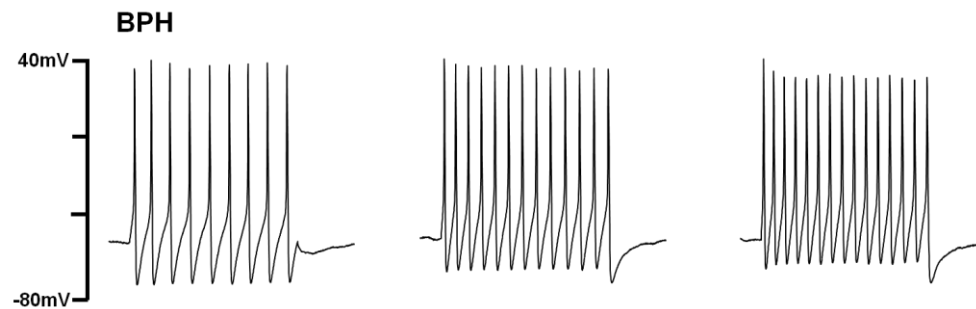
Figure 9. Changes of firing frequency in tonic bladder MPG neurons.

Representative traces of tonic spike firing in MPG neurons from control (A) and BPH (B) rats. All recordings were performed under gramicidin-perforated whole-cell patch-clamp configuration. Tonic firing of MPG neurons was induced by injection of supra-threshold current for 400 ms. Firing frequency of tonic MPG neurons in BPH rats increased compared with control rats. Firing change of tonic MPG neurons represented by the burst spike firing pattern is frequency-dependent on injected current amplitude.

Table 3. Electrophysiological properties in tonic bladder MPG neurons.

	Control (n=4)	BPH (n=5)
Capacitance (pF)	43.8±2.7	55.7±3.7*
R_{in} (MΩ)	482.2±31.1	468.7±26.3
RMP (-mV)	57±3.2	53.1±3.8
Rheobase (pA)	126.6±18.7	56.4±7.1**
AP amplitude (mV)	128.4±4.6	113.5±3.3
AP duration (ms)	1.4±0.2	1.2±0.3
Frequency, Hz-1x	12.5±1.4	15±1.7
Frequency, Hz-2x	18.8±1.8	26.3±2.3**
Frequency, Hz-3x	30±1.4	37.5±2.7**
AHP amplitude (mV)	19.4±1.5	12.4±1.3
AHP duration (ms)	121.5±17.7	89.6±10.7*

Electrophysiological properties of MPG neurons were measured using gramidicin-perforated patch-clamp techniques as described in methods. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. * $p<0.05$; ** $p<0.01$; compared with control. (R_{in}, input resistance; RMP, resting membrane potential; AP, action potential;   AHP, afterhyperpolarization potential)

A
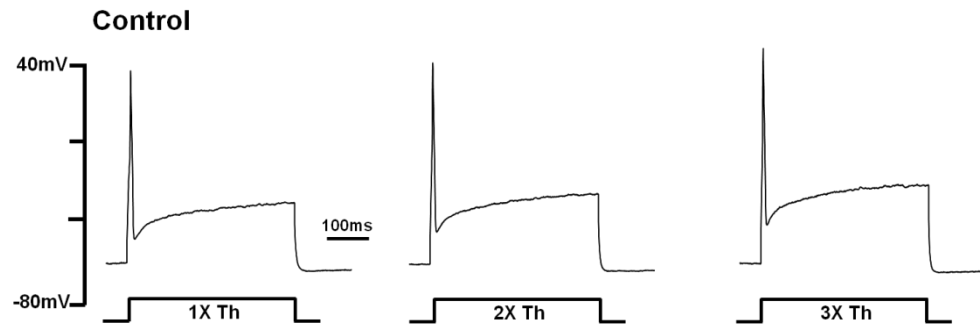
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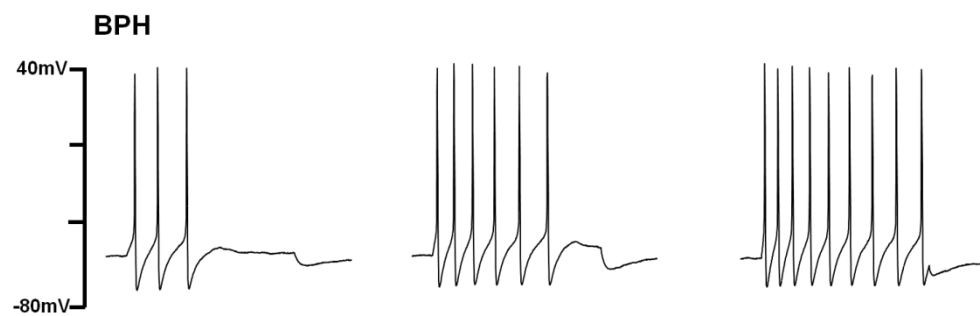
Figure. 10. Alterations of firing pattern in DRG neurons innervating bladder.

Representative traces of spike firing in DRG neurons from control (A) and BPH (B) rats. All recordings were performed under gramicidin-perforated whole-cell patch-clamp configuration. Spike firing of DRG neuron which is TTX-resistant bladder afferent neurons was induced by injection of supra-threshold current for 400 ms. Firing frequency of DRG neuons in BPH rats increased compared with control rats. Firing property of DRG neurons were changed to the burst spike firing pattern which was frequency-dependent on injected current amplitude.

Table.4. Electrophysiological properties in DRG neurons isolated from BPH rat.

	Control (n=3)	BPH (n=3)
Capacitance (pF)	34.4±4.7	44.6±5.4
R_{in} (MΩ)	390.2±23.3	365.6±14.4
RMP (-mV)	51.2±2.2	49.7±3.8
Rheobase (pA)	100.7±16.7	56.7±6.7*
AP amplitude (mV)	112±8.5	98.6±9.3
AP duration (ms)	5.3±0.6	3.4±0.8
Frequency, Hz-1x	3.7±0.7	7.5±1**
Frequency, Hz-2x	4.2±0.6	14.2±0.6***
Frequency, Hz-3x	6.7±0.6	20±1 ***
AHP amplitude (mV)	23.3±2.7	14.±1.7*
AHP duration (ms)	185.2±16.7	105.5±15.5*

All electrophysiological properties of DRG neurons were measured using gramidicin-perforated patch-clamp techniques as described in methods. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; compared with control. R_{in}, input resistance; RMP, resting membrane potential; AP, action potential; AHP, afterhyperpolarization potential.

4.5. Evaluation of BOO rat model

4.5.1. General characteristics of BOO rat model

A BOO rat model was used to study the effect of bladder outlet obstruction on bladder function. Firstly, there was no difference in body weight between control and BOO groups (463.2±5.2 and 444.4±5.6 g, control and BOO groups, respectively, Fig. 11 A). It is known that the BOO rat model shows an increased bladder weight. A significant increase in bladder weight was observed in BOO groups compared to control groups (0.04±0.001 and 0.12±0.006 %, control and BOO groups, respectively, p<0.001, Fig. 11 B). Bladder weight was normalized to body weight.

4.5.2. Histological analysis of bladder in BOO rat model

To analyze pathological changes of bladder by BOO, bladders were sectioned and its histology was examined (Zhang et al., 2004). Compared with control bladder, BOO bladder exhibited hypertrophy of the bladder detrusor muscle (Fig. 12A, C). Both bladder wall thickness and smooth muscle bundles of BPH rats were hypertrophic compared with control bladder (Fig. 12B, D).

4.5.3. Cystometry recordings in BOO rat model

It is known that BOO rat model shows an increase bladder capacity, imcturition pressure, and residual volume. Fig. 13 shows the typical cytometry recordings of

micturion cycles obtained in control and BOO rat. Cystometry was performed under an awaken condition and cystometric parameters were monitored over 50 min recording periods. The voiding patterns were changed in BOO rat model. All volume parameters such as bladder capacity, micturition volume, and residual volume were increased in BOO rat model. Threshold pressure in BOO rats was increased but change of basal and micturition pressure was negligible compared to control rats. Intermicturition oscillatory frequency in BOO rats was increased compared to control rats, while mean intermicturition oscillatory pressure had no difference between groups. Additional cystometric values and n numbers are provided in table 5.
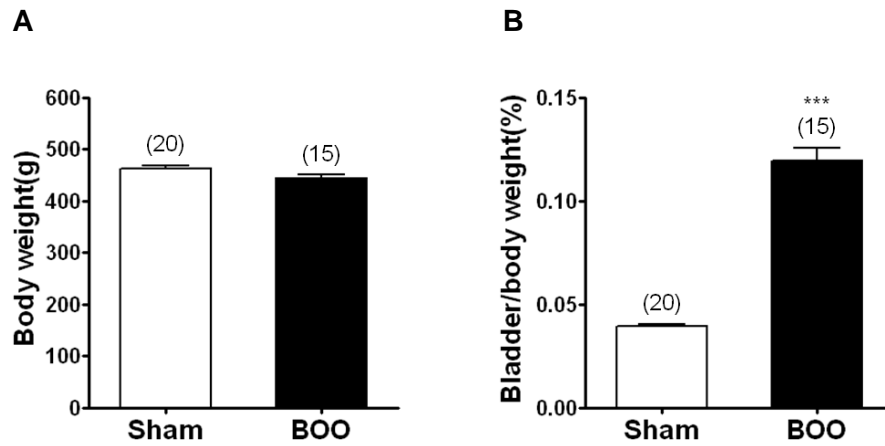
Figure 11. Changes of body and bladder weight in BOO rats.

Mean body weight (A) and bladder weight (B) for experimental groups after 6 weeks under urethral ligation. Bladder weight was normalized to body weight. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. *** P<0.001; compared with control.
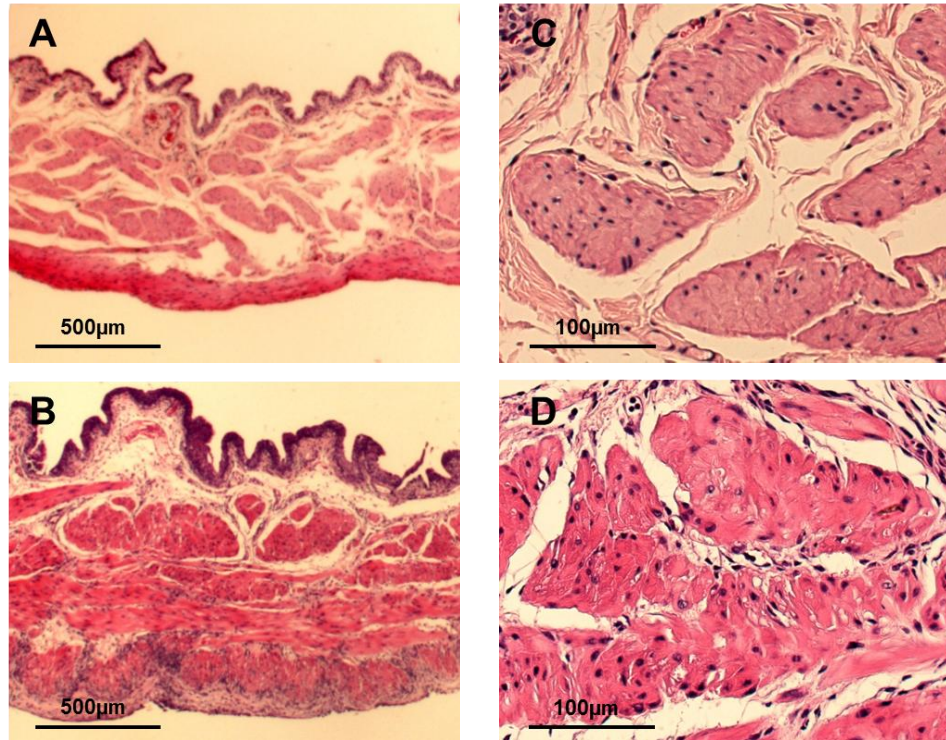
Figure 12. H & E staining of bladder in sham and BOO rat.

H & E staining of sections of bladder from control (sham; A, C) and BOO (B, D) rats.

Badder from BOO rat showed hypertrophic change in smooth muscle layer. H & E

staining of control and BOO bladder shown at X 40 (A, B) or X 100 (C, D) magnification.

A
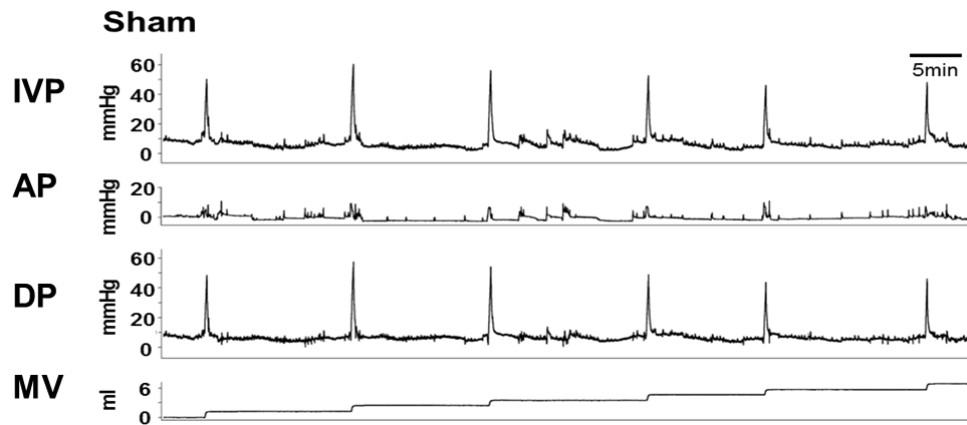
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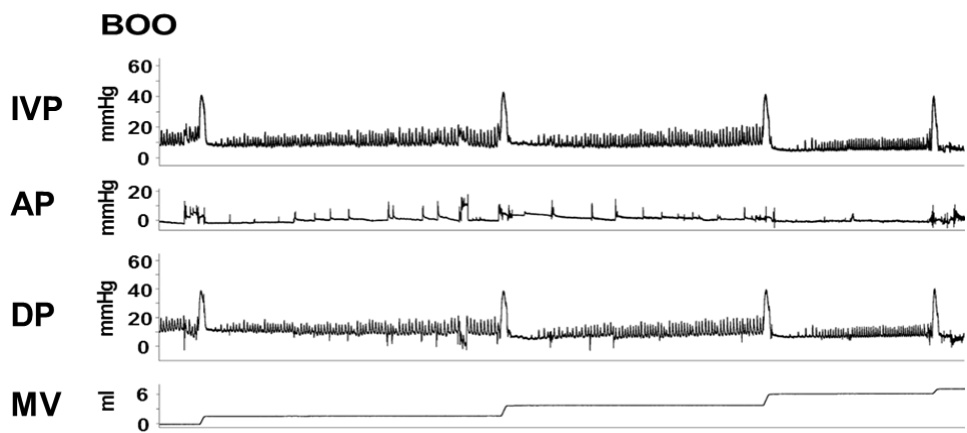
Figure 13. Cystometrical recordings of micturition cycles obtained in sham and BOO rats.

Cystometry recordings in sham control (A) and BOO (B) rats. Warmed saline was infused continuously into the bladder at rate of 6 ml/hr. The following parameters including

intravesical pressure (IVP), intra-abdominal pressure (IAP), detrusor pressure (DP), and micturition volume (MV) were measured. Additional cystometric values and n numbers are provided in table 5.

Table 5. Parameters of cystomety in BOO rats

Parameters	Sham (n=3)	BOO(n=3)
BC (ml)	1.06±0.09	2.69±0.18**
BP (mmHg)	9.9±1.2	9.7±0.9
TP (mmHg)	10.5±0.4	13.5±1.4*
MP (mmHg)	41.7±3.5	48.4±1.0
MV (ml)	1.13±0.05	2.34±0.23**
RV (ml)	0.013±0.004	0.184±0.09*
IOF (Hz)	7.83±3.83	54.32±11.59*
MIOP (mmHg)	3.1±0.2	3.1±0.4

Cystometric parameters; BC, bladder capacity; BP, basal pressure; TP, threshold pressure, MP, micturition pressure; MV, micturition volume; RV, residual volume; IOF, intermicturition oscillatory frequency; MIOP, mean intermicturition oscillatory pressure. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. * $p < 0.05$; ** $p < 0.01$; compared with control.

4.6. Functional changes of nAchR of MPG neurons in the BOO rats

Ach is a major neurotransmitter in ganglionic transmission. nAchR mediates excitatory postsynaptic potential and Ca^{2+} influx in the MPG neurons. As mentioned previously, an $\alpha3\beta4$ subunit combination acts as a major functional nAchR in MPG neurons (Park et al., 2006). Thus, it was tested whether function and expression of nAchR was altered by overactive bladder by BOO. The expression of $\alpha3$ and $\beta4$ subunit of nAchR was examined using quantitative real-time PCR. As shown in Fig. 14, both $\alpha3$ and $\beta4$ subunits of AchR were all upregulated in the MPG of BOO rats. Fold increase of α3 subunit and β4 subunit in BOO rat model was 1.6±0.25 and 2.4±0.3 compared to control rat, respectively. These differences were statistically significant. These data suggest that increased expression of major subunits of nAchR may contribute, at least partly, increase of excitability in MPG neurons of BOO rat.

Expression levels of major nAchR subunits were increased in bladder outlet obstruction by BOO. Thereafter, functional analysis of nAchR was examined using whole cell-ruptured patch-clamp techniques. Ach-induced inward current was evoked by 100 µM ACh in MPG neurons held at -60 mV (Fig. 15A). No significant difference was found in Ach-induced current density of sympathetic MPG neurons from both control and BOOS rats group (279.73±33 pA/pF and 327.33±37.9 pA/pF, control and BOO groups, respectively, Fig. 15B). However, the increase in Ach-induced current density of para sympathetic neurons of BOO rats compared with control rats was significant (201.5±15.3 pA/pF and 249.52±16.9 pA/pF, control and BOO groups, respectively, $p<0.01$, Fig. 15 C). These functional data suggest that increased Ach-induced current density in para sympathetic neurons may contribute augmentation of ganglionic transmission in MPG.
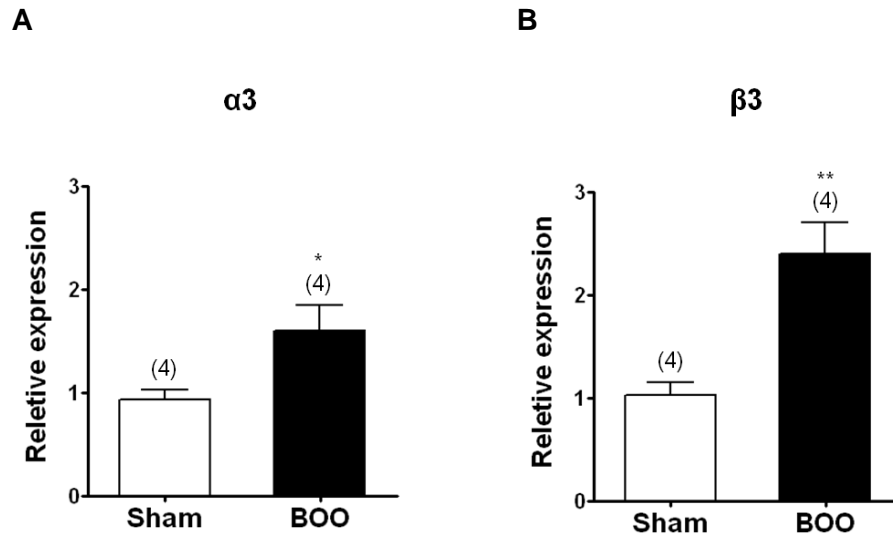
Figure 14.   Expression level of nAChR subunits in BOO rat model measured.

mRNA levels of α3 (A) and β4 (B) nAChR subunits were examined in MPG of control and BPH rats using real-time PCR. The graph shows an average percentage of change in mRNA level relative to control. β-actin mRNA was used as an endogenous control. All values are form triplicate measurements. Similar results were found in three independent experiments. Numbers of cells tested is indicated in parentheses. Data are presented as mean±SEM. *$p<0.05$; ** $p<0.01$; compared with control.

A

Sympathetic   Parasympathetic

Sham

BOO

B   **C**

Sympathetic   Parasympathetic
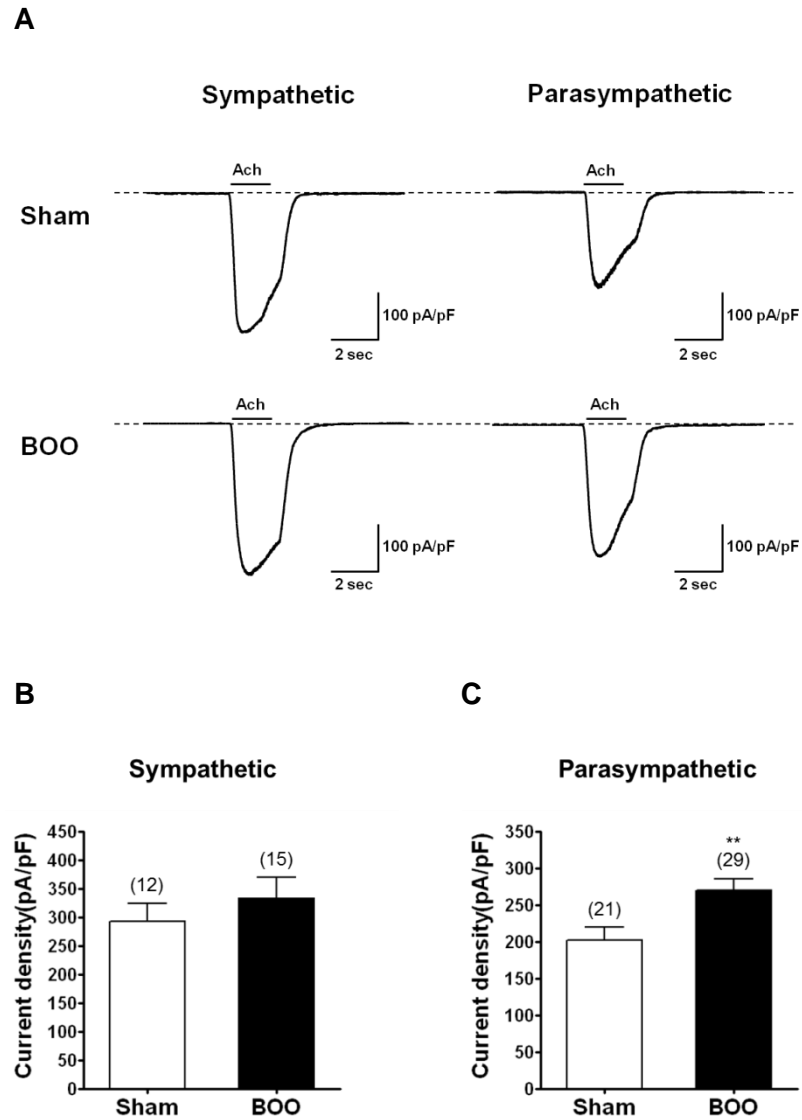
Figure 15. Cell-type specific alterations of Ach-induced current in MPG neurons of the BOO rats

A, representative trace of inward currents evoked by application of 100 μM Ach in both sympathetic and parasympathetic MPG neurons held at -60 mV. An upper and lower

trace was from sham control and BOOS rats, respectively. B and C, Ach-induced current density of MPG neurons innervating bladder was summarized. No significant difference of Ach-induced current density was founded in sympathetic MPG neurons (B). Ach-induced current density was significantly increased in parasympathetic MPG neurons from BOO rats compared with control group (C). Data are presented as mean±SEM. **p < 0.01; compared with control.

4.7. Neuronal excitability of MPG and DRG neurons innervating bladder in BOO rat models

Neuronal excitability was increased by bladder outlet obstruction by BPH (Fig 9-10, table 3, 4). Neuronal excitability of both MPG and DRG neurons in BOO model were examined as well as BPH model. Membrane potential changes were measured under current-clamp mode using gramicidin perforated patch-clamp techniques. As previously mentioned, MPG neurons show both tonic or phasic firing patterns in response to prolonged current injections (Fig. 6B, Fig 16). DRG neurons show only phasic firing pattern (Fig 10A, Fig 17A). In BOO model, neuronal excitability of both MPG and DRG neurons was significantly increased as well as BPH model.

In MPG neurons (Fig. 16, table 6), control group showed tonic firing of 12.5 Hz, 20 Hz, and 30 Hz in response to 1 X, 2 X, and 3 X threshold stimulation, respectively. However, firing rates of BOO groups were increased to 16.7 Hz, 28 Hz, 35 Hz when the same sequence of stimulation was applied. In BOO group, rheobase (pA) was significantly reduced to compared with control group (126.6±18.7 and 56.4±7.1 pA, control and BPH group, respectively, $p<0.01$). In the some way, AHP duration (ms) significantly reduced (131.5±23.3 and 88.3±6.4 ms, control and BPH group, respectively, $p<0.05$) while amplitude had no change (table 6).

In DRG neurons (Fig. 17, table 7), firing frequency in BOO groups was also significantly increased compared with control group. Firing frequencies of control group were 3.7±0.7, 4.2±0.6, and 6.7±0.6 Hz in response to 1 X, 2 X, and 3 X threshold stimulation, respectively. Interestingly, firing pattern was changed from phasic to tonic firing. Firing frequencies of BOO group were 7.5±1, 14.2±0.6, and 20±1 Hz, respectively.

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Rheobase of DRG neurons in BOO group was significantly reduced to 56.7±6.7 pA, compared with control group (100.7±16.7 pA). Furthermore, both amplitude and duration of AHP were significantly reduced compared with control group. Additional values of electrophysiological are provided in table 7. Taken together, these data suggest that neuronal excitability of MPG and DRG neurons innervating bladder of BOO rat was increased.
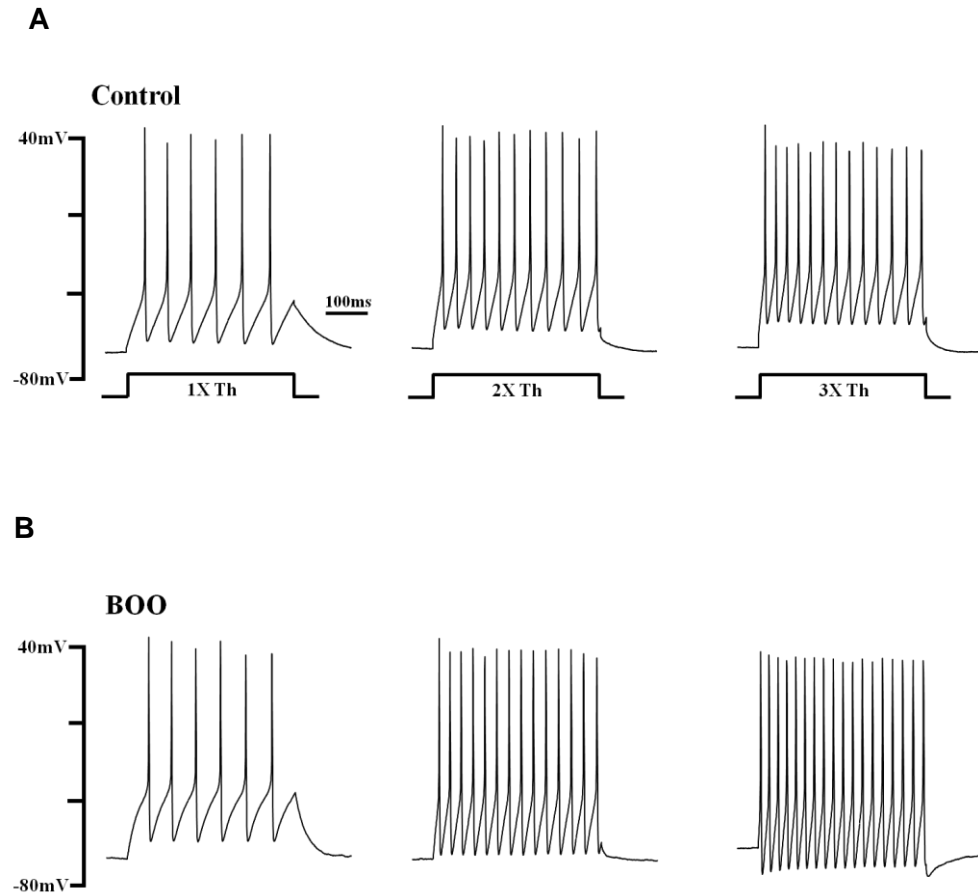
Figure 16. Changes of firing frequency in tonic MPG neurons.

Representative traces of tonic spike firing in MPG neurons from control (A) and BOO (B) rats. All recordings were performed under current-clamp mode using gramicidin-perforated whole-cell patch-clamp configuration. Tonic firing of MPG neurons was induced by injection of supra-threshold current for 400 ms. Firing frequency of tonic MPG neurons in BOO rats increased compared with control rats. Firing change of tonic MPG neurons represented by the burst spike firing pattern is frequency-dependent on injected current amplitude.

Table 6. Electrophysiological properties in tonic MPG neurons isolated from BOO rat

	Control (n=3)	BOO (n=3)
Capacitance (pF)	44.2±4.8	48.7±6.6
R_{in} (MΩ)	532.2±35.4	508.5±25.1
RMP (-mV)	54±4	56.7±3.5
Rheobase (pA)	120±11.5	66.7±6.7*
AP amplitude (mV)	121.4±3.9	103.9±1.8
AP duration (ms)	48.2±3.8	46.8±1.4
Frequency, Hz-1x	12.5±1.4	16.7±0.8
Frequency, Hz-2x	20.8±0.8	28.3±1.6**
Frequency, Hz-3x	30±1.4	35±0.8*
AHP amplitude (mV)	20.3±1.6	15.4±1.6
AHP duration (ms)	131.5±23.3	88.3±6.4*

Electrophysiological properties of MPG neurons were measured by gramidicin-perforated patch-clamp techniques as described in methods. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. * $p<0.05$; ** $p<0.01$; compared with control. (R_{in}, input resistance; RMP, resting membrane potential; AP, action potential; AHP, afterhyperpolarization potential)
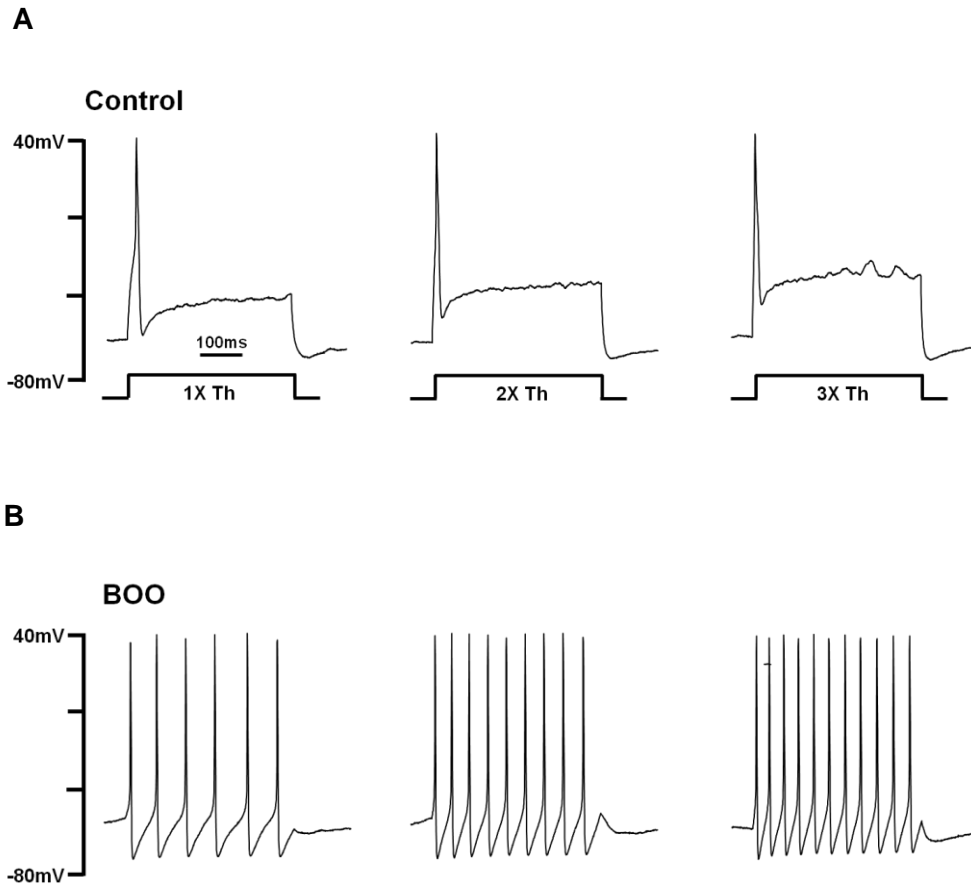
Figure 17. Changes of firing frequency in DRG neurons innervating BOO bladder

Representative traces of spike firing in DRG neurons from control (A) and BOO (B) rats. All recordings were performed under gramicidin-perforated whole-cell patch-clamp configuration. Spike firing of DRG neurons which is TTX-resistant bladder afferent neurons was induced by injection of supra-threshold current for 400 ms. Firing frequency of DRG neuons in BOO rats increased compared with control rats. Firing property of DRG neurons were changed to the burst spike firing pattern which was frequency-dependent on injected current amplitude.

Table.7. Electrophysiological properties in DRG neurons isolated from BOO rat

	Control (n=6)	BOO (n=6)
Capacitance (pF)	31.8±2.4	42.5±3.1*
R_{in} (MΩ)	514.2±18.4	494.5±24.8
RMP (-mV)	51.3±3.1	53.2±3.4
Rheobase (pA)	111.7±16.7	46.7±3.4**
AP amplitude (mV)	108±7.8	102.6±4.5
AP duration (ms)	5.4±0.7	3.5±0.5
Frequency, Hz-1x	3.3±0.4	6.87±1**
Frequency, Hz-2x	5±0.6	18.7±0.8***
Frequency, Hz-3x	5.8±0.8	23.7±1 ***
AHP amplitude (mV)	18.3±3	11.8±1.2*
AHP duration (ms)	179.4±9.7	127.8±12.2**

All electrophysiological properties of DRG neurons were recorded under current-clamp mode using using gramidicin-perforated patch-clamp techniques as described in methods. Numbers of animals tested are indicated in parentheses. Data are presented as mean±SEM. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; compared with control. R_{in}, input resistance; RMP, resting membrane potential; AP, action potential; AHP, afterhyperpolarization potential.

4.8. Properties of voltage-activated Ca^{2+} channel in sympathetic MPG and DRG neurons of BOO rats

Voltage-activated Ca^{2+} channels (VACC) allow Ca^{2+} influx in response to membrane depolarization and mediate diverse neuronal functions, including transmitter release and excitability. Based on their activation threshold, VACC can be divided in two main groups, high-voltage-activated (HVA) and low-voltage-activated (LVA) Ca^{2+} channels. Among autonomic neurons, sympathetic MPG neurons are unique by expressing LVA T-type Ca^{2+} channel whose isoform is α1H (Lee et al., 2002). DRG neurons also express functional α1H isoform of T-type channel. T-type Ca^{2+} currents may contribute to generation of low-threshold spikes in DRG and sympathetic MPG. Thus, the expression change of α1H isoform was examined in BOO model using real-time PCR. As shown in Fig 18, expression of α1H isoform was increased in both MPG and DRG of BOO model compared to control model.

Expression of α1H isoform was increased in bladder outlet obstruction. Next, functional analysis of voltage-activated Ca^{2+} currents was performed using whole-cell ruptured patch-clamp techniques. Ca^{2+} current were evoked by test pulses to -30 mV and +10 mV from a holding potential of -80 mV. T-type current density of BOO group was significantly increased in MPG neurons compared with control group (3.4±0.3 pA/pF and 5.2±0.7 pA/pF, control and BOO group, respectively, $p<0.05$, Fig. 19 A). In DRG neurons, T-type current of BOO group was significantly increased compared with control group (3.9±0.3 pA/pF and 6.1±0.6 pA/pF, control and BOO group, respectively, $p<0.01$, Fig. 19 C). However, N-type currents, a major type of HVA Ca^{2+} current in MPG and DRG neurons, were not changed compared with controls (Fig.19 B, D).
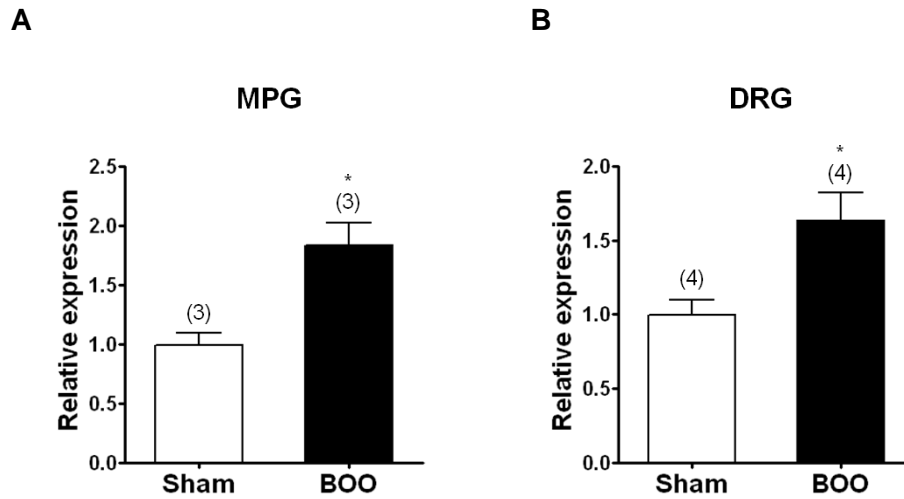
Figure 18. Expression levels of α1H T-type Ca^{2+} channel in MPG and DRG neurons

mRNA level of α1H isoform of T-type Ca^{2+} channel was examined in MPG and DRG of control and BOO rats using real-time PCR. The graph shows an averge pecentage of change in α1H mRNA level relative to control. β-actin mRNA was used as an endogenous control. All values are form triplicate measurements. Similar results were found in three independent experiments Numbers of cells tested is indicated in parentheses. Data are presented as mean±SEM * P<0.05; compared with control.

A

T-type current

B

HAV

C

T-type current

D

HAV
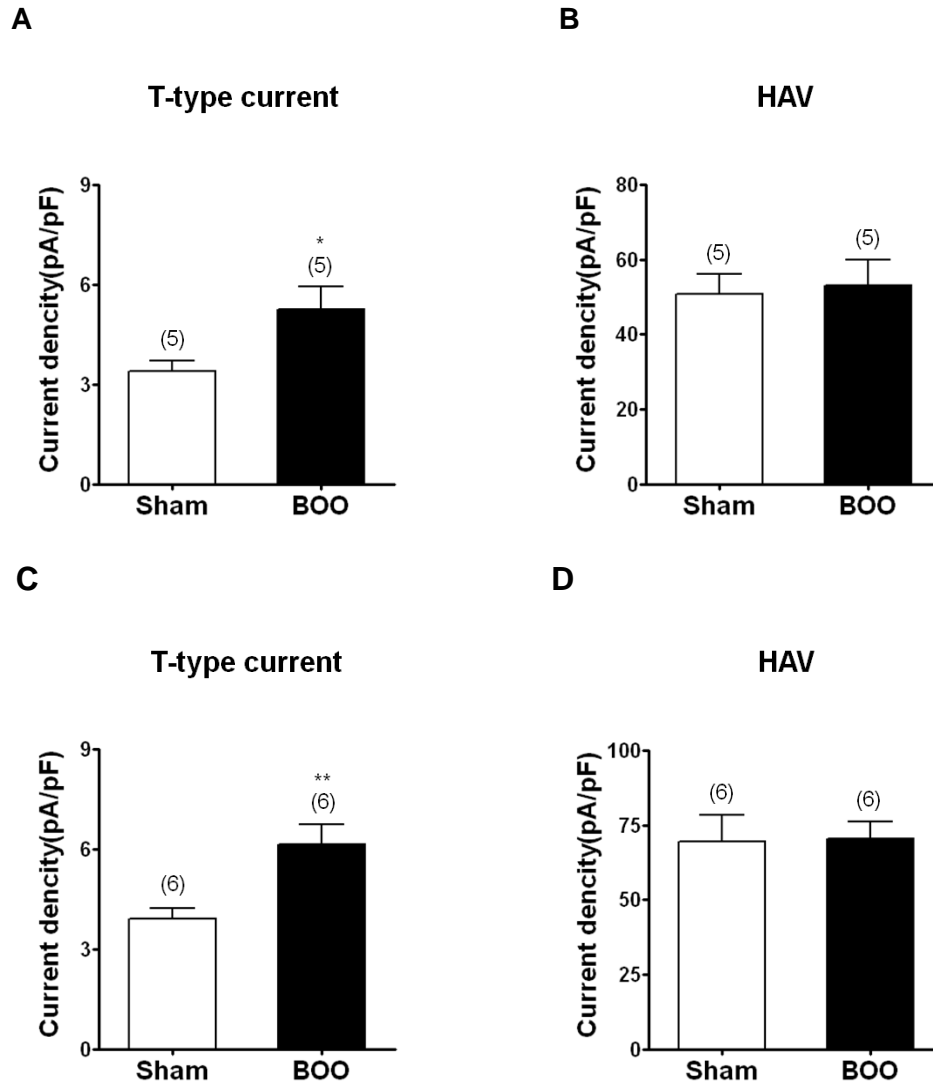
Figure 19. Functional change of voltage-activated Ca^{2+} channel current in MPG and DRG neurons in BOO rat. LVA and HVA Ca^{2+} currents were evoked by test pulses to -30mV and +10mV from a holding potential of -80 mV, respectively. Ca^{2+} currents were recorded in MPG (A, B) and DRG neurons (C, D). HAV is mainly N-type Ca^{2+} current. Numbers of cells tested is indicated in parentheses. Data are presented as mean±SEM. * $P<0.05$; ** $p<0.01$; compared with control.

V. DISCUSSION

BPH results in an age-related and hormone-dependent increase in prostate gland volume, which leads to the LUT symptoms caused by BOO (Irwin et al., 2009). Storage symptoms often persist even after the obstruction is surgically corrected (Seaman et al., 1994), suggesting that some plastic changes in the bladder and neural components controlling the bladder occur during obstruction. To date, the mechanisms underlying the OAB seen in patients with BOO are not fully understood. Thus, the present study was designed to test the hypothesis that OAB is associated with altered functions of MPG and DRG neurons innervating the urinary bladder. In this regard, functional plasticity of the bladder MPG and DRG neurons was extensively investigated at cellular and molecular levels in rat models of BPH and partial urethral obstruction. The key observations in the present study are that : (i) nAchR a3 and b4 subunits were up-regulated in the bladder MPG neurons, which may result in enhanced ganglionic transmission, and (ii) T-type $Ca^{2+}$ channels to increase excitability of sympathetic MPG neurons and capsaicin-sensitive C-fiber DRG neurons innervating the bladder in the BOO rat models.

There appears to be at least three different BOO models: high-fat diet-induced obese rats, hormone-induced BPH rats, and partial urethral obstruction (PUO) rats. The first model is based on the fact that obesity, the principal cause of metabolic syndrome, is a risk factor for urinary storage syndrome and found to be clinically associated with stress urinary incontinence, OAB (Dallosso et al., 2003), and the LUT symptom related to BPH (Rohrmann et al., 2005). As a preliminary experiment in the present study, the high-fat diet-induced obese rat model was tested. However, long-term high-fat feeding

failed to develop BPH and thus OAB in rats as demonstrated in cystometry (see also Tong et al., 2007). Thereafter, hormone-induced BPH and PUO rats were adopted since they show bladder hypertrophy and DO (Levin et al., 1986; Liu et al., 2009; Mattiasson et al., 1982; Tatemichi et al., 2006). Of course, these models do not reproduce all the facets of the human condition. Especially, there is no way of knowing whether rats are experiencing urgency, the key to the diagnosis of OAB. With the inability to quantify urgency in animals, thus, some surrogate markers including DO, voided volume, and intermicturitional contraction on cystometry were alternatively used for validation of the BOO rat models as in other studies (Broderick et al., 1994; Drake et al., 2006; Hayashi et al., 2004; Lluel et al., 1998; Malmgren et al., 1987; Pampinella et al., 1997; Steers et al., 1988; Tatemichi et al., 2006; Wolffenbuttel et al., 2001a; Zvara et al., 2004).

Typical weight gains of dorsal and ventral prostate lobes were observed in BPH rats that were treated with 1:100 estrogen/testosterone, a ratio that is similar to the circulating estrogen/testosterone ratio in older men (Zhou et al., 2009). In parallel with the ventral prostate hyperplasia, DO was observed together with increased VC, RV, TP, MV, and IOF, which are consistent with previous studies (Liu et al., 2009; Tatemichi et al., 2006). Although the urethral pressure or diameters were not measured, the increased urethral resistance (a partial obstruction secondary to BPH) could be indirectly assessed from the decreased flow rate and bladder hypertrophy. In the present study, the cystometry for evaluation of the urodynamics was carried out in conscious rats. However, many researchers have employed cystometrical evaluation of bladder function under anesthesia (Morikawa et al., 1989) producing highly variable results that are markedly affected by the depth of anesthesia. Furthermore, the profile

of the bladder contraction recorded is quite different from that observed in conscious animals. To be convinced with results from the experiments using the hormone-induced BPH rat model, PUO rat model was also evaluated by cystometry. Similar to the BPH rats, PUO increased most of the urodynamic parameters and produced bladder hypertrophy. Compared with the hormone-induced BPH rats, however, the increment of IOF is much higher in the PUO rats. This result suggests that PUO rat models have more severe obstruction than BPH rat model (see Parsons et al., 2011).

Morphological studies have shown that obstructed rat exhibited extensive hypertrophy of rat bladder and bladder MPG neurons (Gabella et al., 1992; Gabella et al., 1993; Steers et al., 1990). Distension of the bladder is known to be a primary stimulus for muscle growth. It has been suggested that increased bladder muscle mass induces neuronal hypertrophy via an increase in neurotrophic factors in the bladder muscle and/or via increased axonal sprouting necessary to innervate the large muscle (Gabella et al., 1992; Gabella et al., 1993). Unexpectedly however, the membrane capacitance of the bladder MPG neurons reflecting cell size was not increased in both BPH and PUO rats. It is unclear where the discrepancy between studies arises. Unlike MPG neurons, DRG neurons were enlarged by BOO and the resultant bladder hypertrophy, which is consistent with previous studies (Steers et al., 1991a; Steers et al., 1996).

As in other autonomic ganglia, fast excitatory synaptic transmission is mediated by Ach which activates the nAChRs composed of the a3 and b4 subunits in the MPG (Park et al., 2006). In the experiments, expression and activity of nAChRs were up-regulated in both sympathetic and parasympathetic bladder MPG neurons in BPH rats. Interestingly, PUO increased expression and activity of nAChRs selectively in the

parasympathetic bladder MPG neurons. To induce BPH, testosterone and 17-b-estradiol was periodically injected. Accordingly, there could be an argument that up-regulation of nAChRs occurs via direct actions of the hormones on MPG neurons but not BOO secondary to prostate hyperplasia. It is unlikely that testosterone affects the bladder MPG neurons which do not express androgen receptors (Keast et al., 1998; Schirar et al., 1997). On the other hand, estrogen is suggested to act on MPG neurons in a phenotype-non-specific manner (Purves-Tyson et al., 2007). Thus, it remains to test whether estrogen receptors are expressed and their activation increases nAChRs in the sympathetic bladder MPG neurons. In addition, it might be interesting whether nAChRs are up-regulated in sympathetic bladder MPG neurons of BPH rats induced by testosterone alone. For a long time, the cholinergic synapse in rat MPG has been considered to have a very strong synaptic strength partly due to the large amount of transmitters released from the preganglionic terminal varicosities (Warren et al., 1996). However, not all MPG neurons appear to have such a high safety factor. An electrophysiological study has demonstrated that parasympathetic MPG neurons receive relatively weak (subthreshold) synaptic inputs when compared with sympathetic MPG neurons (Kanjhan et al., 2003). Accordingly, up-regulation of nAChRs in the parasympathetic MPG neurons innervating the bladder may enhance ganglionic transmission and discharge of parasympathetic bladder neurons, which may potentially contribute to induction of OAB in PUO rats.

Bladder hyperactivity after BOO is accompanied by an increase in ACh and adenosine triphosphate (ATP) release from bladder epithelium. Increased ACh and ATP activate afferent terminals in the bladder wall to facilitate the micturition reflex (de Groat 2006). In addition, these afferents can be activated by chemical irritants of the

bladder mucosa (Habler et al., 1993). Under a normal condition, Ad bladder afferent fibers respond to both passive distension and active contraction of the bladder (Janig et al., 1986; Torrens and Morrison 1987). However, C-fiber afferent neurons are known to play critical roles in inducing OAB during pathophysiological conditions such as chronic bladder inflammation and spinal cord injury (Choo et al., 2000; de Groat 1993). About 70% of bladder afferent neurons in the rat are the C-fiber neurons based on responsiveness to capsaicin. Under the BOO conditions, most bladder DRG neurons appears to be capsaicin-sensitive. Consistent with the previous studies, capsaicin-sensitive bladder DRG neurons exhibited TTX-sensitive phasic firing in response to prolonged depolarizing current injection. However, the firing pattern became tonic in the bladder DRG neurons of the BPH and PUO rats. Previous studies have also reported the increased excitability of bladder afferent neurons after chronic bladder inflammation (Hayashi et al., 2009; Sculptoreanu et al., 2005; Yoshimura et al., 1999) and spinal cord injury (Yoshimura et al., 1997b) which elicit OAB. BPH and PUO also increased excitability of sympathetic and parasympathetic MPG neurons which show tonic and phasic firing patterns, respectively.

Several mechanisms have been proposed to account for the induction of hyperexcitability in DRG neurons. They include changes in expression of voltage-dependent Na^+ (Black et al., 2004; Moore et al., 2002; Yoshimura et al., 1997b) and A-type K^+ channels encoded by Kv1.4 (Hayashi et al., 2009). In the present study, BOO produced common changes in active properties of the bladder afferent and efferent neurons; decreases in rheobase and AHP duration. Indeed, the decreased threshold for generation of action potentials might be primarily due to up-regulation of low-threshold T-type Ca^{2+} channel expression. T-type Ca^{2+} channels had never been

revealed in autonomic ganglia until described in rat MPG neurons (Zhu et al., 1995). Interestingly, the expression of T-type Ca^{2+} channels is phenotype-specific within MPG, providing a reliable electrophysiological marker for distinguishing the sympathetic one from the parasympathetic population (Zhu et al., 1997; Zhu et al., 1995). The large window currents of T-type Ca^{2+} channels may regulate intracellular Ca^{2+} concentration contributing to activation of Ca^{2+} dependent ion channels or other signaling under resting conditions in MPG neurons. The Ca^{2+} influx via T-type Ca^{2+} channels may also contribute the amplitude and frequency of action potentials. Thus, overexpression of T-type Ca^{2+} channels may underlie the hyperexcitability of bladder DRG and MPG neurons of PUO rats, which may enhance micturition reflex. A previous study using streptozotocin-induced diabetic rats has shown up-regulation of T-type Ca^{2+} channels in DRG neurons which may contribute to the development of pain in diabetic neuropathy (Cao et al., 2011; Jagodic et al., 2007). Thus, it might be interesting to investigate whether up-regulation of T-type Ca^{2+} channel activity in nociceptive C-fiber neurons (i.e., capsaicin-sensitive DRG neurons) is associated with the bladder pain caused by OAB (see Chancellor et al., 1999; Yoshimura et al., 2000).

The sympathetic and parasympathetic MPG neurons have tonic and phasic firing patterns, respectively. In many neurons, Ca^{2+}-activated ion channels play important roles in controlling spike firing by forming prolonged AHP of the membrane potential. In the present study, BOO significantly decreased AHP duration, which may enhance spike firing in both types of MPG neurons. However, the ionic mechanisms underlying the changes in AHP duration might be different in sympathetic and parasympathetic MPG neurons. In a previous study (Won et al., 2006), AHP duration was found to be determined by Ca^{2+}-activated Cl^- (CACC) and K^+ (SK1 and SK2) in sympathetic and

parasympathetic MPG neurons, respectively. This study suggests that Ca^{2+} influx through L- and N-type HVA Ca^{2+} channels during action potential seems to directly activate certain types of SK and/or CACC in the MPG neurons. In the present study, HVA Ca^{2+} channels were not affected in MPG neurons of BOO rats. Thus, it might be interesting to know whether CACC and SK can be regulated in MPG neurons of BOO rats.

NGF levels in the bladder and/or spinal cord as well as in lumbosacral DRG were increased in animals with spinal cord injury or chronic cystitis that exhibited bladder overactivity (Seki et al., 2002; Seki et al., 2004; Vizzard 2000). Some studies have shown that autoimmunization against NGF suppresses bladder overactivity and somal hypertrophy in bladder afferents neurons in animal with BOO (Steers et al., 1996; Steers et al., 1991b). In addition, intrathecal delivery of NGF reduced bladder overactivity and hyperexcitability of bladder afferent neurons (Yoshimura et al., 2006). Indeed, these studies propose that NGF one of key molecules responsible for hyperexcitability of the C-fiber afferent neurons in different pathophysiological conditions such as spinal cord injury, chronic cystitis, and BOO. To date, it is unclear that NGF can regulate excitability of MPG neurons as well as expression of T-type Ca^{2+} channels in both DRG and MPG neurons. In a series of experiments, neuregulin, but not NGF is capable of up-regulating nAChRs in MPG neurons. Thus, it should be tested whether NGF can regulate expression of T-type Ca^{2+} channels and neuregulin increase in MPG neurons and the bladder of BOO rats and regulate nAChRs.

VI. CONCLUSIONS

The major findings were as follow: (i) BPH and PUO-induced BOO caused OAB, (ii) nAChRs were upregulated in bladder MPG neurons from the BOO rats, (iii) BOO enhanced excitability of MPG and DRG neurons innervating the bladder via up-regulation of T-type Ca^{2+} channels as well as reduction in AHP duration implicating altered expression of SK channels. In conclusions, BOO-induced OAB might be associated with enhanced ganglionic transmission and excitability of motor and sensory neurons innervating the bladder in the pathophysiological condition of BOO. To date, antimuscarinic agents have been considered as an primary option for pharmcotherapy of OAB (Erdem 2006). Unfortunately, however, these agents are limited by undesirable antocholinergic adverse effects such as dry mouth, constipation, sedation, impaired cognitive function, tarchycardia, and blurred vision (Erdem 2006). Thus, use of T-type Ca^{2+} channel blockers might be an alternative option of pharmacologic management of OAB.

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ABSTRACT IN KOREAN

과민성방광 쥐 모델에서 자율골반신경절의
기능적 가소성

말초 자율골반신경절 신경세포는 저장 및 배뇨반사의 매개하는 중요한 역할을 한다. 척수손상이나 만성방광염 같은 병태생리학적 상황에서 골반신경절의 가소성 변화는 방광의 기능이 비정상적으로 변한다. 중년과 노년 남성에 영향을 주는 전립선비대는 요도주위를 둘러싸고 있는 전립선의 비정상적 증식에 의해 나타나는데 급뇨, 빈뇨, 야뇨, 잔뇨, 소변속도약화 등의 하부요로증상을 야기한다. 과민성 방광은 빈뇨와 야뇨를 동반한 요절박의 특징을 가지는 증상인데 현재까지 과민성 방광을 일으키는 신경성 기전에 대해서는 거의 알려져 있지 않았다. 본 연구에서는 방광출구폐쇄에 의한 과민성 방광은 방광배뇨근을 지배하는 골반신경절과 후근신경절의 기능의 변화에 의해 나타난다는 가설을 세웠다. 이 가설을 검증하기 위해 부분방광출구폐쇄가 골반신경절 내 신경전달에 관여하는 니코틴성 아세틸콜린 수용체의 발현과 활성을 변화시키는 지 방광을 지배하는 감각신경인 후근신경절 및 골반신경절의 흥분성을 변화시키는 지 확인하였다. 이를 위해 요도부분결찰법으로 부분방광출구폐쇄 쥐 모델을, 테스토스테론과 17-베타 에스트라디올을 피하주사하여 전립선비대 쥐 모델을 각각 만들었다. 두

모델에서 방광 배뇨근의 비후를 확인하였으며 방광계검사에 의해 부분방광출구폐쇄 및 전립선 비대 쥐 모델의 방광 용량, 배뇨를 위한 역치압력, 배뇨량, 배뇨후잔류량, 배뇨간수축빈도 등의 측정에 의해 과민성 방광이 유도되었음을 확인하였다. 실시간 중합효소연쇄반응 분석결과 방광출구폐쇄 쥐의 골반신경절에서 니코틴성 아세틸콜린 수용체 알파3 베타4 서브유닛의 발현이 증가하였다. 다음은 니코틴성 전류를 다이아이로 염색된 방광배뇨근을 지배하는 골반신경세포에서 측정한 결과 부교감신경에서만 선택적으로 증가되는 것이 관찰되었다. 이러한 결과는 방광출구폐쇄의 의한 과민성 방광이 골반신경세포 표현형 선택적인 니코틴성 아세틸콜린 수용체의 발현 증가에 의해 야기된다는 사실을 시사하였다. 패치클램프를 이용한 전류고정 방법으로 대조군과 실험군의 후근신경세포 및 골반신경세포에서 활동전압을 기록하였다. 그 결과 방광출구폐쇄는 두 종류의 신경세포에서 활동전압의 역치와 후관부극 기간을 감소시켜 신경의 흥분성을 항진시킨다는 것을 확인하였다. 과흥분성의 이온메커니즘의 일부를 규명한 결과 방광출구폐쇄는 배뇨근을 지배하는 후근신경 및 교감골반신경 세포에서 티형 칼슘채널의 발현을 증가시키는 것을 확인하였다. 한편, 후과분극 기간의 감소는 후근신경 및 교감골반신경 세포에서 칼슘의존성 클로라이드 또는 포타슘 채널의 발현을 변화시키는 것으로 추측하였다. 이상의 연구결과로 부터 방광출구폐쇄에 의한 과민성방광은 방광을 지배하는 내장성 자율운동신경과 감각신경절에서 시냅스전달과 흥분성 항진과 유관하여 나타난다고 결론을 내렸다. 임상적인 관점에서 티형 칼슘채널 차단제의 사용에 의해 과민성방광의 약리학적 치료가 잠재적으로 가능할 수 있다고 사료된다.

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