Effects of bladder outlet obstruction on properties of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in rat bladder

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Kita M, Yunoki T, Takimoto K, Miyazato M, Kita K, de Groat WC, Kakizaki H, Yoshimura N. Effects of bladder outlet obstruction on properties of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in rat bladder. Am J Physiol Regul Integr Comp Physiol 298: R1310–R1319, 2010. First published March 3, 2010; doi:10.1152/ajpregu.00523.2009.—In this study, we investigated the effects of bladder outlet obstruction (BOO) on the expression and function of large conductance (BK) and small conductance (SK) Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in detrusor smooth muscle. The bladder from adult female Sprague-Dawley rats with 6-wk BOO were used. The mRNA expression of the BK channel α-subunit, β1-, β2-, and β4-subunits and SK1, SK2, and SK3 channels were investigated using real-time RT-PCR. All subunits except for the BK-β2, SK2, and SK3 channels were predominantly expressed in the detrusor smooth muscle rather than in the mucosa. The mRNA expression of the BK channel α-subunit was not significantly changed in obstructed bladders. However, the expression of the BK channel β1-subunit and the SK3 channel was remarkably increased in obstructed bladders. On the other hand, the expression of the BK channel β4-subunit was decreased as the severity of BOO-induced bladder overactivity progressed. In detrusor smooth muscle strips from obstructed bladders, blockade of BK channels by iberiotoxin (IBT) or charybdotoxin (CTX) and blockade of SK channels by apamin increased the amplitude of spontaneous contractions. These blockers also increased the contractility and affinity of these strips for carbachol during cumulative applications. The facilitatory effects elicited by these K\textsuperscript{+} channel blockers were larger in the strips from obstructed bladders compared with control bladders. These results suggest that long-term exposure to BOO for 6 wk enhances the function of both BK and SK types of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in the detrusor smooth muscle to induce an inhibition of bladder contractility, which might be a compensatory mechanism to reduce BOO-induced bladder overactivity.

bladder outlet obstruction; Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels; spontaneous contraction; muscarinic stimulation

BLADDER OUTLET OBSTRUCTION (BOO) causes various structural and functional changes in the lower urinary tract and induces not only obstructive but also detrusor overactivity, as well as irritative symptoms, such as urgency, urge incontinence, urinary frequency, and nocturia. This situation has been simulated using animal models with partial urethral obstruction, and numerous mechanisms including myogenic and neurogenic changes, have been proposed as a source of detrusor overactivity. In the case of BOO, there is evidence that detrusor overactivity is closely related to increased excitability of detrusor smooth muscle (1, 53).

One of the important functions of K\textsuperscript{+} channels is to stabilize the membrane potential and reduce the excitability of nerves and muscle cells (38). The relationship between detrusor overactivity and K\textsuperscript{+} channels has been reported (18, 31, 33, 45, 46), and several kinds of modulators of these K\textsuperscript{+} channels have been developed in an attempt to manage detrusor overactivity (10, 27, 34). Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels are one of the major groups of six/seven transmembrane potassium-selective channels. These channels are divided into several groups according to the genetic, electrical, and structural properties of each channel (50). Large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (BK) channels, which are activated by membrane potential depolarization and by increases in cytosolic Ca\textsuperscript{2+} concentration (50), mainly mediate repolarization phases of detrusor smooth muscle cells (13, 15, 46). BK channel knockout mice show reduced bladder capacity, increased amplitude of spontaneous contractions, and increased responses to electrical and carbachol-induced stimulation (31, 46). The BK channels consist of pore-forming α-subunits associated with accessory β-subunits, which contribute to the functional diversity of the channels (5, 11, 12, 29, 30, 33, 51). Four different β-subunits with tissue-specific distribution have been cloned (5, 21, 30, 49). The smooth muscle-specific β1-subunit was first identified in bovine tracheal and aortic smooth muscle (21). The detrusor smooth muscle from β1-subunit knockout mice shows altered phasic contractions (33). A recent study has also demonstrated the presence of β4-subunits in the rat detrusor smooth muscle both at mRNA and protein levels (7), although β4-subunits were thought to be expressed mainly in neurons (11, 30, 51). Small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (SK) channels, which are Ca\textsuperscript{2+} sensitive, but not voltage sensitive, are also important determinants of detrusor smooth muscle excitability and contractility. They are divided into three subtypes (SK1, SK2, and SK3). Genetic modification of these channels in mice has shown critical roles of these channels in the control of detrusor smooth muscle activity (18, 45).

In this study, we examined how BOO affects the expression of BK and SK channels and investigated the impact of altered expressions of these channels on the contractile properties of detrusor smooth muscle in rats.

MATERIALS AND METHODS

Animals. A total of 63 adult female Sprague-Dawley rats, weighing 200–250 g, were used. All experimental procedures were in accor-
Partial urethral obstruction. Partial urethral obstruction was produced as previously described (8, 20). The bladder and proximal urethra were exposed via a lower abdominal midline incision under isoflurane anesthesia. A 4–0 silk ligature was placed around the proximal urethra, and the abdomen was closed with sutures. A PE-50 transducer (World Precision Instruments, Sarasota, FL) and a syringe pump for recording intravesical bladder pressure and infusing saline into the bladder, respectively. Intravesical pressure changes were recorded by the PowerLab system (AD Instruments, Castle Hill, NSE, Australia), and data were analyzed using data acquisition software (AD Instruments). Saline at room temperature was infused into the bladder at 2.4 ml/h in control rats. In BOO rats, because of the large bladder capacity, the infusion rate of saline was adjusted to 3–15 ml/h to obtain an intercontraction interval of 10–20 min. Saline was infused until rhythmic bladder contractions became stable. Parameters listed in Table 1 were determined from cystometrograms as previously described (20). Nonvoiding contractions (NVCs) were defined as bladder contractions without voiding prior to micturition, the amplitudes of which were more than 4 cmH2O.

RNA extraction and cDNA synthesis. After cystometric evaluation, rats were killed by carbon dioxide inhalation. Immediately after death, the whole bladder above the ureteral orifices was dissected out and weighed. Some bladders (n = 7 in the control group; n = 13 in the BOO group) were cut into small pieces containing all layers of the bladder wall, whereas others (n = 5 in each group) were placed in Krebs-Henseleit solution aerated with 95% oxygen and 5% carbon dioxide followed by separation of the bladder into the mucosa and muscle layers under a dissecting microscope, which were then cut into small pieces. These specimens were frozen in liquid nitrogen immediately after preparation and stored at −80°C until further processing. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), according to manufacturer’s instructions. All samples were treated with DNase (Promega, Madison, WI) to prevent the contamination of genomic DNA followed by cleaning up with an RNeasy mini kit (Qiagen, Valencia, CA) or TRIzol reagent. RNA was quantified by a spectrophotometer (Biochrom, Cambridge, UK). Five micrograms total RNA from whole bladder tissues and 1 μg from separated bladder tissues were reverse-transcribed using Thermoscript with oligo (dt) primers (Invitrogen) following manufacturer’s instructions.

Real-time RT-PCR. Real-time PCR was performed in 96-well reaction plates using the MX3000P (Stratagene, La Jolla, CA). The reaction mixture contained 1 μl diluted cDNA, 12.5 μl 2 × SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) or PrimerQuest free software (Integrated DNA Technologies, Coralville, IA) or PrimerQuest free software (Integrated DNA Technologies, Coralville, IA) or PrimerQuest free software (Integrated DNA Technologies, Coralville, IA) or PrimerQuest free software (Integrated DNA Technologies, Coralville, IA).

Table 1. Effects of BOO on characteristics of the bladder and cystometrical parameters

<table>
<thead>
<tr>
<th>Control</th>
<th>m-BOO</th>
<th>s-BOO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>BW, mg</td>
<td>79.4 (3.62)</td>
<td>281.5 (31.5)</td>
</tr>
<tr>
<td>MVP, cmH2O</td>
<td>24.9 (1.28)</td>
<td>112.2 (9.45)</td>
</tr>
<tr>
<td>TP, cmH2O</td>
<td>6.05 (0.65)</td>
<td>6.54 (1.13)</td>
</tr>
<tr>
<td>BP, cmH2O</td>
<td>1.31 (0.72)</td>
<td>2.79 (0.38)</td>
</tr>
<tr>
<td>BC, ml</td>
<td>0.83 (0.09)</td>
<td>1.79 (0.31)</td>
</tr>
<tr>
<td>VE, %</td>
<td>89.0 (5.39)</td>
<td>70.1 (6.79)</td>
</tr>
</tbody>
</table>

m-BOO, mild bladder outlet obstruction; s-BOO, severe bladder outlet obstruction; BW, bladder weight; MVP, maximal voiding pressure; TP, threshold pressure; BP, baseline pressure; BC, bladder capacity; VE, voiding efficacy. Values are expressed as means ± SE. *P < 0.01, † P < 0.001 vs. control group. ‡ P < 0.05 versus m-BOO group.

Table 2. Characteristics of primers

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Primer Sequence</th>
<th>Accession Number</th>
<th>Amplicon Sizes</th>
<th>Annealing Tm, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium large-conductance calcium-activated channel, subfamily M, α member 1 (BK-α)</td>
<td>Kcnm1</td>
<td>AGGGCTGTGGATGGAGGGGGTAC</td>
<td>NM_013828</td>
<td>250</td>
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<tr>
<td>Potassium large-conductance calcium-activated channel, subfamily M, β member 1 (BK-β1)</td>
<td>Kcnmb1</td>
<td>AGGCGATGGGAGGGGGTTAAT</td>
<td>NM_019273</td>
<td>236</td>
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<tr>
<td>Potassium large-conductance calcium-activated channel, subfamily M, β member 2 (BK-β2)</td>
<td>Kcnmb2</td>
<td>TGGGCTGTGTTTATAGACACAA</td>
<td>NM_176861</td>
<td>231</td>
</tr>
<tr>
<td>Potassium large-conductance calcium-activated channel, subfamily M, β member 4 (BK-β4)</td>
<td>Kcnmb4</td>
<td>TGGGCTGTGTTTATAGACACAA</td>
<td>NM_023960</td>
<td>107</td>
</tr>
<tr>
<td>Potassium intermediate/small-conductance calcium-activated channel, subfamily N, member 1 (SK1)</td>
<td>Kcn1</td>
<td>ACGGCGGCAGGGGATGAGT</td>
<td>NM_019313</td>
<td>213</td>
</tr>
<tr>
<td>Potassium intermediate/small-conductance calcium-activated channel, subfamily N, member 2 (SK2)</td>
<td>Kcn2</td>
<td>TGGGCTGTGTTTATAGACACAA</td>
<td>NM_019314</td>
<td>240</td>
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<tr>
<td>Potassium intermediate/small-conductance calcium-activated channel, subfamily N, member 3 (SK3)</td>
<td>Kcn3</td>
<td>TGGGCTGTGTTTATAGACACAA</td>
<td>NM_019315</td>
<td>228</td>
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<tr>
<td>Ribosomal protein L13a (Rp131a)</td>
<td>Rpl13a</td>
<td>ACGGGCGGCAGGGGATGAGT</td>
<td>NM_173340</td>
<td>172</td>
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<tr>
<td>Hypoxanthine phosphoribosyltransferase 1 (HPRT)</td>
<td>Hp1</td>
<td>TGGGCTGTGTTTATAGACACAA</td>
<td>NM_012583</td>
<td>105</td>
</tr>
</tbody>
</table>

*a Abbreviation used in this article (abb.). b Sequences of forward (F) and reverse (R) primers. c Accession number of GenBank (http://www.ncbi.nlm.nih.gov/). d Amplicon length in base pairs (bp). e Annealing temperature (Tm). f Sequences referenced to Bonefeld et al. (2). g Sequences referenced to van Wijngaarden et al. (47).
DNA Technologies, Coralville, IA), except where referenced otherwise. These primers were tested in silico for specificity against sequences for Rattus norvegicus using BLAST software (National Center for Biotechnology Information, Bethesda, MD) and synthesized by Integrated DNA Technologies. The cycle conditions comprised 15-min polymerase activation and 40 cycles with denaturation at 94°C for 15 s, annealing at 55°C or 58°C for 30 s and extension at 72°C for 30 s followed by dissociation from 58°C to 95°C. Five-fold dilution series of cDNA were used to establish standard curves.

**Tissue preparations for isolated detrusor smooth muscle studies.** The bladders from separate groups of control and BOO rats were removed immediately after death with carbon dioxide inhalation and placed in the aerated Krebs-Henseleit solution. One × four millimeter detrusor muscle strips, in which the mucosa was removed, were cut out longitudinally from the bladder body above the ureteral orifices under a dissecting microscope. Each strip was mounted in an 8-ml organ bath containing aerated 37°C Krebs-Henseleit solution with one end fixed to a stationary rod and the other to a transducer. Tension equipment as cystometry. The initial load was set to 10 mN. After a 60–90 min equilibration period accompanied by periodical changes of KCl, test drugs were applied. All procedures were conducted in the presence of 1 μM tetrodotoxin to reduce the influence of nerve activity. At the end of the experiment, wet weight was recorded for each strip.

**Evaluation of spontaneous contractions in bladder strips.** To stabilize spontaneous contractions, the concentration of K+ in the Krebs-Henseleit solution was increased to 20 mM by equimolar substitution of NaCl with KCl as previously described (10, 43). After the equilibration period, 10, 30, and 100 nM iberiotoxin (IbTx) and charybdo-toxin (CTx), which are blockers of BK channels, were applied cumulatively at 20-min intervals. Amplitudes and frequency of spontaneous contractions during the last 5 min of each application period were measured.

**Evaluation of carbachol-induced contractions.** After the equilibration period, carbachol was applied in increasing cumulative concentrations of 10–8, 3 × 10–8, 10–7, 3 × 10–7, 10–6, 3 × 10–6, 10–5, 3 × 10–5, 10–4, and 3 × 10–4 M. When contraction force reached the maximum at one concentration, the next concentration was added. After conducting the first concentration-response study, carbachol was washed out by changing the Krebs-Henseleit solution of the organ bath 5 times at 10-min intervals. Following the washout period, 100 nM of each K+ channel blocker was added to the bath, and after a 20-min incubation time, the second carbachol concentration-response study was conducted.

**Drugs.** Krebs-Henseleit solution contained: 143 mM Na+, 5.9 mM K+, 2.5 mM Ca2+, 1.2 mM Mg2+, 127.7 mM Cl−, 1.2 mM SO42−, 1.2 mM PO43−, 25 mM HCO3−, and 11 mM glucose. Tetrodotoxin and carbachol were purchased from Sigma-Aldrich (St. Louis, MO). IbTx, CTx, and apamin were purchased from Alomone (Jerusalem, Israel). All drugs were reconstituted in distilled water as concentrated stock solutions and diluted with Krebs-Henseleit solution immediately before use.

**Data analysis.** To evaluate the expression level of K+ channel mRNA, we used ribosomal protein L13a (Rpl13a) and hypoxanthine guanine phosphoribosyl-transferase-1 mRNA as internal controls, which were not significantly different in control and BOO groups, according to an algorithm proposed previously (48). This was necessary because some studies have reported increased expression of GAPDH and β-actin in the hypertrophied bladder (3, 28). Also in our preliminary study, the mRNA levels of GAPDH were markedly increased in mucosa-intact obstructed bladders and in mucosa-reMOVED smooth muscle layers of obstructed bladders (n = 7 in both...

Fig. 1. Relative mRNA expression levels of large-conductance (BK) channel α-subunit (BK-α) (A), BK channel β1-subunit (BK-β1) (B), BK channel β2-subunit (BK-β2) (C), BK channel β4-subunit (BK-β4) (D), small-conductance (SK)1 channel (SK1) (E), SK2 channel (SK2) (F), and SK3 channel (SK3) (G) in mucosa-intact whole bladders from control (n = 7), mild bladder outlet obstruction (m-BOO) (n = 6), and severe bladder outlet obstruction (s-BOO) (n = 7) groups. Each value is expressed as a percentage of the expression level of the control group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control group. §P < 0.05 vs. m-BOO group.
groups, \( P < 0.001; n = 5 \) in both groups, \( P < 0.01 \), respectively). The expression of \( \beta \)-actin was also significantly increased in mucosa-removed smooth muscles of obstructed bladders \( (n = 5 \) in both groups, \( P < 0.01 \) (data not shown).

In the mucosa-removed muscle strip study, responses to carbachol are expressed as a percentage of the maximum force (Emax) induced by the first application of carbachol. \( \Delta \)Emax was obtained by subtracting the Emax value in the first carbachol application from that in the second application. Cumulative concentration-response curves were analyzed by nonlinear regression in each individual experiment using GraphPad Prism 4.03 (GraphPad Software, San Diego, CA). EC\(_{50}\) values were calculated for concentration-response curves before and after a test drug application, and are presented as \(-\log EC_{50}\). \( \Delta -\log EC_{50}\) was then obtained by subtracting the \(-\log EC_{50}\) value of the first carbachol application from that of the second carbachol application.

All data are expressed as means \( \pm SE \). Student’s \( t \)-test or one-way ANOVA followed by Bonferroni’s multiple-comparison test was used to determine significance among two groups or more than two groups, respectively. For all statistical tests, \( P < 0.05 \) was considered significant. In the isolated muscle strip study, \( n \) represents the number of strips and \( N \) represents the number of animals.

**RESULT**

**Cystometry.** Six weeks after urethral ligation, bladder weight was significantly increased in BOO rats \( (79.4 \pm 3.62 \text{ mg in control rats vs. } 559.8 \pm 104.6 \text{ mg in BOO rats}) \). However, the degree of hypertrophy and an occurrence of NVCs were variable among animals. Therefore, to investigate the influence of severity of altered properties induced by BOO on K\(^+\) channel function, BOO rats were divided into mildly obstructed \((m\text{-BOO})\) and severely obstructed \((s\text{-BOO})\) groups, based on the absence and presence of NVCs greater than 4 cmH\(_2\)O during cystometry, respectively. Bladder weight and bladder capacity in the s-BOO group were more than 10 times larger than those in the control group (Table 1). Significantly increased maximum voiding pressure was noted in both BOO groups although the value in the s-BOO group was lower compared with the m-BOO group. Voiding efficacy was also significantly lower in the s-BOO group than in the m-BOO group. These results indicate that s-BOO bladders exhibit more pronounced detrusor overactivity \((i.e., \text{NVCs})\) along with decompensated contractile properties during voiding compared with m-BOO rats.

**mRNA expressions.** In our preliminary experiments, mRNA expression of \( \beta_1\)-, \( \beta_2\)-, and \( \beta_4\)-subunits, but not \( \beta_3\)-subunits, of the BK channel was detected in the bladder tissue as previously described \((35)\). Therefore, we investigated the BK channel \( \alpha\)-subunit, \( \beta_1\)-, \( \beta_2\)-, and \( \beta_4\)-subunits and all 3 types of SK channels in this study. To evaluate the influence of BOO on the expression of each channel transcript, we compared the mRNA expression in control, m-BOO and s-BOO groups. There were no significant differences of the BK channel \( \alpha\)-subunit transcripts expression among groups, although the expression in the m-BOO group tended to be higher compared with other groups \((Fig. 1A)\). Similar patterns were seen in the expression of the SK1 and SK2 channel transcripts \((Fig. 1, E \text{ and } F)\). However, BOO markedly increased the expression of
the BK channel β1-subunit and the SK3 channel transcripts regardless of the severity of BOO (Fig. 1, B and G). On the other hand, the expression of the β4-subunit transcripts was significantly decreased in parallel with the severity of BOO (Fig. 1D).

To examine mRNA expression in different layers of the bladder, RT-PCR was performed on the mucosa and smooth muscle layers of bladder tissues from control and BOO rats, which showed increased bladder weight (659.2 ± 99.0 mg) and NVCs during cystometry similar to s-BOO rats. Muscle and mucosa exhibited marked differences in expression of the different genes; BK-β1, SK2 and SK3 transcripts, as well as the β1 and β4 subunit transcripts, were more prominently expressed in muscle. Among these channels and subunit transcripts in the muscle layer, changes in expression similar to the whole bladder experiment were observed after BOO except the SK2 channel, which was significantly increased compared with controls (Fig. 2).

Spontaneous activity in muscle strips. To investigate whether functional changes are elicited by the altered expression of BK channel subunits and SK channel transcripts following BOO, we examined responses of detrusor smooth muscle strips, in which the mucosa was removed, to BK channel blocking agents, IbTx and CTx, and a SK channel blocker, apamin.

One series of experiments investigated the influence of blockers on spontaneous bladder contractions. Before application of K+ blocking agents, the amplitude of spontaneous contractions was significantly higher in the control group compared with the BOO group when values were corrected with wet weight (wt wt) of muscle strips (0.20 ± 0.012 vs. 0.12 ± 0.011 mN/mg wt wt, P < 0.001). The frequency of spontaneous contractions was also greater in the control group than in the BOO group. (0.154 ± 0.006, n = 35 vs. 0.040 ± 0.002 Hz, n = 40, P < 0.001). All blocking agents increased the amplitude and frequency of spontaneous contractions. Figure 3A shows representative responses to a range of CTx concentrations in control and BOO groups. CTx application at 100 nM elicited larger changes in amplitude of spontaneous contractions in the BOO group compared with the control group (235.3 ± 20.8% vs. 146.9 ± 4.3% of predrug values, P < 0.001, Figs. 3A and 4B). IbTx application at 100 nM also showed significantly larger responses in the BOO group compared with the control group (191.2 ± 9.6% vs. 155.0 ± 6.0% of predrug values, P < 0.01, Fig. 4A). Apamin also increased the amplitude of spontaneous activity, and this increase was greater in the BOO group compared with the control group at concentrations of 10 and 100 nM (309.5 ± 39.2% vs. 135.5 ± 9.0% at 10 nM, P < 0.001, 335.7 ± 44.8% vs. 136.8 ± 10.0% at 100 nM, P < 0.001, Fig. 4C). Meanwhile, although the frequency of spontaneous contractions also tended to be increased by all blockers, the magnitude of this effect was not significantly different between control and BOO rats.

Carbachol evoked contractions. The influence of K+ blocking agents on the contractions evoked by carbachol, a cholinergic agonist, were also examined in control and BOO strips (Fig. 3B and Fig. 5). To quantify the effect of multiple applications of carbachol on muscle strips’ contractility, we inves-
igated carbachol-evoked contractions of time-matched controls (TMC) in the absence of blocking agent.

Before the blocker application, maximum force (Emax) values corrected for wet weight of strips were larger in the control group than in the BOO group (16.4 ± 0.71 mN/mg wet wt, n = 40 vs. 11.6 ± 0.83 mN/mg wet wt, n = 37, P < 0.001). The EC50 values in the control group are smaller than in the BOO group (309.0 ± 20.2 nM vs. 526.0 ± 46.28 nM, P < 0.001). These results indicate decreased contractility and affinity to carbachol of the BOO smooth muscle.

Emax values at the second carbachol application were reduced by 3.29 ± 1.36% without blocker application in the control group (Figs. 5A and 6A). IibtX, CTx, and apamin treatments tended to prevent this spontaneous decline in Emax, but differences in ΔEmax with and without blocker treatments were not statistically significant. On the other hand, significant but differences in treatments tended to prevent this spontaneous decline in Emax, and 6A control group (Figs. 5A and 6A). The apamin treatment showed a larger but insignificant increase in ΔEmax (12.36 ± 3.42%, P < 0.05 and 12.26 ± 2.01%, P < 0.05, respectively) (Figs. 5, F and G and 6A), compared with the changes (1.89 ± 0.79%) in the TMC of BOO rats (Figs. 5E and 6A). The apamin treatment showed a larger but insignificant increase of ΔEmax (5.22 ± 1.61%, Figs. 5H and 6A) compared with the TMC.

The potassium channel blockers also influenced the EC50 values. The ΔlogEC50 values in the TMC of control and BOO groups were decreased to an equal degree (i.e., decreased affinity) at second applications of carbachol without blockers, while the ΔlogEC50 tended to increase after blocker application (i.e., increased affinity). In both groups, the Δ-logEC50 obtained after IibtX or CTx treatment was larger than that seen in the TMC (Fig. 6B). Furthermore, the CTx treatment showed a greater increase in the Δ-log EC50 than in the IibtX treatment in the BOO group. However, changes in the Δ-log EC50 after the IibtX or CTx treatment were larger in the BOO group than in the control group. On the other hand, there is no significant difference in the Δ-logEC50 obtained after apamin treatment between control and BOO groups, although the apamin treatment showed a significant increase compared with the TMC in the BOO model.

**DISCUSSION**

The present study describes the impact of BOO on function and expression of Ca2+-activated K+ channels. BOO for 6 wk greatly influenced the mRNA expression of SK2 and SK3 channels, as well as β1 and β4 modulatory subunits of the BK channel. Functional studies using detrusor smooth muscle strips also revealed enhanced effects of BK and SK channel blockers in the detrusor smooth muscle of the obstructed bladder, suggesting an up-regulated inhibitory function of BK and SK channels after BOO.

In this study, we found that mRNA expression of BK modulatory β1 and β4 subunits were increased and decreased, respectively, in the detrusor, while there was no change in the BK α-subunit expression 6 wk after BOO. The BK channel β1-subunit, which is mainly expressed in the smooth muscle dramatically increases Ca2+ and voltage sensitivity of the BK α-subunit, resulting in facilitation of the BK channel function (5, 29, 33). On the other hand, the β4-subunit, which is known to be expressed mainly in neurons, but was proven to be present also in bladder smooth muscle (7), decreases the voltage sensitivity and slows activation kinetics, thereby being called a “down regulator” of the BK channel (5, 51). Therefore, it is assumed that increased and decreased mRNA expression...
of the BK β1- and β4-subunits, respectively, contributes to the facilitated inhibitory function of BK channels in the detrusor smooth muscle of BOO rats, even though the BK channel α-subunit expression was not changed.

We also detected changes in expression of SK2 and SK3 channel transcripts in the BOO bladder. However, there was discordance in the expression level of the SK2 channel transcripts between mucosa-attached and mucosa-removed tissues (Figs. 1F and 2F). Because the mucosal expression of SK2 channel mRNA was minimal (Fig. 2F), the discrepancy is likely to be related to the detrusor muscle expression. One possible explanation for this discrepancy might be the difference in severity of bladder hypertrophy induced by BOO because the bladder weight of BOO bladders used for mucosa-removed preparations was lower (660 vs. 790 mg) than the weight of s-BOO bladders used for whole bladder experiments although both groups of BOO rats showed NVCs during cystometry. Thus, it is possible that the expression of SK2 channel transcripts in the detrusor is sensitive to the bladder condition after BOO, although mRNA and protein expression of SK2 channels might not correlate with each other after BOO. Nevertheless, the functional study using a SK channel blocker, apamin, showed overall SK channel activity is enhanced in the detrusor smooth muscle of obstructed bladders.

In this study, we did not evaluate protein expression of BK and SK channel and subunits due to the lack of high-quality antibodies against Ca2+-activated K+ channels, especially β-subunits. Therefore, we instead performed muscle strip ex-
periments to examine whether changes in BK and SK channel transcripts are correlated with functional changes in muscle activity. Smooth muscle strips are known to develop spontaneous contractions. These contractions are initiated by action potentials and associated Ca$^{2+}$ transients through L-type Ca$^{2+}$ channels (13, 14). This Ca$^{2+}$ entry through L-type Ca$^{2+}$ channels can activate both BK and SK channels (17), which regulates the repolarization phase and after hyperpolarization phase of the action potential, respectively (15, 17, 46). Thus BK and SK channel blockers can increase the amplitude of spontaneous bladder contractions (17, 44). In the present study, this effect of IbTx, CTx, and apamin was enhanced in BOO detrusor muscle strips, indicating that both BK and SK channel activity is enhanced following BOO and suppresses spontaneous contractions induced by Ca$^{2+}$ influx through the L-type Ca$^{2+}$ channel.

The present study also showed that BOO enhanced the modulatory influence of BK and SK channel on pharmacomechanical coupling in the detrusor smooth muscle as evidenced by increased effects of BK and SK channel blockers on muscle contraction induced by the cholinergic receptor agonist, carbacol. Although the mammalian bladder expresses M2 and M3 muscarinic receptors among five subtypes (M1-M5), physiological bladder contractions largely depend on the M3 receptor subtype activation, which induces an increase of the intracellular Ca$^{2+}$ concentration essential for initiating the smooth muscle contraction, as well as Ca$^{2+}$ sensitization due to activation of Rho kinase pathways (4, 32). The proposed cellular mechanisms for muscarinic M3 receptor-mediated increase in intracellular Ca$^{2+}$ concentrations involve not only Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels but also Ca$^{2+}$ release from intracellular stores, which elicits local increase of Ca$^{2+}$ concentrations in the cytosol (36, 39, 40, 52).

The increased intracellular Ca$^{2+}$ concentration following muscarinic receptor activation also activates Ca$^{2+}$-activated K$^{+}$ channels. In this study, BK channel blockers increased the Emax and $\Delta$–log EC50 in response to carbacol stimulation as reported previously (16), and the upregulated inhibitory function of BK channels after BOO is indicated by the larger changes of Emax and $\Delta$–log EC50 by BK blockers in obstructed bladders vs. normal bladders. The current study also revealed that the contractile response to carbacol was reduced in the BOO group, as shown by decreased Emax and increased EC50 values compared with the control group. Because BK blockers partially restored Emax and EC50 of carbacol responses in BOO bladders, it seems that enhanced BK channel activity contributes at least, in part, to the reduction in muscarinic receptor-mediated contractility after BOO. However, the effect of apamin on muscarinic receptor-mediated contractility was much smaller compared with the effect of BK channel blockers. This smaller contribution of SK channels to carbacol-induced detrusor contraction might be explained by the previous findings that Ca$^{2+}$ sparks derived from intracellular Ca$^{2+}$ stores have smaller effects on SK channel activation compared with BK channels (17).

In contrast to the results of our study, a previous study using 6-wk BOO rats with NVCs reported decreased expression of the BK channel α-subunit and SK channel transcripts in obstructed bladders by using semiquantitative RT-PCR techniques with β-actin as an internal control (24). Although the reason for this discrepancy is unclear, experimental models of BOO in rats are known to yield a range of morphological and functional changes, even if the same surgical procedures are used (41). In our study, we also found a discrepancy in the expression of SK2 channel transcripts in different groups of BOO rats used for whole bladder or detrusor preparations. Therefore, different conditions of experimental models could be one of the reasons of this discrepancy. Another reason that should be considered is the difference of internal controls used to evaluate and standardize the results of the PCR investigation. As the real-time PCR has proven valuable for accurate expression profiling of selected genes, stability of commonly used housekeeping genes adopted from literature as reference genes has been argued (2, 19, 47, 48). In the case of the hypertrophied bladder, several studies reported increased expression of commonly used housekeeping genes such as β-actin and GAPDH at mRNA or protein levels (3, 28). Therefore, in this study, we carefully assessed the variability of several candidates of housekeeping genes and used two selected genes, which showed no significant differences of mRNA expression between control and BOO groups, as internal controls according to an algorithm proposed previously (48).

In animal models of BOO, NVCs are often seen in cystometry, and these observations are regarded as evidence of bladder instability, which is thought to contribute to irritative symptoms (26). Therefore, it is reasonable to speculate that the excitability of detrusor smooth muscle is closely related to bladder instability. However, in the present study, the amplitude and frequency of spontaneous contractions of muscle strips from obstructed rats were smaller than those from control rats. At the cellular level, an increase in excitability of detrusor smooth muscle cell membranes from the obstructed bladder has also been difficult to demonstrate consistently. In terms of spontaneous activity of detrusor muscle, several studies using
microelectrode recording in intact bladder tissues reported reduced or unchanged smooth muscle cell excitability in the obstructed bladder (22, 42). On the other hand, increased spontaneous electrical activity was detected using patch-clamp recording techniques in isolated smooth muscle cells from the obstructed bladder (23). Thus, there is the possibility that bladder instability induced by BOO might be related to other factors than increased detrusor smooth muscle excitability. In this regard, recent studies have demonstrated enhanced cell-to-cell interactions of smooth muscle as evidenced by increased expression of gap-junction proteins such as connexin 43 or the increased number of interstitial cells in obstructed bladders (8, 22, 53). Therefore, the enhanced activity of BK and SK channels shown in this study could be a compensatory mechanism to suppress bladder instability induced by BOO. Similar compensatory reactions have been reported in vascular smooth muscle of spontaneous hypertensive rats (6, 9, 25, 37).

These findings support our conclusion that Ca\(^{2+}\)-activated K\(^+\) channels act in a direction to suppress the excitability of bladder smooth muscle cells in response to either spontaneous or muscarinic stimulation in the BOO condition. Moreover, BOO rats with NVCs (s-BOO group) had reduced voiding efficiency and micturition pressure compared with those without (m-BOO group) in this study. Thus, although inhibition of spontaneous activity might contribute to suppression of BOO-induced bladder instability during the storage phase, a reduction due to BK channel upregulation in muscarinic receptor-mediated contractility, which is directly related to voiding bladder contractions, could be involved in the emergence of voiding dysfunction including decreased voiding efficiency and increased residual volume in BOO.

**Perspectives and Significance**

Long-term BOO for 6 wk induces changes in the subtype expression of BK and SK channel transcripts of Ca\(^{2+}\)-activated K\(^+\) channels, which contribute to the enhanced function of these channels in the detrusor smooth muscle. The BOO-induced changes in Ca\(^{2+}\)-activated K\(^+\) channel activity might be a compensatory mechanism to reduce bladder overactivity following BOO.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

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