5-Hydroxytryptamine-induced bladder hyperactivity via the 5-HT_{2A} receptor in partial bladder outlet obstruction in rats

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Sakai T, Kasahara K, Tomita K, Ikegaki I, Kuriyama H. 5-Hydroxytryptamine-induced bladder hyperactivity via the 5-HT_{2A} receptor in partial bladder outlet obstruction in rats. Am J Physiol Renal Physiol 304: F1020–F1027, 2013. First published January 23, 2013; doi:10.1152/ajprenal.00365.2012.—We investigated the effects of partial bladder outlet obstruction (BOO) on the function and gene expression of 5-hydroxytryptamine (5-HT) receptor subtypes in rat bladder. Isometric contractions of the isolated bladders from sham-operated control and BOO rats were examined. The contractile responses to 5-HT were significantly increased in BOO rat bladder strips, while the responses to KCl, carbachol, or phenylephrine were not different from the control. The 5-HT-induced hypercontraction in BOO rat bladder strips was inhibited by ketanserin, a 5-HT_{2A} receptor antagonist. The contractile responses to 5-HT in bladder strips were not affected by urothelium removal from the intact bladder. The gene expression of 5-HT receptor subtypes in the bladders was analyzed by RT-PCR. The mRNA expression of the 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{4A}, and 5-HT_{7} receptors was detected in both the control and BOO rat bladders. Quantitative RT-PCR analysis showed there was a significant increase of 5-HT_{2A} receptor mRNA in the BOO rat bladder compared with the control bladder. On the other hand, the gene expression of the 5-HT_{4} receptor was not changed in the BOO rat bladder. These results suggest that the increased contractile responses to 5-HT in BOO rat bladder may be partly caused by 5-HT_{2A} receptor upregulation in the detrusor smooth muscles.

In both humans and animals, 5-hydroxytryptamine (5-HT) receptor subtypes have been identified in the lower urinary tract. Investigations have found that 5-HT shows excitatory effects in both unstimulated and electrically stimulated detrusor strips in both humans and animals (15, 23, 24). The contractile responses to 5-HT in detrusor strips are thought to be mediated via both direct and/or indirect effects on the detrusor smooth muscle cells. While 5-HT receptor antagonists can inhibit 5-HT-induced contraction of the detrusor strips, neither α- or β-adrenergic receptor blockers nor cholinergic receptor antagonists have any effect (23). In addition, increased contractile responses to 5-HT in aged rat bladders, along with increased 5-HT_{1A} receptor agonist-induced bladder contraction, have also been reported in BOO rats (30, 38). Furthermore, an upregulation of 5-HT binding sites in the detrusor has also been reported in the rabbit model of BOO (21). While these studies have documented the possibility that 5-HT might have some role on detrusor overactivity, the specific 5-HT receptor family subtypes that are related to the action of 5-HT have yet to be elucidated.

The present study was designed to both examine how BOO affects the expression of 5-HT receptor subtypes and investigate the impact of altered expressions of 5-HT receptors on the contractile properties of bladder in rats.

MATERIALS AND METHODS

Animals. All animal experiments were approved by the Committee on Ethics in Animal Experiments of Asahi Kasei Pharma and followed the National Institutes of Health guidelines. Female Sprague-Dawley rats were obtained from Charles River (Tokyo, Japan). Rats were housed in an air-conditioned room, fed a standard laboratory diet (CRF-1; Oriental Yeast, Tokyo, Japan), and given water ad libitum. Studies were conducted from 8 to 12 wk of age in animals weighing 200–250 g.

Partial bladder outlet obstruction. Each rat was anesthetized with pentobarbital sodium (30–40 mg/kg ip). A 22-G Surflo intravenous catheter (Terumo, Tokyo, Japan) was inserted into the urethra, with the bladder neck urethra then exposed via a lower abdominal incision. A 4–0 silk ligature was placed around the proximal urethra, and a 1.1-mm diameter steel rod that was placed adjacent to the urethra. After the ligature was tightly tied, the steel rod was carefully removed. The abdominal wound was closed, and ampicillin (50 mg/kg·day^{-1}) was administered to all animals via the drinking water to control any postoperative infections. Sham operations were performed using the same procedure with the exception of the placement of the ligation around the urethra. In a preliminary study, we confirmed that bladders from the sham group were the same as those from the non-operated group, in terms of bladder weight, bladder pathology, and response to KCl and carbachol, as well as to 5-HT.

Tissue preparation for the pharmacological studies in the BOO rats. One week after the partial BOO procedure, all animals were anesthetized using pentobarbital sodium and then euthanized via exsanguination. Bladders from the control and BOO rats were isolated immediately after death and placed in ice-cold Krebs-Henseleit solu-

BENIGN PROSTATIC HYPERPLASIA (BPH) afflicts patients with bothersome lower urinary tract symptoms that involve voiding and storage symptoms, such as incomplete bladder emptying, weak urine stream, urgency, urge incontinence, urinary frequency, and/or nocturia (28). It is clear that storage symptoms in men with BPH are related to the static and dynamic prostatic enlargement; detrusor; overactive bladder; LUTS; benign prostatic hyperplasia; obstruction or pharmacological treatment with 5-HT blockers to relax prostatic and urethral smooth muscles still have some role on detrusor overactivity, the specific 5-HT receptor family subtypes that are related to the action of 5-HT have yet to be elucidated.

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tion. Under the dissecting microscope, four longitudinal bladder muscle strips that were approximately the same size (6 × 2 mm) were prepared from a bladder body, after which the strips were mounted in a 10-ml organ bath containing 37°C Krebs-Henseleit solution aerated with a 95% O2-5% CO2 gas mixture. Strip tension was measured isometrically using a TB-612T force displacement transducer (Nihon Koden, Tokyo, Japan). An initial tension of 9.8 mN was applied to the tissue, with the tissues being allowed to equilibrate for at least 90 min before any experimental procedure was begun. After the equilibration period, contractile responses to 40 mM KCl were challenged three times in each strip, with 30- to 40-min intervals between the individual challenges. Strips exhibiting three repeated equivalent contractions (97% of the strips) were used in the subsequent experiments. After confirmation of the responses to KCl, stimulants, such as 5-HT, carbachol, or phenylephrine, were applied in increasing cumulative concentrations. The 5-HT receptors antagonists were preincubated for 10 min before the application of 5-HT to evaluate their antagonistic activities. At the end of the experiment, the wet weight of each strip was measured. The wet weights of bladder strips from sham and BOO rats were 7.1 ± 0.3 and 10.3 ± 0.3 mg, respectively.

Bladder strip contraction in intact and urothelium-denuded bladder. The bladders from non-operated normal female Sprague-Dawley rats were removed immediately after death as per the procedure previously described for the BOO rats. After each bladder was cut in half, one-half was left intact while the other was carefully urothelium denuded by a small scissors under a dissecting microscope. After urothelium removal, the contractile responses to 5-HT were evaluated as described in Tissue preparation for the pharmacological studies in the BOO rats.

Histological examination of the bladders. For light microscopy, the bladders were immersed in 10% buffered formaldehyde solution and then embedded in paraffin. Subsequently, 5-μm slices were cut and the specimens were stained with hematoxylin-eosin. The slides were then examined and photographed under a light microscope at a magnification of ×100.

RNA extraction and cDNA synthesis. One week after the partial BOO procedure, the bladders from the control and BOO rats were immediately removed as per the previously described tissue preparation method. The bladders were weighed and cut into small pieces, with each piece containing all layers of the bladder wall. These specimens were immediately placed in RNAlater (Invitrogen, Carlsbad, CA) to prevent RNA degradation and then stored at −20°C until further processing was performed. Total RNA was extracted using RNeasy fibrosis tissue kit (Qiagen, Valencia, CA) in accordance with the manufacturer’s instructions. All samples were treated with DNase to remove residual genomic DNA contamination. Extracted RNA concentrations were quantified with a spectrophotometer (Thermo Fisher Scientific, Waltham, MA). cDNA was synthesized from 0.5 μg of DNase-treated total RNA using a SuperScript VILO cDNA synthesis kit (Invitrogen) in accordance with the manufacturer’s instructions. A fivefold dilution of the cDNA reaction products was used for the PCR analyses.

Gene expression analysis by PCR and quantitative real-time PCR. Gene expression analysis for the 5-HT receptor subtypes (1A, 2A, 2B, 2C, 3A, 4, 5A, 6, and 7) was performed by PCR using Platinum Blue PCR SuperMix (Invitrogen). The reaction mixture contained 2 μl of diluted cDNA and 10 μM of the primer pair and Platinum Blue PCR SuperMix in a 50-μl total volume. Forty cycles of PCR were performed with a thermal cycler (Takara, Shiga, Japan). Annealing temperatures used and the primer sequence design were based on the work of previous studies that are listed in Table 1. PCR products obtained from all of the primer sets were resolved by electrophoresis using 2% agarose gel and ethidium bromide staining, with visualization of the PCR products by ultraviolet light. Real-time quantification of 5-HT2A and 5-HT3 mRNA was performed with the TaqMan assay using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). The specific TaqMan probes were designed by Primer Express version 2.0 (Applied Biosystems), with the specificity against each sequence confirmed by BLAST software (National Center for Biotechnology Information, Bethesda, MD). The expression of ribosomal protein 19 (RPL19) was measured and used as the internal control for the sample to sample variation. The sequence of the TaqMan probes and primers are listed in Table 2.

Drugs. Carbacholylcholine chloride (carbachol), 5-HT, and phenylephrine hydrochloride (phenylephrine) were purchased from Sigma-Aldrich (St. Louis, MO). Ketanserin was purchased from Wako Pure Chemical (Osaka, Japan), and 4-(4-fluoronaphthalen-1-yl)-6-propan-2-ylpyrimidin-2-amine hydrochloride (RS127445) was purchased from Tocris Bioscience (Bristol, UK). All drugs were dissolved in distilled water as a concentrated stock solution and then diluted with Krebs-Henseleit solution immediately before use. The Krebs-Henseleit solution contained 118 mM NaCl, 4.7 mM KCl, 2.4 mM

<table>
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<th>Product size, bp</th>
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<td>5′-TGC AGC CAA ACT AGC CTC CTT CA-3′</td>
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<td>5-HT2B</td>
<td>NM107250</td>
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5-HT, 5-hydroxytryptamine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; bp, base pair; Ref., reference.
**Results**

**Status of obstructed bladder.** Bladder weights were significantly increased in partial BOO after 1 wk in female rats (Fig. 1A). The BOO rat bladder exhibited hypertrophy of the smooth muscle cells and hyperplasia of the transitional urothelium compared with the sham-operated control rats (Fig. 1, B and C). Infiltration of inflammatory cells, such as macrophages and mast cells, along with some fibroblasts were observed in the BOO rat bladder. Bladder weights and histology of the sham-operated rats were almost the same as those observed in the non-operated normal rats.

**Change in bladder contractility to 5-HT.** The contractile responses to 5-HT, carbachol, phenylephrine, and KCl in the BOO rat bladder were investigated. Cumulative applications of 5-HT induced concentration-dependent contraction in both the sham-operated control and BOO rat bladder strips (Fig. 2, A and B).

CaCl₂, 1.18 mM MgSO₄, 1.18 mM KH₂PO₄, 24.9 mM NaHCO₃, and 11.1 mM glucose.

Statistical differences between two groups were analyzed by the unpaired Student's t-test. A P value < 0.05 was considered statistically significant.

### Table 2. TaqMan probe and primers for real-time PCR

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<tr>
<td>RPL19</td>
<td>NM031103</td>
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<td>101–120</td>
<td>Reverse 5'-TCA GGC CAT GTT TGA TCA GGT T-3'</td>
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<td>155–176</td>
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<td>124–148</td>
<td>TaqMan probe 5'-PM-GGC CAA TGC CAA TCG TCA ACA G TAMRA-3'</td>
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Annealing temperature used for each of the primers was 60°C.

Compared with the controls, 5-HT-induced bladder contraction was significantly increased ∼2.8-fold in the BOO rats. The maximum tensions induced by 5-HT in the control and BOO rat bladders were 0.85 ± 0.12 and 2.35 ± 0.21 mN/mg tissue, respectively (Fig. 2C). On the other hand, the contractile responses to carbachol, which is an acetylcholine analog, were not increased in the BOO rat bladder compared with the control (Fig. 2D). The contractile responses to phenylephrine, an α₁-adrenergic receptor agonist, or KCl, a receptor-independent stimulant, also exhibited no increases in the BOO rat bladder (Fig. 2, E and F). Compared with the controls, the contractile responses to carbachol, phenylephrine, or KCl tended to decrease in the BOO rat bladder strips. The percentage of the maximum tension of 5-HT to that of carbachol at 10⁻⁵ M was increased in the BOO rats (38.4%) compared with the control rats (17.5%). The maximum tension of 5-HT was >10 times stronger than that seen for phenylephrine in the BOO rats.

**Effects of 5-HT receptor antagonists.** Ketanserin, a 5-HT₂ₐ receptor antagonist, attenuated the 5-HT-induced contraction in a concentration-dependent manner from 0.01 to 0.1 µM in the BOO rat bladder. The strong inhibitory effects of ketanserin on 5-HT-induced contraction were observed with high concentrations (10⁻⁴ and 10⁻³ M) of 5-HT (Fig. 3A). Although RS127445, a selective 5-HT₂₉ receptor antagonist, did not attenuate the 5-HT-induced contraction at 0.1 µM in the BOO rat bladder, it did seem to attenuate the 5-HT-induced contraction at a low concentration (10⁻⁷ M) of 5-HT (Fig. 3B).

**Detrusor muscle-mediated contraction to 5-HT.** The contractile responses to 5-HT were examined in both the intact and urothelium-denuded bladder strips in the same animal. In the urothelium-denuded bladder strips, the contractile responses to 5-HT and KCl were significantly increased compared with the intact bladder strips when corrected for the weight of each bladder strip (Fig. 4, A and B). On the other hand, when the contractile responses to 5-HT were expressed as the percentage of the 40-mM KCl-induced contraction, no differences were observed between the intact and urothelium-denuded bladder strips (Fig. 4C). The wet weights of strips from the intact and urothelium-denuded bladders were 10.1 ± 0.6 and 7.0 ± 0.5 mg, respectively.

**5-HT receptor family mRNA expressions in the rat bladder.** Gene expression profiles of the 5-HT receptor family in the rat bladder were screened by RT-PCR using subtype specific primers that were based on previously published sequences (Table 1). Electrophoresis of the PCR products for the 5-HT₂ₐ, 5-HT₂₉, 5-HT₂C, 5-HT₄, and 5-HT₇ receptors resulted in predicted sizes (Fig. 5A). While no PCR products were obtained...

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*Fig. 1. Partial bladder outlet obstruction (BOO)-induced bladder hypertrophy in female rats. A: bladder weight of sham-operated (control, n = 6) and 1 wk partial BOO (n = 10) rats. Line represents mean bladder weight in each group. B: histology of bladder sections stained with hematoxylin and eosin in control (B) and BOO rats (C). Arrowheads indicate mast cells, and scale bars indicate 200 µm. Each picture is representative of 5 rats that exhibited similar results. **P < 0.01 vs. control group.*
for the 5-HT$_{1A}$, 5-HT$_{3A}$, or 5-HT$_{5A}$ receptors, we did confirm PCR products for these three 5-HT receptors in a simultaneous investigation performed in rat spinal cord cDNA (data not shown). Since these results show that there were no problems associated with the PCR method for these receptors, our findings suggest that 5-HT$_{1A}$, 5-HT$_{3A}$, and 5-HT$_{5A}$ receptors genes are not expressed in the rat bladder. For the 5-HT$_{6}$ receptor, only a very faint band was obtained. Additionally, the gene expression pattern of the 5-HT receptor family in the BOO rat bladder was the same as that seen for the control bladder (data not shown).

Quantitative real-time PCR was conducted to compare the gene expression levels of 5-HT receptors in the sham-operated control and BOO rat bladders. Gene expression levels of the 5-HT$_{2A}$ receptor were significantly increased by 3.2-fold in the BOO rat bladder compared with the control (Fig. 5B). In contrast to the 5-HT$_{2A}$ receptor, the expression levels of the 5-HT$_{4}$ receptor were equivalent for the BOO and control bladders (Fig. 5C). The gene expression levels of RPL19, which was used as the internal control gene, were equivalent for the BOO and control bladders.

**DISCUSSION**

In this study, we showed that partial BOO influenced both the function and gene expression of the 5-HT receptor subtypes in the rat bladder. Contractile responses to 5-HT were significantly increased in the BOO rat bladder strips. In contrast to the responses to 5-HT, there was no increase in the cholinergic responses to carbachol, adrenergic responses to phenylephrine, and receptor-independent responses to KCl in the BOO rat bladders. Previous studies suggested that pathophysiological changes in the BOO models were different for the periods of BOO (5, 6, 31, 42). Takahashi et al. (42) reported that tonic contraction to carbachol in isolated bladder was increased at 4 wk after BOO in rats, even though the phasic contraction was not changed. For shorter periods of BOO, Barendrecht et al. (5) reported the contractile responses to carbachol or KCl in isolated bladder were reduced after 1 day of BOO and fully recovered after 7 days. The BOO model also showed gradual denervation with increasing duration of BOO. In addition, Barendrecht et al. (6) reported no functionally relevant differences in the $\alpha_{1}$-adrenergic receptor agonists-induced contraction in 7-day BOO. The results of our current 7-day BOO study were approximately the same as those reported in this previous study. The contractile responses to carbachol, KCl, and phenylephrine were not changed from the control, and morphological characteristics such as hyperplasia of the urothelium and mild hypertrophy of the smooth muscle layer were observed. Thus the supersensitivity to 5-HT could have occurred in the mild bladder hypertrophy with denervation in the early phase of BOO.

By convention, 5-HT receptors are classified into seven subfamilies (2, 33). Pharmacological studies that used 5-HT

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**Fig. 2.** Representative tracing charts of contractile responses to 5-hydroxytryptamine (5-HT) in sham-operated control (A) and BOO rats (B). Concentration-response curve for contractile responses to 5-HT (C), carbachol (D), phenylephrine (E), and 40 mM KCl (F) in bladder strips from sham-operated control and BOO rats. Contractile responses are expressed as the actual force (mN/mg tissue). Each value represents means ± SE from 6–10 animals.
receptor subtypes-selective agonists or antagonists have reported that 5-HT1A, 5-HT2A, 5-HT2C, 5-HT3, 5-HT4, and 5-HT7 receptors contribute to bladder contraction (7, 16, 23, 30, 36, 40). Our study revealed that there was gene expression of 5-HT2A, 5-HT2B, 5-HT2C, 5-HT3, 5-HT4, and 5-HT7 receptors in the rat bladder. Our identification of the 5-HT2B receptor gene expression in rat bladder is a novel finding. The activation of the 5-HT2B receptor has been shown to cause contraction in both the stomach fundus and the small intestine (8, 11). Our results suggest that the expression profile of the 5-HT receptor subtypes in the bladder is similar to that found in the gastrointestinal tract. On the other hand, we could not confirm 5-HT1A and 5-HT3 receptor gene expressions. At the present time, it is still unclear whether the 5-HT1A and 5-HT3 receptors exist in the rat bladder. A previous study has reported that only high concentrations (over 10 \mu M) of 8-OH-DPAT, a selective 5-HT1A receptor agonist, causes isolated bladder contraction in both control and BOO rats (25, 30). We also confirmed these results (data not shown). The effects of 8-OH-DPAT at high concentrations are considered to be a nonselective effect, since the pK_i value for the 5-HT1A receptor is 8.5 (12). Additionally, we have also demonstrated that \( \text{N}(-2-[4-(2-	ext{methoxyphenyl}) \text{piperazin}-1-	ext{yl}[\text{ethyl}]-\text{N}-\text{pyridin-2-yl-cyclohexanecarboxamide (WAY-100635)}, \) a selective 5-HT1A receptor antagonist, did not inhibit 5-HT-induced bladder contraction in both control and BOO rats even at 0.1 \mu M, which is an adequately effective concentration (data not shown). Thus the 5-HT1A receptor activation does not have direct effects on bladder contraction at least in rats. With regard to the role of the 5-HT3 receptor on bladder contraction, the potentiating effect of 5-HT on electrically stimulated bladder strip contraction has been shown to be inhibited by the 5-HT3 receptor antagonists in mice and rabbits but not in humans (7, 14, 15). Although the 5-HT3 receptor could have potentially functioned mainly via the prejunctional transmitter release, our current study was not designed to investigate the prejunctional effects. Further studies are necessary to clarify the role of the 5-HT3 receptor on bladder contraction in rats.

The direct effects of 5-HT on bladder contraction may involve both 5-HT2A and 5-HT2B receptor-mediated components, with the 5-HT2A receptor playing the dominant role in the 5-HT-induced BOO rat bladder contraction. Ketanserin, a 5-HT2A receptor antagonist with a pK_i value of 8.1–9.7 (2), strongly attenuated the 5-HT-induced BOO rat bladder hypercontraction when high concentrations of 5-HT were used. Previous studies have also reported that ketanserin inhibits 5-HT-induced normal bladder contraction in both humans and rats (22, 23). On the other hand, the role of the 5-HT2B receptor on the 5-HT-induced BOO rat bladder contraction appears to be restricted, since RS127745, which is a selective 5-HT2B receptor antagonist with a pK_i value of 9.5 that has more than a 1,000-fold selectivity to 5-HT2A and other 5-HT receptor subtypes (9), only attenuated 5-HT-induced weak bladder contractions at 10^{-7} M of 5-HT. Thus the incomplete attenuation by ketanserin to the weak bladder contractions at 10^{-7} M of 5-HT may be due to the contribution of the 5-HT2B receptor. The suggestions that 5-HT2A and 5-HT2B receptors contribute to the direct effects on bladder contraction are reasonable, since both are seven-transmembrane receptors coupled with the \( \text{G}_\text{q} \) protein, which upon activation increases \( \text{Ca}^{2+} \) in the cells (18, 32, 39). The 5-HT-induced bladder contraction was observed in urotheleium-denuded bladder strips. This suggests that the detrusor smooth muscle cells express 5-HT receptors and induce bladder contraction in response to 5-HT. The contractile responses to 5-HT and KCl in the urotheleium-denuded bladder strips were increased compared with those observed for the intact strips when responses were corrected for the weight of each bladder strip. These results suggest two possibilities. First, the urotheleial cells might be negatively regulating the bladder contractions induced by 5-HT and KCl. Alternatively, only the smooth muscle cells might be related to the 5-HT-induced contraction. In the latter case, an increase in the ratio of the weight of the smooth muscle cells would be expected, with a correction of the tension according to the tissue weight thereby leading to enhancement in the denuded strips. In either case, this suggests that the 5-HT induces bladder contraction via a direct action on the detrusor smooth muscles in rats. Therefore, the 5-HT2A and 5-HT2B receptors could be expressed in the detrusor smooth muscle cells in the rat bladder.

The gene expressions of the 5-HT2A receptor were significantly increased in the BOO rat bladder compared with the control. On

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**Fig. 3.** Effects of 5-HT receptor antagonists, ketanserin (A) and RS127445 (B) on 5-HT-induced contraction in BOO rat bladder strips. Contractile responses are expressed as the actual force (mN/mg tissue). Each value represents the means ± SE from 6–7 animals.
the other hand, the gene expressions of 5-HT4 receptor, another 5-HT receptor subtype, which is known to be expressed in detrusor smooth muscle cells at least in human (16), were not changed from the control rat bladder. Although it is assumed that alterations of cell types, such as hyperplasia of the transitional urothelium and infiltration of the inflammatory cells, affect the overall gene expressions in the BOO rat bladder, the gene expressions of RPL19, a ubiquitously expressed internal control gene, were not changed from the control rat bladder. This suggests that the increased gene expressions of 5-HT2A receptor in the BOO rat bladder are not caused by alterations of cell types. The results of contractile responses in intact and urothelium-denuded bladder

![Fig. 4. Contractile responses to 5-HT (A and C) and 40 mM KCl (B) in intact and urothelium-denuded bladder strips. Contractile responses to 5-HT are expressed as the actual force (mN/mg tissue; A and B) and as a percentage of the maximum response obtained in response to 40 mM KCl (C) for each strip. After cutting each bladder in half, one-half was left intact while the other was urothelium-denuded. Each value represents the means ± SE from 7 animals.](image)

![Fig. 5. Expression of 5-HT receptor subtype mRNA in whole bladder from sham-operated control rats and 1 wk partial BOO rats. A: 2% agarose gel electrophoresis of PCR-amplified products from control bladder. Data are representative of 3 rats that exhibited similar results. 5-HT receptor subtype-specific primers were designed as described in Table 1. The predicted sizes for 5-HT1A, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT3A, 5-HT4, 5-HT5A, 5-HT6, 5-HT7, and GAPDH were 388, 465, 222, 248, 285, 583, 370, 383, and 532 base pairs, respectively. B and C: relative expression levels of 5-HT2A and 5-HT4 receptor mRNA in the bladder of control (n = 6) and BOO (n = 5) rats are shown. Relative expression levels of 5-HT2A, and 5-HT4 receptors were analyzed by quantitative real-time PCR as described in MATERIALS AND METHODS. Values were normalized to the ribosomal protein 19 (RPL19) levels. Each value represents means ± SE. **P < 0.01 vs. control group.](image)
strips suggests that the 5-HT\textsubscript{2A} receptor expresses in the detrusor smooth muscle cells in rats. Thus the increased gene expressions of 5-HT\textsubscript{2A} receptor might be related to the hypertrophy of detrusor smooth muscle cells in the BOO rat bladder.

Approximately 50% of BPH patients present with storage symptoms in addition to the obstructive voiding symptom. The storage symptoms, which include urinary frequency, nocturia, and urgency, are all associated with detrusor overactivity (3). Although antimuscarinic drugs are the first-line therapy for overactive bladder (OAB), there has been reluctance to use these drugs for BPH patients due to the risk of precipitating urinary retention. The contractile responses to 5-HT via the 5-HT\textsubscript{2A} receptor in the BOO rat bladder were potent, since the maximum contractile responses to 5-HT were ~40% of those for the cholinergic responses. Additionally, the contractile responses to 5-HT were quite potent compared with those for other bladder contracting factors, such as ATP, PGE\textsubscript{2}, and phenylephrine (4, 43). Furthermore, it has been reported that there is an increased contractility in response to 5-HT in aged or diabetic rat bladder (24, 38). Although BPH occurs solely in males, our present findings were obtained in female rats. However, it has been previously reported that there are not many differences in the cholinergic or adrenergic responses between male and female rats at least in isolated bladder strips (19, 27). While the effects of gender differences need to be considered, the present findings in isolated bladder strips of female rats might be adaptable to the BOO-associated functional changes of the bladder in BPH patients.

The source of 5-HT in the bladder may be the essential factor that is the key to understanding the physiological role of peripheral 5-HT on bladder function. In the central nervous system, 5-HT exists abundantly and the cerebrospinal 5-HT has been previously shown to control the micturition reflex (17). On the other hand, the peripheral source of 5-HT has yet to be elucidated, as 5-HT neurons have not been found in the bladder. We hypothesize that platelets or mast cells could be one of the sources of peripheral 5-HT in obstructed bladder, as it is well known that platelets and mast cells contain 5-HT. The histological examination of the BOO rat bladder showed that there was infiltration of inflammatory cells in the bladder, including mast cells. BOO causes ischemic reperfusion and oxidative stress within the bladder, which can then induce bladder inflammation (26, 35). Previous studies have demonstrated that ischemia and inflammation cause platelet activation and the release of 5-HT (34, 37). BPH patients often show prostatic inflammation, and it has been suggested that an inflammatory process contributes to lower urinary tract symptoms (41). In addition, mast cells have also been observed in the bladder of BPH patients. Thus platelets or mast cells could be a potent source for peripheral 5-HT. Overall, 5-HT may have a role in the development of OAB in BOO. Further studies are required to clarify the peripheral role of 5-HT on the development of OAB and the medicinal potential of using 5-HT\textsubscript{2A} receptor antagonists for OAB treatment.

In conclusion, BOO rat bladder shows hypercontractility in response to 5-HT through the upregulation of 5-HT\textsubscript{2A} receptor expression in the detrusor smooth muscle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.S., K.K., and I.I. conception and design of research; T.S. and K.T. performed experiments; T.S. and K.T. analyzed data; T.S. and K.K. interpreted results of experiments; T.S. prepared figures; T.S. drafted manuscript; T.S. and K.K. edited and revised manuscript; K.K., I.I., and H.K. approved final version of manuscript.

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