Jpn. J. Phramacol. **89**, 349 – 355 (2002)

5-Hydroxytryptamine Receptors, Especially the 5-HT_4 Receptor, in Guinea Pig Urinary Bladder

Akira Yoshida[1,2], Yasuko S.-Yamashita[2], Muneshige Kaibara[2], Kohtaro Taniyama[2,*] and Nobuyuki Tanaka[1]

[1]*Department of Rehabilitation and Physical Medicine, Faculty of Medicine, Kagoshima University, Kagoshima 890-8520, Japan*
[2]*Department of Pharmacology, Nagasaki University School of Medicine, Nagasaki 852-8523, Japan*

Received February 6, 2002 Acceped April 30, 2002

ABSTRACT—The function of 5-hydroxytryptamine (5-HT) receptors, especially the 5-HT_4 receptor, in the urinary bladder were examined in preparations isolated from the guinea pig by in vitro receptor autoradiography and determinations of mechanical activity and acetylcholine (ACh) release. Specific [^{125}I]SB207710 binding sites were detected evenly throughout the urinary bladder. 5-HT ($3 \times 10^{-8} – 10^{-4}$ M) caused contractions of strips of the urinary bladder, in a concentration dependent manner. Ketanserin antagonized the 5-HT-induced contractions, while granisetron and SB204070 antagonized the contractions induced by high concentrations of 5-HT. Atropine inhibited the contractions induced by high concentrations of 5-HT. Ketanserin prevented the 5-HT-induced contractions in the presence of atropine, but granisetron and SB204070 did not affect the contractions under such a condition. 5-HT enhanced the electrically-stimulated (5 Hz, 0.5 ms) outflow of [^{3}H]acetylcholine from strips preloaded with [^{3}H]choline, and the enhancement was antagonized by granisetron and SB204070. Thus, the contractile response to 5-HT was mediated by activations of 5-HT_2, 5-HT_3 and 5-HT_4 receptors. The 5-HT_2 receptor may be a property of high affinity to 5-HT and located on the smooth muscle cells. The 5-HT_4 as well as 5-HT_3 receptor may be a property of low affinity to 5-HT and located on the cholinergic neurons.

Keywords: 5-HT receptor, Guinea pig urinary bladder, Receptor autoradiography, Acetylcholine release

The bladder functions are controlled by the integration of excitatory, inhibitory and sensory nerve activity in the spinal cord, pons and forebrain; and 5-hydroxytryptamine (5-HT) has been identified as an inhibitory neurotransmitter in the micturition reflex pathway at spinal and supraspinal sites (1). In vivo studies have shown that the 5-HT_{1A} receptor of some 5-HT receptor subtypes are important in the micturition reflex pathway (2 – 4), especially at the spinal site (4). In vitro study has shown that 5-HT causes contractions of urinary bladder preparations from several mammals (5). In most species, activation of 5-HT_2 receptor has been reported to cause contractions due to direct stimulation of smooth muscle cells of cat (6, 7) and human (8) urinary bladder. Activation of 5-HT_3 receptor causes neurogenic contractions mediated by stimulation of the receptor located on the excitatory neurons in the cat (7), mouse (9), guinea pig (10), rabbit (11, 12), and human (13, 14). The 5-HT_4 receptor has been suggested to be located on the excitatory neurons of urinary bladder and its activation facilitates cholinergic transmission in humans (14, 15), while in the monkey urinary bladder, the 5-HT_4 receptor is located post-junctionally and its activation causes inhibition of neurogenic contractions (16). In the guinea pig urinary bladder, it has been reported by mechanical experiments that 5-HT_{2A} and 5-HT_4 receptors are involved in enhancing purinergic transmission and the 5-HT_3 receptor is involved in enhancing cholinergic transmission (10).

In order to elucidate the function of 5-HT receptor subtypes, especially 5-HT_4 receptor in the urinary bladder, and interaction between localization and function of 5-HT_4 receptor, we examined effects of 5-HT and 5-HT antagonists on the responses to 5-HT using mechanical activity, acetylcholine (ACh) release and in vitro receptor autoradiography.

MATERIALS AND METHODS

The study has been carried out in accordance with the Guide for the Care and Use of Laboratory Animals of Nagasaki University, as adopted and promulgated by the notification of the Director-General of the Science and

*Corresponding author. FAX: +81-95-849-7048
E-mail: taniyama@net.nagasaki-u.ac.jp

International Affairs Bureau of the Japanese Ministry of Education, Culture, Sports, Science and Technology, Japan. Male guinea pigs, weighing between 300 and 500 g, were killed by cervical dislocation. The urinary bladder was immediately excised and mucosa-free strips (1 cm × 0.2 cm) were prepared by cutting in a longitudinal fashion.

Receptor autoradiography

Five animals were used for the receptor autoradiographic experiments. The mucosa-free preparation from urinary bladder was immediately immersed in isopentane at –30°C. Frozen tissues were cut into 20-μm-thick sections on a cryostat at right angles to the long axis, thaw-mounted onto gelatin-coated glass slides and stored overnight under vacuum at 4°C. After preincubation in the incubation buffer of the following composition: 50 mM Tris-HCl buffer (pH 7.4) containing 4 mM $MgCl_2$, 0.3% bovine serum albumin, 0.2 mM ascorbic acid and 10 μM pargyline, at 23°C for 30 min, tissue sections were incubated in 2 ml of incubation buffer containing [^{125}I]SB207710 at concentration of 10 pM at 23°C for 2 h. Consecutive tissue sections were labelled to characterize [^{125}I]SB207710 binding in the presence of 1 μM unlabelled SB204070 (non specific binding). Then, the labelled sections were washed three times (for 1 min each) at 4°C in 50 mM Tris-HCl buffer (pH 7.2), tapped in ice-cold distilled water, and then dried under a stream of cold air. To obtain autoradiograms of a higher resolution, the dry-labelled sections were apposed against Hyperfilm-[^{3}H] (Amersham, Little Chalfont, UK) for 1 week and the films were developed with a D19 developer (Eastman Kodak, Rochester, NY, USA) for 7 min at 4°C.

Measurements of the mechanical activity

The strips isolated from the urinary bladder were placed in a 20-ml organ bath in the presence of Krebs-Ringer solution of the following composition: 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.19 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 1.18 mM KH_2PO_4 and 11 mM glucose, which was continuously gassed with 95% O_2 – 5% CO_2 and maintained at 34 – 36°C and pH 7.4. Approximately 1 g of resting tension was applied and was kept constant by re-adjustment during the equilibration period. Mechanical responses to a single dose of 5-HT were recorded by means of an isometric transducer (SD-1T; Nihon Kohden, Tokyo). After a stabilization of contraction induced by 5-HT (10^{-5} M), an interval of 15 – 20 min (with intervening washings) between single doses of 5-HT completely prevented any desensitization. 5-HT was aplied to the organ in the absence and presence of antagonists for 5-HT receptors, atropine or tetrodotoxin. The concentration-response curves of 5-HT in the absence and presence of antagonists for 5-HT receptors, atropine or tetrodotoxin were obtained using the same strips.

Measurement of [^{3}H]ACh outflow

The methods of incubation and superfusion were as described by Kusunoki et al. (17) and Shirakawa et al. (18). The strips of urinary bladder were incubated at 37°C for 60 min with [^{3}H]choline at a final concentration of 200 nM in Krebs solution of the following composition: 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.19 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 1.18 mM KH_2PO_4 and 11 mM glucose. After washing in fresh Krebs solution for 30 min, the strips were mounted in the superfusion apparatus and superfused at 0.8 ml/min with Krebs solution gassed with 95% O_2 – 5% CO_2, maintained at 35 – 37°C. The superfusion medium used was Krebs solution containing 10 μM hemicholinium-3 to prevent the uptake of choline formed from ACh. Two parallel platinum electrodes were used to stimulate intramural nerves. The strips were stimulated electrically at parameters of 0.5-ms duration, 15 V intensity, and a frequency of 5 Hz for 30 s. The strip was stimulated successively four times (S1, S2, S3 and S4) at 30-min intervals. The superfusate was collected every 1 min and the radioactivity of the superfusates and that of the tissue dissolved in Soluene at the end of the release experiment were counted in a liquid scintillation spectrometer (Packard Instrument Co., Downers Groves, IL, USA). Experiments were begun 60 min after the spontaneous [^{3}H] outflow had approached a plateau.

The validity of assuming total [^{3}H] as a measure of [^{3}H]ACh outflow under the present experimental conditions has been documented in our previous studies (13, 14). The outflow of [^{3}H] was represented as the fractional rate obtained by dividing the amount of [^{3}H] in the perfusate by the respective amount of [^{3}H] in the tissue. The [^{3}H] content of the tissue at each period was calculated by adding cumulatively the amount of each fractional [^{3}H] outflow, to the [^{3}H] content of the tissue at the end of the experiment. From each of the outflow curves obtained by plotting the fractional outflow of [^{3}H] against time, the peak outflow of [^{3}H] evoked by stimulation with 5-HT in each condition was calculated from the difference between the peak [^{3}H] outflow and the basal outflow. When electrical transmural stimulation was applied successively four times to the preparation at 30-min intervals, the stimulation-evoked outflow of [^{3}H] markedly decreased or increased from the S1 to S2, whereas there were no significant differences between the S2 to S4; therefore, the ratio of S3/S2 calculated from the S2 and S3 without substances was used as a control, and the effects of substances on the electrically evoked outflow were evaluated by the ratio of S3/S2 calculated from the S3 in the presence of substances. To exclude the effect of mechanical distortion accompanied with 5-HT-induced contraction on the

[^{3}H]ACh outflow, the perfusion solution contained 10^{-6} M ketanserin. It was preliminarily confirmed that there were no significant differences in the [^{3}H] outflow in the presence of 5-HT at 10^{-5} M among the S2, S3 and S4 and that the effect of 5-HT was reversible. Data were analyzed using Student *t*-test and Dunnett's *t*-test, and a *P* value of 0.05 or less was considered statistically significant.

Chemicals

Substances used were as follows: [^{125}I]SB207710 (74 TBq/mmol) (Amersham); [^{3}H]choline (3.33 TBq /mmol) (New England Nuclear, Boston, MA, USA); 5-hydroxytryptamine (5-HT) creatinine sulfate, hemicholinium-3 and EGTA (Sigma, St. Louis, MO, USA); soluene (Packard); ketanserine tartrate (Research Biochemicals Int., Natick, MA, USA); and atropine sulfate and tetrodotoxin (Wako, Osaka). Other chemicals used were of reagent grade. SB204070 (1-*n*-butyl-4-piperidinyl) methyl-8-amino-7-chloro-1,4-benzodioxane-5-carboxylate and granisetron (BRL43694) were generously provided by Smith Kline Beecham, UK.

RESULTS

In vitro receptor autoradiography

Fig. 1A and B show typical autoradiograms of the [^{125}I]SB207710 binding sites in the absence and presence of unlabelled 1 μM SB204070 in the smooth muscle layer of body of guinea pig urinary bladder, respectively. The [^{125}I]SB207710 binding was visible (Fig. 1A), and the [^{125}I]SB207710 binding densities were abolished by addition of unlabelled 1 μM SB204070 (Fig. 1B). When the distribution of [^{125}I]SB207710 binding sites was compared with the consecutive sections stained with hematoxylin (Fig. 1C), the distribution of [^{125}I]SB207710 binding sites was even throughout the urinary bladder (Fig. 1A).

Mechanical response to 5-HT of urinary bladder strips in the presence of antagonists for 5-HT receptor subtypes

5-HT at $3 \times 10^{-8} - 3 \times 10^{-4}$ M caused contractions of strips in a concentration dependent manner (Fig. 2A). The maximum response to 5-HT was obtained with 10^{-4} M and the EC_{50} value was 2.9×10^{-6} M. Pretreatment with ketanserin at 10^{-7} M for 20 min inhibited the contractions induced by 5-HT at 10^{-7} M to 10^{-4} M (Fig. 2B). Pretreatment with granisetron at 3×10^{-7} M for 20 min inhibited the contractions induced by 5-HT at 10^{-5} M and over (Fig. 2C). Pretreatment with SB204070 at 3×10^{-7} M for 20 min inhibited the contractions induced by 5-HT at 10^{-5} M and over (Fig. 2D). The effects of ketanserin at 10^{-7} M, granisetron at 3×10^{-7} M and SB204070 at 3×10^{-7} M were reversible 30 min after removing these antagonists.

Effects of tetrodotoxin and atropine on 5-HT-induced contractions

Pretreatment with tetrodotoxin at 3×10^{-7} M for 20 min did not affect the 5-HT-induced contractions (Fig. 3A), but pretreatment with atropine at 10^{-7} M for 20 min inhibited the contractions induced by 5-HT at 10^{-5} M and over (Fig. 3B). In the presence of atropine at 10^{-7} M, ketanserin at 10^{-7} M prevented the 5-HT-induced contractions (Fig. 3C), while granisetron at 3×10^{-7} M and SB204070 at 3×10^{-7} M did not affect the 5-HT-induced contractions (Fig. 3D).

Effect of 5-HT on the outflow of [^{3}H]ACh from the urinary bladder

The spontaneous outflow of [^{3}H]ACh from strips preloaded with [^{3}H]choline reached a steady level to a single exponential curve, 30 min after start of the superfusion. Electrical transmural stimulation (5 Hz, 15 V, 0.5 ms, for 30 s) induced an increase in the outflow of [^{3}H]ACh from strips. Superfusion with the Ca^{2+}-free medium or with the medium containing 3×10^{-7} M tetrodotoxin prevented the

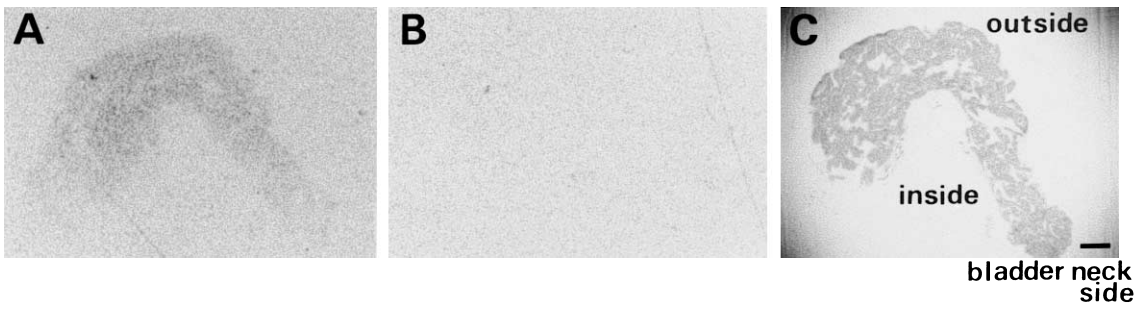


Fig. 1. Typical receptor autoradiographic localization of [^{125}I]SB207710 binding (A and B) sites and hematoxylin staining (C) in the smooth muscle layer of urinary bladder of guinea pig. Consecutive, 20-μm-thick sections at right angles to the long axis were labeled with 10 pM [^{125}I]SB207710 in the absence (total binding) (A) and presence of 1 μM SB204070 (non-specific binding) (B), in vitro. The [^{125}I]SB207710 binding (A) was abolished by addition of unlabelled 1 μM SB204070 (B). Scale bar = 50 μm.

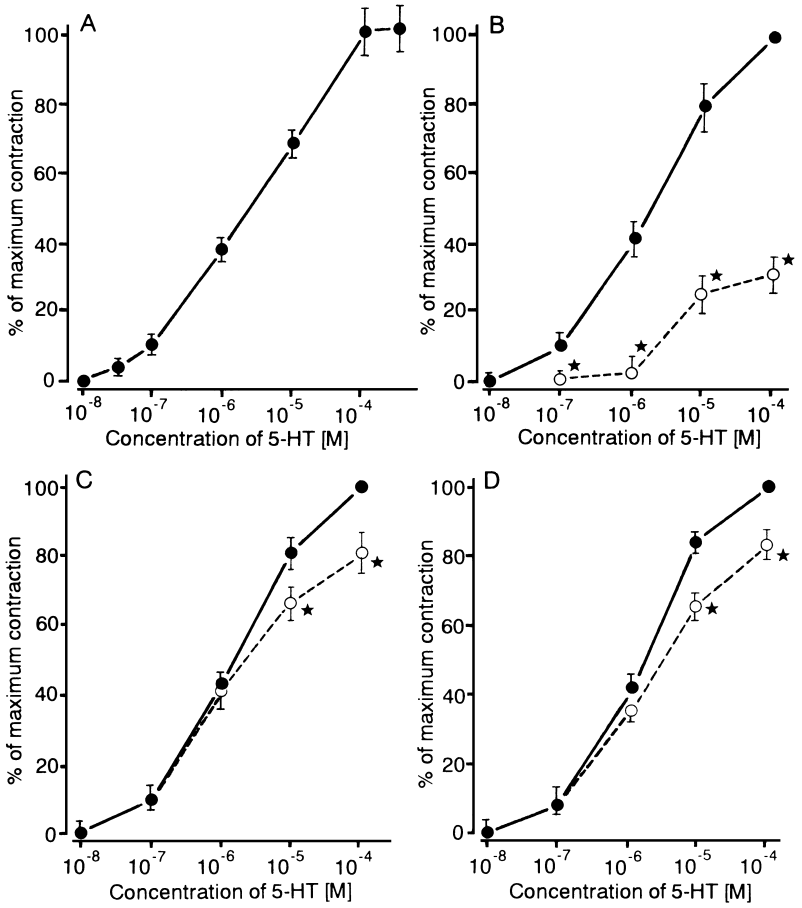


Fig. 2. Concentration-response curves of 5-HT in the absence (A) and presence of ketanserin (B), granisetron (C) and SB204070 (D). Ketanserine at 10^{-7} M, granisetron at 3×10^{-7} M and SB204070 at 3×10^{-7} M were applied 20 min before and during addition of 5-HT. Each point represents the mean ± S.E.M. of 6 animals in A and 7 animals in B, C and D. *Significance of difference from the value in the absence of antagonists (closed circle) was calculated by Student *t*-test, at the $P<0.05$ level of probability.

stimulation-evoked outflow of [^{3}H]ACh (data not shown).

To exclude the effect of mechanical distortion accompanied with 5-HT-induced contraction on the [^{3}H]ACh outflow, the perfusion solution contained 10^{-6} M ketanserin. Additions of 5-HT at 3×10^{-6} M and 10^{-5} M 30 s before and during the third stimulation (S3) enhanced the electrical transmural stimulation-evoked outflow of [^{3}H]ACh from the strips (Fig. 4A). The 5-HT (3×10^{-6} M)-induced enhancement was antagonized by 10-min pretreatment with either granisetron at 3×10^{-7} M or SB204070 at 3×10^{-7} M (Fig. 4B). The antagonisms by granisetron and SB204070 were to the same degree. Simultaneous pretreatment with granisetron at 3×10^{-7} M and SB204070 at 3×10^{-7} M completely inhibited the 5-HT (3×10^{-6} M)-induced enhancement of [^{3}H]ACh outflow (Fig. 4B). The effects of granisetron at 3×10^{-7} M and SB204070 at 3×10^{-7} M were reversible 30 min after removing these antagonists.

DISCUSSION

We found the presence and function of 5-HT$_4$ receptors in the guinea pig urinary bladder. The specific binding sites of [^{125}I]SB207710 were detected in the smooth muscle layer of urinary bladder. [^{125}I]SB207710 is a radioligand of high specific activity and selectivity for 5-HT$_4$ receptors (19) and the [^{125}I]SB207710 binding was abolished by addition of unlabelled SB 204070, a selective antagonist for the 5-HT$_4$ receptors (20); therefore, specific [^{125}I]SB207710 binding sites may demonstrate the presence of 5-HT$_4$ receptors. The 5-HT$_4$ receptor was evenly distributed throughout the smooth muscle layer of the urinary bladder.

5-HT caused contractions of strips of urinary bladder in a concentration dependent manner. Ketanserin, a 5-HT$_2$ antagonist, inhibited the contractions induced by 5-HT at all concentrations used in the present experiment, while granisetron, a 5-HT$_3$ antagonist and SB204070, a 5-HT$_4$ antagonist, inhibited the contractions induced by high

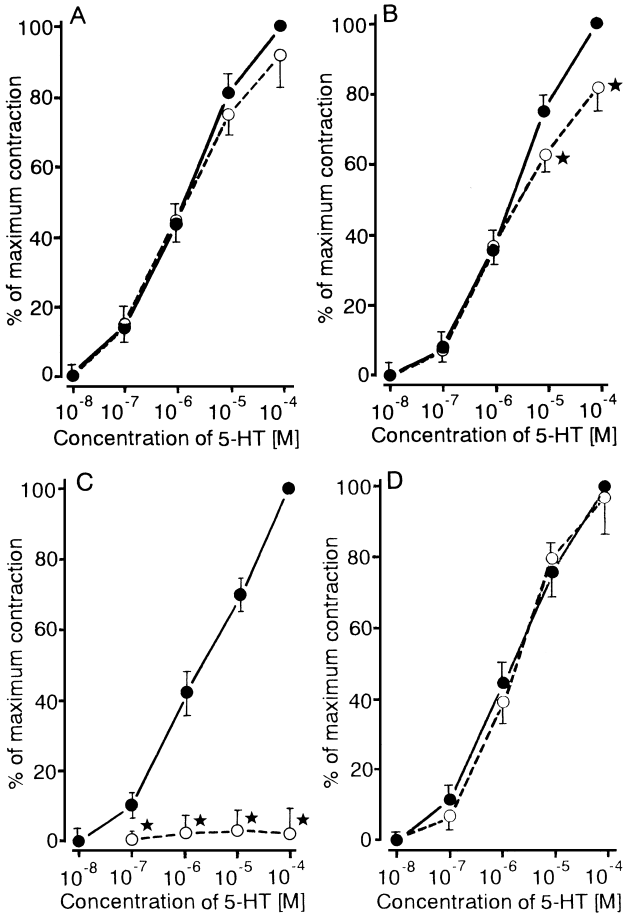
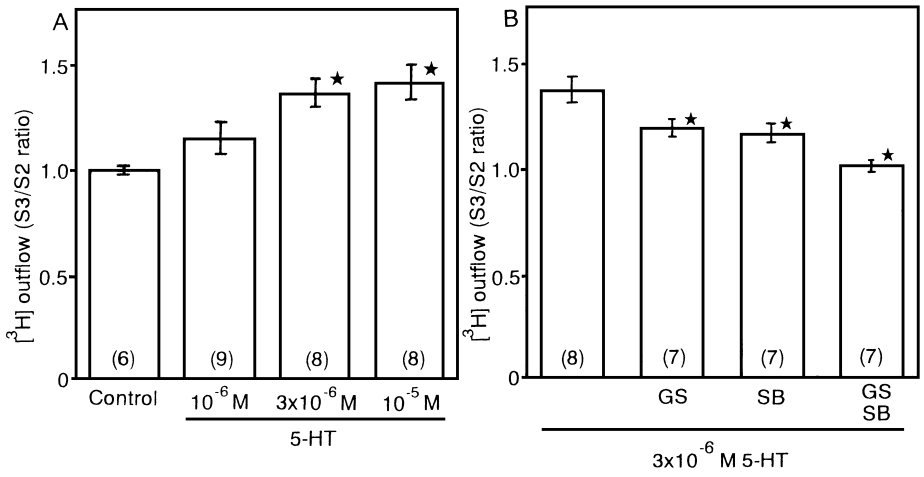


Fig. 3. Concentration-response curves of 5-HT in the absence and presence of tetrodotoxin (A), atropine (B), ketanserin in the presence of atropine (C) and granisetron and SB204070 in the presence of atropine (D). Tetrodotoxin at 3×10^{-7} M, atropine at 10^{-7} M, ketanserine at 10^{-7} M, granisetron at 3×10^{-7} M and SB204070 at 3×10^{-7} M were applied 20 min before and during addition of 5-HT. Each point represents the mean ± S.E.M. of 6 animals in A, 9 animals in B, 5 animals in C and 4 animals in D. *Significance of difference from the value in the absence of antagonists (closed circle) was calculated by Student *t*-test, at the $P<0.05$ level of probability.

concentrations of 5-HT, with no effect on the contractions induced by low concentrations of 5-HT. These results indicate that 5-HT may cause the contractions of urinary bladder due to activations of 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors, although 5-HT$_3$ and 5-HT$_4$ receptors may be activated by high concentrations of 5-HT. Atropine did not affect the contractions induced by low concentrations of 5-HT, but partially inhibited the contractions induced by high concentrations of 5-HT. Furthermore, atropine did not affect the 5-HT-induced contractions in the condition of blocking both the 5-HT$_3$ and 5-HT$_4$ receptors. Thus, the 5-HT$_3$ and 5-HT$_4$ receptors may be located on the cholinergic neurons, and activations of these receptors by high concentrations of 5-HT may enhance the release of ACh. Tetrodotoxin did not affect the contractions induced by all concentrations of 5-HT used in the present study, and furthermore, ketanserin prevented the 5-HT-induced contractions in the presence of atropine; therefore, the 5-HT$_2$ receptor may be located on the smooth muscle cells, but not the cholinergic neurons, and activation of the 5-HT$_2$

Fig. 4. 5-HT-induced enhancement of electrically-stimulated [^{3}H] outflow from the strips of urinary bladder preloaded with [^{3}H] choline in the absence and presence of SB204070. Parameters of electrical stimulation were 0.5-ms duration, 15 V intensity, and a frequency of 5 Hz for 30 s. The [^{3}H] outflow was represented as a ratio of the release evoked by the third stimulation (S3) to that by the second stimulation (S2). A: 5-HT was present 30 s before and during the third stimulation (S3). Each column represents the mean from the number of animals given in columns with S.E.M. of the mean shown by vertical lines. *Significance of difference from the value in the absence of substance (control) calculated by Dunnett's *t*-test, at the $P<0.05$ level of probability. B: 5-HT (3×10^{-6} M), granisetron (GS, 3×10^{-7} M) and SB204070 (SB, 3×10^{-7} M) were present 30 s, 10 min, and 10 min before and during the third stimulation (S3). Each column represents the mean from the number of animals given in columns with S.E.M. of the mean shown by vertical lines. *Significance of difference from the value in the presence of 5-HT only calculated by Dunnett's *t*-test, at the $P<0.05$ level of probability.



receptor by 5-HT may cause contractions due to direct stimulation of smooth muscle cells.

The localization of 5-HT_3 and 5-HT_4 receptor at the cholinergic neurons was confirmed by ACh release experiments. 5-HT at high concentrations enhanced the electrically stimulated release of ACh from strips of urinary bladder. Since the 5-HT_2 receptor was blocked by ketanserin, the strips did not cause the contractile responses to 5-HT, and thus the increase in [^{3}H]ACh outflow is not attributed to mechanical distortion accompanied with 5-HT-induced contraction. The enhancement by 5-HT was inhibited by either granisetron or SB204070, although the inhibitions by both antagonist were to the same degree, and the enhancement was completely prevented by simultaneous treatment with both antagonists. Thus, both 5-HT_3 and 5-HT_4 receptors may be located on the cholinergic neurons and the activation of these receptors with 5-HT increases the release of ACh.

The present study demonstrated that the contractile response to 5-HT was mediated by activations of 5-HT_2, 5-HT_3 and 5-HT_4 receptors. When focusing on the 5-HT_4 receptors, receptor autoradiographic study showed the presence of 5-HT_4 receptors throughout the urinary bladder, and the functional study indicated the localization of 5-HT_4 receptor on the cholinergic neurons. Thus, activation of 5-HT_4 receptor causes contractions of urinary bladder mediated by stimulation of the cholinergic neurons. Similar concepts are proposed in human detrusor muscle (14, 15), in which 5-HT or 5-HT_4 agonist enhanced the cholinergic contractions to electrical field stimulation and the effect was antagonized by 5-HT_4 antagonist. In the guinea pig urinary bladder, it has been reported that the cholinergic transmission is mediated by 5-HT_3 receptors and the 5-HT_4 receptor is involved in enhancing purinergic transmission (10). In the present study, the function of 5-HT_3 receptor was agreement with the previous reports, but the function of 5-HT_4 receptor was different. The present study directly demonstrated that the 5-HT-induced enhancement of ACh release was antagonized by a 5-HT_4 antagonist. There may be differences in the experimental conditions, such as parameters of stimulation to nerve activation. It can not be excluded that the 5-HT_4 receptor is involved in other neurons besides cholinergic neurons.

The 5-HT_4 receptor was detected evenly throughout the smooth muscle layer of the urinary bladder by the receptor autoradiography. The 5-HT-induced contractions were partially atropine-sensitive, but tetrodotoxin-resistant. It is well known that tetrodotoxin blocks the nerve conduction to the nerve terminals of the impulse, which was generated in the soma-dendritic regions of neurons, but does not affect the release of the transmitter substances from nerve terminals induced by local depolarizing stimulation (21 – 23); therefore, there is possibility that the 5-HT_4 receptor is located on the cholinergic nerve terminals, although it cannot be elucidated whether all of the 5-HT_4 receptors observed by receptor autoradiography correspond to the 5-HT_4 receptor located on the cholinergic nerve terminals. Thus, 5-HT may act at the 5-HT_4 receptors located on the nerve terminals of cholinergic neurons and evokes the release of ACh.

Acknowledgments

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

1 de Groat WC and Yoshimura N: Pharmacology of the lower urinary tract. Annu Rev Pharmacol Toxicol **41**, 691 – 721 (2001)
2 Testa R, Guarneri L, Poggesi E, Angelico P, Velasco C, Ibba M, Cilia A, Motta G, Riva C and Leonardi A: Effect of several 5-hydroxytryptamine$_{1A}$ receptor ligands on the micturition reflex in rats: comparison with WAY 100635. J Pharmacol Exp Ther **290**, 1258 – 1269 (1999)
3 Conley RK, Williams TJ, Ford APDW and Ramage AG: The role of α_1-adrenoceptors and 5-HT_{1A} receptors in the control of the micturition reflex in male anaesthetized rats. Br J Pharmacol **133**, 61 – 72 (2001)
4 Kakizaki H, Yoshiyama M, Koyanagi T and de Groat WC: Effects of WAY100635, a selective 5-HT_{1A}-receptor antagonist on the micturition-reflex pathway in the rat. Am J Physiol (Integrative Comp Physiol) **280**, R1407 – R1413 (2001)
5 Anderson K-E: Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol Rev **45**, 253 – 308 (1993)
6 Cohen ML: Canine, but not rat bladder, contracts to serotonin via activation of 5-HT_2 receptors. J Urol **143**, 1037 – 1040 (1990)
7 Saxenathe PR, Heiligers J, Mylecharane EJ and Tio R: Excitatory 5-hydroxytryptamine receptors in the cat urinary bladder are of M- and 5-HT_2-type. J Auton Pharmacol **5**, 101 – 107 (1985)
8 Klarskov and Horby-Petersen JP: Influence of serotonin on lower urinary tract muscle in vitro. Br J Urol **58**, 507 – 513 (1986)
9 Holt SE, Cooper M and Wyllie JH: On the nature of the receptors mediating the action of 5-hydroxytryptamine in potentiating responses of the mouse urinary bladder strip to electrical stimulation. Naunyn Schmiedebergs Arch Pharmacol **334**, 333 – 340 (1986)
10 Messori E, Rizzi CA, Candura SM, Lucchelli A, Balestra B and Tonini M: 5-Hydroxytryptamine receptors that facilitate excitatory neuromuscular transmission in the guinea-pig isolated detrusor muscle. Br J Pharmacol **115**, 677 – 683 (1995)
11 Chen H-I: Evidence for the presynaptic action of 5-hydroxytryptamine and the involvement of purinergic innervation in the rabbit lower urinary tract. Br J Pharmacol **101**, 212 – 216 (1990)
12 Barras M, Van der Graaf PH and Angel I: Characterisation of the 5-HT receptor potentiating neurotransmission in rabbit bladder. Eur J Pharmacol **318**, 425 – 428 (1996)
13 Corsi M, Pietra C, Toson G, Trist D, Tuccitto G and Artibani: Pharmacological analysis of 5-hydroxytryptamine effects on

electrically stimulated human isolated urinary bladder. Br J Pharmacol **104**, 719 – 725 (1991)

14 Tonini M, Messori E, Franceschetti GP, Rizzi CA, Castoldi AF, Coccini T and Candura SM: Characterization of the 5-HT receptor potentiating neuromuscular cholinergic transmission in strips of human isolated detrusor muscle. Br J Pharmacol **113**, 1 – 2 (1991)

15 Candura SM, Messori E, Franceschetti GP, D'Agostino G, Vicini D, Tagliani M and Tonini M: Neural 5-HT_4 receptors in the human isolated detrusor muscle: effects of indole, benzimidazolone and substituted benzamide agonists and antagonists. Br J Pharmacol **118**, 1965 – 1970 (1996)

16 Waikar MV, Ford APDW and Clarke DE: Evidence for an inhibitory 5-HT_4 receptor in urinary bladder of Rhesus and Cynomolgus monkeys. Br J Pharmacol **111**, 213 – 218 (1994)

17 Kusunoki M, Taniyama K and Tanaka C: Neuronal GABA release and GABA inhibition of ACh release in guinea-pig urinary bladder. Am J Physiol **246**, R502 – R509 (1984)

18 Shirakawa J, Nakanishi T, Taniyama K, Kamidono S and Tanaka C: Regulation of the substance P-induced contraction via the release of acetylcholine and γ-aminobutyric acid in the guinea-pig urinary bladder. Br J Pharmacol **98**, 437 – 444 (1989)

19 Brown AM, Young TJ, Patch TL, Cheung GW, Kaumann AJ, Gaster L and King FD: [^{125}I]-SB 207710, a potent, selective radioligand for 5-HT_4 receptors. Br J Pharmacol **110**, 10P (1993)

20 Wardle KA, Ellis ES, Baxter GS, Kennett GA, Gaster LM and Sanger GJ: The effects of SB 204070, a highly potent and selective 5-HT_4 receptor antagonist, on guinea pig distal colon. Br J Pharmacol **112**, 789 – 794 (1994)

21 Gonella J, Niel JP and Roman C: Mechanism of the noradrenergic motor control on the lower oesophageal sphincter in the cat. J Physiol (Lond) **306**, 251 – 260 (1980)

22 Starke K: Presynaptic receptor. Annu Rev Pharmacol Toxicol **21**, 7 – 30 (1981)

23 Sawa T, Mameya S, Yoshimura M, Itsuno M, Makiyama K, Niwa M and Taniyama K: Differential mechanism of peptide YY and neuropeptide Y in inhibiting motility of guinea-pig colon. Eur J Pharmacol **276**, 223 – 230 (1995)