

Original Article: Laboratory Investigation**Serotonin in the rat prefrontal cortex controls the micturition reflex through 5-hydroxytryptamine 2A and 5-hydroxytryptamine 7 receptors**Hiroki Chiba,¹ Takeya Kitta,¹ Yu Ohmura,² Madoka Higuchi,¹ Masafumi Kon,¹ Michiko Nakamura,¹ Mitsuhiro Yoshioka² and Nobuo Shinohara¹Departments of ¹Renal and Genitourinary Surgery, and ²Neuropharmacology, Graduate School of Medicine, Hokkaido University, Sapporo, Hokkaido, Japan**Abbreviations & Acronyms**

5-HT = 5-hydroxytryptamine
CMG = cystometrography
CNS = central nervous system
fMRI = functional magnetic resonance imaging
GABA = gamma-aminobutyric acid
ICI = intercontraction interval
IL = infralimbic cortex
MVP = maximal voiding pressure
PAG = periaqueductal grey
PET = positron emission tomography
PFC = prefrontal cortex
PL = prelimbic cortex
SNRI = serotonin noradrenalin reuptake inhibitor
SPECT = single-photon emission computed tomography
SSRI = selective serotonin reuptake inhibitor

Correspondence: Takeya Kitta M.D., Ph.D., Department of Renal and Genitourinary Surgery, Graduate School of Medicine, Hokkaido University, North-15, West-7, Kita-Ku, Sapporo, Hokkaido 060-8638, Japan. Email: kitta@fb3.so-net.ne.jp

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Objectives: To identify the types of serotonin (5-hydroxytryptamine) receptors of the prefrontal cortex related to the micturition reflex.

Methods: Female Sprague–Dawley rats and a microinjection method were used for this study. Stainless steel guide cannulas were implanted bilaterally into the prefrontal cortex, and a polyethylene catheter was inserted into the bladder. Cystometric parameters (intercontraction interval and maximum voiding pressure) were measured before and after injection of any one of six specific antagonists of 5-hydroxytryptamine receptors (5-hydroxytryptamine 1A, 5-hydroxytryptamine 2A, 5-hydroxytryptamine 2C, 5-hydroxytryptamine 3, 5-hydroxytryptamine 4 and 5-hydroxytryptamine 7) into the prefrontal cortex. The prefrontal cortex was divided into two regions, namely the prelimbic cortex and the infralimbic cortex. The experiments were carried out in conscious and free-moving rats.

Results: The intercontraction interval value increased significantly after injection of the 5-hydroxytryptamine 2A receptor antagonist, MDL11939, into the prelimbic cortex of the rat prefrontal cortex (7.68 ± 1.28 vs 9.02 ± 1.41 min, $P < 0.05$), whereas the intercontraction interval value decreased significantly after injection of the 5-hydroxytryptamine 7 antagonist SB269970 into the prelimbic cortex (9.42 ± 0.39 vs 8.14 ± 0.71 min, $P < 0.05$). The intercontraction interval was unaffected by injection of either of these two antagonists into the infralimbic cortex. The other four antagonists (5-hydroxytryptamine 1A, 5-hydroxytryptamine 2C, 5-hydroxytryptamine 3 and 5-hydroxytryptamine 4) had no effect on the intercontraction interval after injection into the prelimbic cortex and the infralimbic cortex. The maximum voiding pressure was unaffected by injection of any one of the six 5-hydroxytryptamine antagonists into the prelimbic cortex and infralimbic cortex.

Conclusions: In the rat prefrontal cortex 5-hydroxytryptamine 2A receptors excite the micturition reflex, whereas 5-hydroxytryptamine 7 receptors inhibit this reflex.

Key words: central nervous system, microinjection, micturition reflex, prefrontal cortex, serotonin.

Introduction

The CNS, consisting of the brain and spinal cord, controls lower urinary tract functions, such as urine storage and voiding. Although the relationship between the brain and the micturition reflex has been established, the underlying mechanisms have not been fully elucidated. Since the late 1990s, functional brain imaging studies, such as SPECT, fMRI and PET, have been used to examine the human brain's role in this reflex.^{1–3} These studies suggested that several regions of the brain become activated during each of the urine storage and voiding phases, and a working model has been proposed for brain control of the micturition reflex.⁴ According to this model, several regions of the brain are involved in the micturition reflex and make complicated connections with each other.

One of these regions, namely the PFC, is one of the most important with respect to facilitating neural control of the micturition reflex. The PFC plays roles in executive functions,

such as learning, memory, inhibitory control, cognition, planning and decision-making.⁵ We previously reported a role for the PFC in the control of micturition using a microdialysis study in rats.⁶ In that study, it was found that the PFC suppressed neural control of the micturition reflex through serotonin (5-HT). However, the types of 5-HT receptors in the PFC that are relevant to the control of this reflex are unknown. There are seven general 5-HT receptor classes, including 14 subtypes. The aim of the present study was to determine the 5-HT receptor subtypes in the PFC related to the micturition reflex using a microinjection method.

Methods

Animals

A total of 40 female Sprague–Dawley rats (bodyweight 236–308 g) were used in this study. They were supplied by Japan SLC (Hamamatsu, Japan). All rats had free access to food and water, and were kept under a 12-h light/dark cycle with a constant temperature of 21°C. The animals were treated in accordance with the National Institutes of Health Animal Care Guidelines, and all animal experiments were approved by the Institutional Animal Experiment Committee of our institution (protocol number: 15-0042).

Surgical procedure

Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and fixed in a stereotaxic frame (Narishige, Tokyo, Japan). Then, bilateral implantation of stainless steel, 24-gauge, 9-mm long guide cannulas was carried out into the medial PFC (3.2 mm anterior and 0.7 mm lateral from the bregma, and 2.0 mm ventral from the dura) according to the atlas of Paxinos and Watson.⁷ Then, into the guide cannulas, 30-gauge dummy cannulas were inserted. After brain surgery, a midline abdominal incision was made to expose the bladder, followed by insertion of a polyethylene catheter (PE-50; Clay-Adams, Parsippany, NJ, USA) into the bladder dome. The catheter tip was then sealed and buried under the back skin. The rats were individually housed, and a 7-day period of recovery from surgery was allowed.

Microinjection protocol

At 7 days after surgery, microinjection was carried out with rats conscious and able to move freely. A bladder catheter was connected to a pressure transducer and to a syringe pump (Model STC-523; Terumo, Tokyo, Japan). AcqKnowledge 3.7.1 software (Biopac Systems, Santa Barbara, CA, USA) was used to measure the ICI and the maximum voiding pressure.

First, the bladder was infused with physiological saline at 0.2 mL/min for 60 min, and several micturition reflexes were observed. Measurements of the ICI and maximum voiding pressure were carried out as control data. Then, after the dummy cannulas were removed, they were replaced with stainless steel, 30-gauge injection cannulas. The rat PFC was divided into two regions, the PL on the dorsal side and the IL on the ventral side of the PFC (Fig. 1a,b). Injection

cannulas were 9.8-mm long for the PL and 11.3-mm long for the IL, and each cannula was connected to polyethylene tubes. The solution containing each 5-HT antagonist was infused at 0.5 μ L/min into the PFC (PL or IL) using a microinjection pump (Carnegie Medicine, Stockholm, Sweden), as previously reported.⁸ To facilitate diffusion of the 5-HT antagonist, the injection cannulas were kept in place for 1 min following each injection. After injection of each 5-HT antagonist, physiological saline was again infused into the bladder at 0.2 mL/min for 60 min. The micturition parameters before and after injection of each 5-HT antagonist were compared. When each experiment was completed, pentobarbital was used to deeply anesthetize the rats. Brains were then removed and fixed with 10% formalin. Toluidine blue was injected before the brain was removed in order to stain each 60- μ m thick coronal section, and the placement of the injection cannula was confirmed (Fig. 2a,b). The data were excluded if cannulas were determined to have been incorrectly placed. Finally, two of 40 rats were excluded due to incorrect insertion. In this protocol, a single drug was injected to the rat PFC, and PL and IL were not injected simultaneously. If we injected the same rat twice, the first

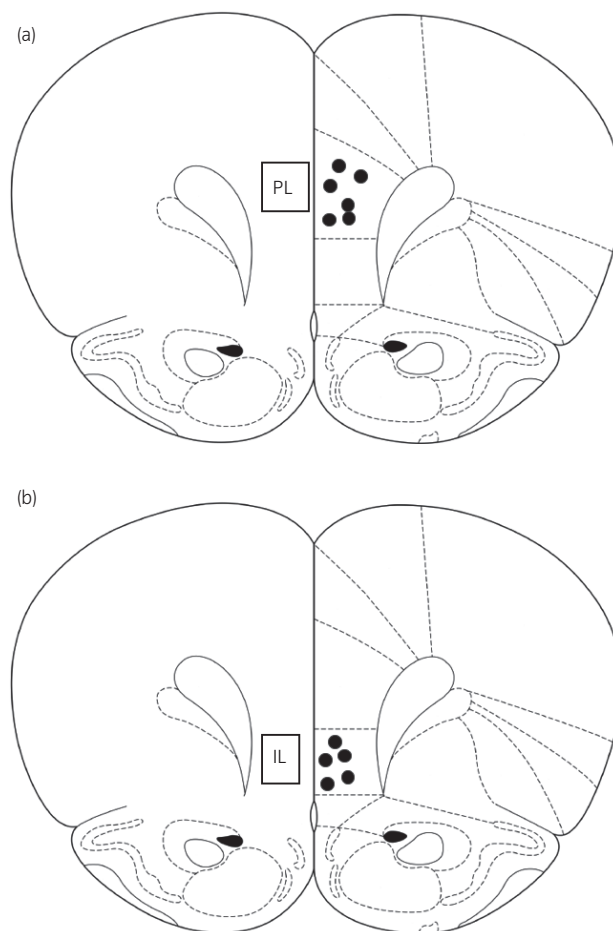


Fig. 1 Coronal section (3.2-mm anterior from the bregma) from the atlas of Paxinos and Watson (1997). The black dots represent the injection points of (a) PL ($n = 6$) and (b) IL ($n = 5$), respectively. These figures show the results of microinjection of MDL11939 as an example.

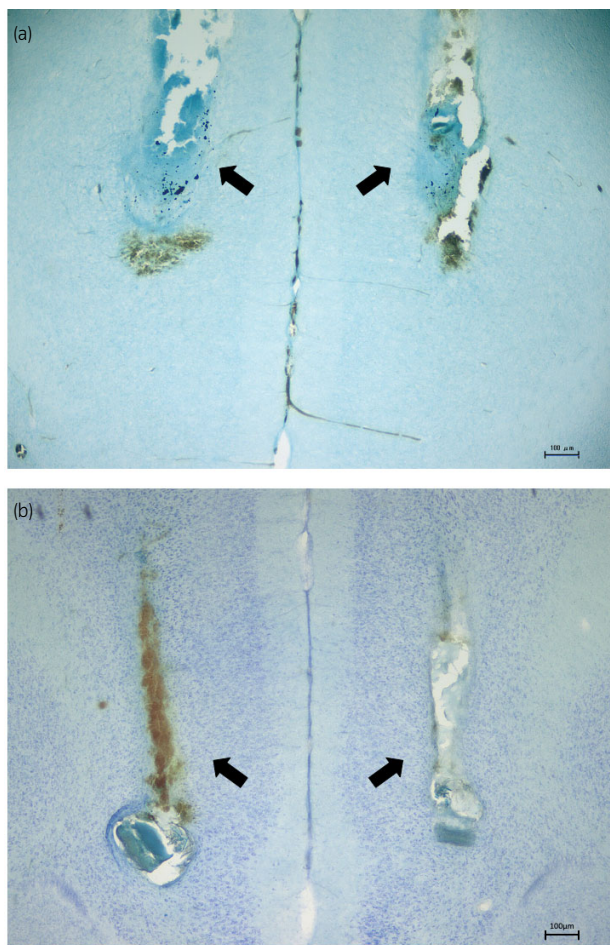


Fig. 2 Photomicrographs of coronal sections of the PFC staining by toluidine blue. The two arrows indicate the path of the bilateral microinjection cannulas and infusion sites. (a) PL. (b) IL.

injection was carried out to the PL, and after an interval of >24 h, the second injection was carried out to the IL.

5-HT antagonists

The 5-HT receptors antagonists used were: 5-HT_{1A} receptors antagonist WAY100635 (300 ng/0.5 µL per side, $K_i = 0.84$ nmol/L), 5-HT_{2A} receptors antagonist MDL11939 (300 ng/0.5 µL per side, $K_i = 6.06$ nmol/L), 5-HT_{2C} receptors antagonist SB242084 (300 ng/0.5 µL per side, $K_i = 9.0$ nmol/L), 5-HT₃ receptors antagonist ondansetron (1000 ng/0.5 µL per side, $K_i = 6.16$ nmol/L), 5-HT₄ receptors antagonist GR113808 (500 ng/0.5 µL per side, $K_i = 57$ pmol/L) and 5-HT₇ receptors antagonist SB269970 (1000 ng/0.5 µL per side, $K_i = 8.9$ nmol/L). Each of MDL11939, SB242084 and GR113808 was dissolved in pure dimethylsulfoxide and diluted with physiological saline (<5% dimethylsulfoxide). The other antagonists were dissolved in physiological saline. The doses of antagonists were determined based on our preliminary results and previous reports.^{9–11} It has been proved that all 5-HT receptors that were used in the present study are expressed in PFC.^{12,13}

Statistical analysis

All data are expressed as the mean \pm standard error of the mean. Micturition parameters before and after injection into the PFC of each 5-HT antagonist were compared using Student's paired *t*-test. A *P*-value <0.05 was considered significant.

Results

The ICI increased significantly after injections of MDL11939, the 5-HT_{2A} receptors antagonist, into the PL region of the rat PFC (7.68 ± 1.28 vs 9.02 ± 1.41 min, $P < 0.05$; Fig. 3a; Table 1). The ICI decreased significantly after injection of SB269970, the 5-HT₇ receptors antagonist, into the PL region (9.42 ± 0.39 vs 8.14 ± 0.71 min, $P < 0.05$; Fig. 3b; Table 1). The ICI was not changed after injection of MDL11939 and SB269970 into the IL region. Injection of any of the other four antagonists into the both PL and IL region had no significant effect on the ICI value. Maximum voiding pressure was not significantly affected by injection of any one of the six 5-HT antagonists into the PL or IL region. ICI seems to be increased after injection of 5-HT_{1A} antagonist (WAY100635); however, it was not statistically significantly different.

Discussion

In the present study, injection of the 5-HT_{2A} receptors antagonist into the PL led to an increase in the ICI, whereas injection of the 5-HT₇ antagonist led to a decrease in the ICI. These results suggest that these two 5-HT receptor types in the PFC have opposite roles with respect to controlling the micturition reflex, and this is especially true for the PL region.

5-HT is a multifunctional biogenic amine acting as a neurotransmitter in the CNS and a signal molecule in the gastrointestinal system. In the CNS, the production of 5-HT occurs in the raphe nuclei, which send axons to most brain regions, including the PFC, and the spinal cord. It is widely known that levels of 5-HT in the CNS are related to mood disorders, especially depression. 5-HT also contributes to the control of the micturition reflex not only in the CNS, but also in the peripheral organs, such as the bladder and urethra, according to previous studies.¹⁴ There are 14 5-HT receptor subtypes in the CNS that have been classified into seven receptor families (5-HT_{1–7}) by their characteristics (structural, functional and pharmacological).¹⁵ Six 5-HT receptor subtypes that we used in the present study had been reported relating to the micturition reflex. In addition, these six receptors are confirmed to be distributed in the PFC.

It is considered that the PFC plays an important role in the control of the micturition reflex, but this has not been sufficiently elucidated. In particular, a few studies have investigated which neurotransmitter types in the PFC regulate the micturition reflex and probed the underlying mechanisms. Although the relationship between 5-HT receptors in the CNS and control of the micturition reflex has been reported, few studies have investigated the relevant areas of the brain;

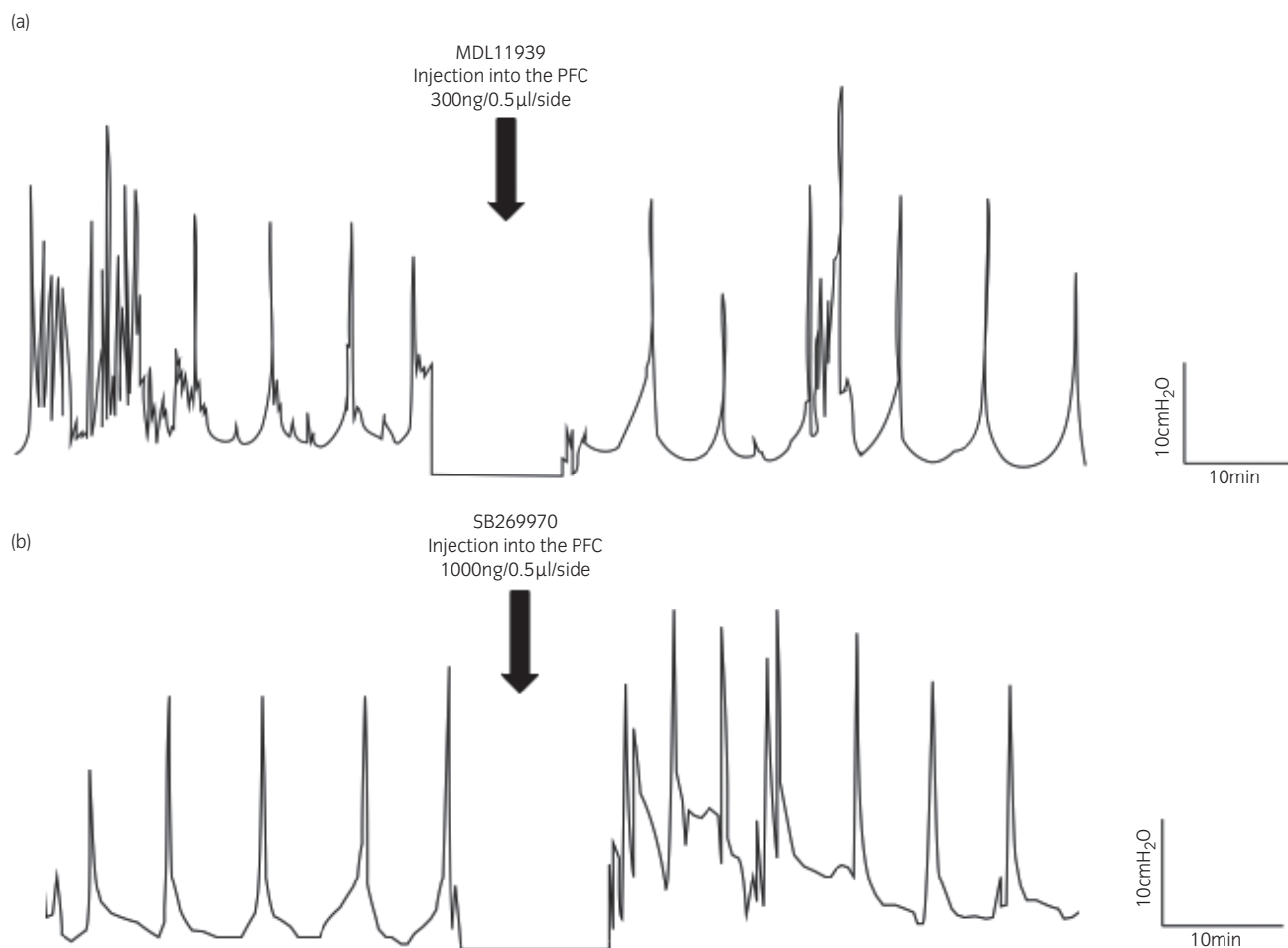


Fig. 3 Representative trace of CMG in free-moving rats, showing the effects of microinjection of 5-HT antagonist into the PFC. (a) ICI was increased after injection of 5-HT_{2A} antagonist, MDL11939 (300 ng/0.5 μL/side), into the PFC (prelimbic region). (b) ICI was decreased after injection of 5-HT₇ antagonist, SB269970 (1000 ng/0.5 μL/side), into the PFC (prelimbic region).

for example, PFC. In the present study, a microinjection technique, which is a major tool in modern neuroscience, was used to evaluate the function of particular regions in the brain with pinpoint accuracy.¹⁶ The advantage of this method is to evaluate the pharmacological effects of agonists or antagonists in the awake condition without the effect of anesthetics. The rat PFC can be divided into two regions; namely, the PL and IL, each of which has a different role in executive functions according to neuropharmacological studies.^{17,18} Therefore, 5-HT receptor antagonists were injected into both regions (Fig. 1a,b).

The present results show that 5-HT_{2A} receptors and 5-HT₇ receptors in the PL, but not the IL, are related to control of the micturition reflex. Each of these receptors is highly expressed in the PFC and involved in executive functions.^{19,20} Interestingly, these two receptors have diametrically opposite functions for regulating micturition; that is, excitatory and inhibitory effects, respectively.

Regarding the function of 5-HT_{2A} receptors in micturition, some studies were previously reported. 5-HT_{2A} receptors have

an excitatory effect on the micturition reflex by activating the external urethral sphincter, whereas 5-HT_{2C} receptors inhibit micturition in rats.²¹ In addition, intrathecal infusion of ketanserin, a 5-HT_{2A} receptors antagonist, was shown to inhibit the function of the external urethral sphincter, thereby reducing voiding efficiency.²² Furthermore, intracerebroventricular injection of a 5-HT_{2A/2B/2C} receptors agonist, α -methyl-5-hydroxytryptamine, enhanced the micturition reflex caused by bladder filling.²³ In the present study, injection of MDL11939, a selective 5-HT_{2A} receptors antagonist, into the PL suppressed the micturition reflex, and this result coincides with previous reports. Furthermore, 5-HT₇ receptors are also involved in controlling the micturition reflex. In a rat model of spinal cord injury, administration of LP44 or LP211, selective 5-HT₇ receptors agonists, improved voiding efficiency by stimulating the external urethral sphincter.^{24–26} In a study by Shimizu *et al.*, SB269970 was found to attenuate the ICI reduction induced by intracerebroventricularly-administered bombesin, suggesting that brain 5-HT₇ receptors play an excitatory role in bladder function control;²⁷ however, in the present study, the ICI was

Table 1 Effects of 5-HT receptor antagonists on the ICI and MVP after injection into the PL or IL

	Vehicle	WAY100635	MDL11939	SB242080	Ondansetron	GR113808	SB269970
ICI (min)							
PL							
Before injection	9.19 ± 1.72	6.92 ± 1.80	7.68 ± 1.28	9.01 ± 1.07	10.7 ± 0.45	11.18 ± 0.88	9.42 ± 0.39
After injection	9.78 ± 1.57	9.31 ± 1.44	9.02 ± 1.41*	9.07 ± 0.71	10.05 ± 0.71	11.38 ± 1.23	8.14 ± 0.71*
	n = 4	n = 7	n = 6	n = 5	n = 4	n = 4	n = 9
IL							
Before injection	10.92 ± 0.26	8.00 ± 1.02	7.32 ± 0.81	10.28 ± 1.48	12.99 ± 1.26	11.08 ± 0.74	10.09 ± 0.63
After injection	10.11 ± 0.15	7.22 ± 1.42	8.03 ± 0.91	10.81 ± 1.31	12.72 ± 1.59	11.25 ± 0.95	10.48 ± 0.73
	n = 4	n = 5	n = 5	n = 5	n = 4	n = 4	n = 4
MVP (cmH ₂ O)							
PL							
Before injection	31.8 ± 1.93	23.22 ± 2.24	27.60 ± 1.76	31.58 ± 1.50	28.28 ± 3.42	30.61 ± 3.14	30.09 ± 2.09
After injection	33.83 ± 5.13	25.38 ± 1.42	27.38 ± 1.80	28.07 ± 2.33	26.39 ± 2.74	29.86 ± 2.84	26.94 ± 1.37
	n = 4	n = 7	n = 6	n = 5	n = 4	n = 4	n = 9
IL							
Before injection	27.62 ± 0.55	24.07 ± 3.57	27.87 ± 1.97	25.99 ± 3.98	28.96 ± 3.37	29.42 ± 2.10	28.51 ± 2.38
After injection	24.58 ± 1.08	27.17 ± 4.20	29.87 ± 1.23	27.63 ± 4.47	29.90 ± 2.59	30.89 ± 3.45	29.12 ± 2.83
	n = 4	n = 5	n = 5	n = 5	n = 4	n = 4	n = 4

Data represent the mean ± standard error. GR113808, 5-HT₄ receptor antagonist; MDL11939, 5-HT_{2A} receptor antagonist; ondansetron, 5-HT₃ receptor antagonist; SB242080, 5-HT_{2C} receptor antagonist; SB269970, 5-HT₇ receptor antagonist; WAY100635, 5-HT_{1A} receptor antagonist. **P* < 0.05 (*n* = 4–9).

decreased by SB269970 injection into the PL. It is possible that the difference of the drug administration route might contribute to the difference in the role of 5-HT₇ receptors for the micturition reflex. 5-HT₇ receptors antagonist was administered into the cerebral ventricle in a study by Shimizu *et al.*,²⁷ however, it was administered into the PFC directly in the present study.

As aforementioned, the serotonergic nervous system for controlling the micturition reflex is complicated, because 5-HT can have opposing effects depending on the types of receptors present and the target organs. In the clinical setting, there is the possibility that unselective serotonergic drugs, such as SSRI or SNRI, cause urinary hesitancy or urinary retention. Under the circumstances that we prescribe such kinds of drugs for patients, for instance, with depression, we should be aware of these lower urinary symptoms. In the current study, regarding the rat PFC (especially the PL), it was found that 5-HT_{2A} receptors had an excitatory effect on micturition, whereas that of 5-HT₇ receptors was inhibitory. These results suggest that 5-HT in the PFC is related to the neural switching system for micturition; that is, 5-HT₇ receptors exerts its effect during the storage phase, whereas 5-HT_{2A} receptors affects voiding. In the present study, there was no significant change in micturition parameters after injection of 5-HT receptor antagonists into the IL region. Although the PL and IL regions are adjacent to each other, their psychological and physiological functions are different.²⁸ Therefore, it is reasonable that the responses to injection of 5-HT_{2A} and 5-HT₇ antagonists were different in both regions.

The present study had several limitations. First, certain other neurotransmitters, such as glutamate, dopamine and GABA, were not investigated. 5-HT in the PFC is associated with modulation of the impulse-dependent release of glutamate and GABA.²⁹ Therefore, it is possible that these neurotransmitters, in addition to 5-HT, are involved in regulating micturition. Second, we have not checked the other regions of the brain that are correlated with controlling the micturition reflex. It is

considered that several regions in the brain are connected for controlling the micturition. Third, we have not tried to inject 5-HT receptor antagonists with different doses. It is possible that some antagonists might have some effects for the micturition reflex using a higher dose than that of the present study. Fourth, 5-HT agonists were not injected in this study. There could be stronger evidence if we carry out microinjection using 5-HT agonists. Further studies are required to elucidate the neural network, including the PFC, that controls the micturition reflex.

In conclusion, the present study showed that 5-HT in the rat PFC controls the micturition reflex through 5-HT_{2A} and 5-HT₇ receptors. 5-HT_{2A} receptors have an excitatory role in micturition, whereas 5-HT₇ receptors have an inhibitory role. These findings might facilitate the development of new treatments for lower urinary tract symptoms.

Conflict of interest

None declared.

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Editorial Comment

Editorial Comment to Serotonin in the rat prefrontal cortex controls the micturition reflex through 5-hydroxytryptamine 2A and 5-hydroxytryptamine 7 receptors

Chiba *et al.* beautifully reported the effect of serotonin (5-hydroxytryptamine 2A [5-HT_{2A}] and 5-hydroxytryptamine 7 [5-HT₇] receptors) on micturition through the prefrontal cortex (PFC) in rats.¹ They concluded that, using microinjection of 5-HT agonists directly into the rat brain (particularly the PFC), 5-HT_{2A} receptors excited the micturition reflex, whereas 5-HT₇ receptors inhibited this reflex. In contrast, 5-HT_{1A}, 5-HT_{2C}, 5-HT₃ and 5-HT₄ agonists had no effect.

PFC is a key area to regulate the micturition function, such as “on-off” initiation, known as executive function and decision-making. Dense pathways exist from the PFC to the basal ganglia (ventral striatum) and the hypothalamus, both further sending fibers, either directly or indirectly, to the periaqueductal gray that involves the spinobulbosacral micturition reflex. Not many studies have been carried out to examine which neurotransmitter is involved above the spinobulbosacral micturition reflex. The finding of Chiba *et al.* showed that 5-HT_{2A} and 5-HT₇ receptors are involved, either facilitatory or inhibitory, depending on the receptor subtypes. As serotonin positron emission tomography has become available to detect brain neurotransmitter changes, we expect

that such change relevant to micturition might be visualized in the human brain *in vivo* in the future.²

There are at least seven subtypes of serotonin receptors (5-HT₁ to 5-HT₇),³ which distribute widely in the brain (neurons from the brainstem raphe nucleus),³ spinal cord and the lower urinary tract.⁴ Clinically, depletion of brain serotonin is known to cause depression. Approximately one-third of depressive patients have lower urinary tract symptoms (mostly urgency/frequency [in some, voiding difficulty]),⁵ whereas antidepressant medication is known to cause urinary retention. Therefore, the study by Chiba *et al.* suggests that we should keep in mind the net effects of serotonergic drugs with adverse events, but they also have the potential to be a central remedy for lower urinary tract symptoms.

Ryuji Sakakibara M.D., Ph.D.
Neurology Division, Department of Internal Medicine,
Sakura Medical Center, Toho University, Sakura, Chiba,
Japan
sakakibara@sakura.med.toho-u.ac.jp

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