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Shimizu T[1], Shimizu S[2], Wada N[3], Takai S[3], Shimizu N[3], Higashi Y[2], Kadekawa K[3], Majima T[3], Saito M[2], Yoshimura N[3]

1. *Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, USA and Department of Pharmacology, Kochi Medical School, Kochi University, Nankoku, Japan,* ***2.*** *Department of Pharmacology, Kochi Medical School, Kochi University, Nankoku, Japan,* ***3.*** *Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, USA*

PHARMACOLOGICAL CHARACTERIZATION OF BRAIN SEROTONIN RECEPTOR SUBTYPES INVOLVED IN BOMBESIN-INDUCED FREQUENT URINATION IN RATS

Hypothesis/aims of study

Stress plays an important role in exacerbation of urinary bladder dysfunction including overactive bladder and bladder pain syndrome. Stress-related information is conveyed to the brain, and then the brain recruits neuronal and neuroendocrine systems for adaptation to stressful conditions. However, the brain pathophysiological mechanisms underlying stress-induced effects on bladder function are unclear. Recently, we reported that bombesin (BB)-like peptides, which are stress-related neuropeptides, centrally induce facilitation of the micturition reflex in rats [1]. Under stress conditions, brain BB-like peptides can modulate activity of the serotoninergic nervous system [2], which has also been shown to modulate micturition [3]. In this study, therefore, we examined the brain mechanisms for the BB-induced frequent urination in rats, focusing on serotonin (5-HT) receptor subtypes, 5-HT_{1A}, 5-HT_2 and 5-HT_7, reported to control micturition in the central nervous system [3].

Study design, materials and methods

In urethane anesthetized (1.0-1.2 g/kg, ip) male Sprague-Dawley rats (300-350 g), a catheter was inserted into the bladder from the dome in order to perform cystometry (12 ml/h saline infusion). Three hours after the surgery, BB or each 5-HT receptor antagonist was icv administered. Saline infusion into the bladder, and intercontraction intervals (ICI) and maximal voiding pressure (MVP) were evaluated 1 hour before the icv administration. (1) In some rats, *p*-chlorophenylalanine (PCPA), which depletes brain 5-HT by inhibiting of the rate-limiting enzyme for 5-HT biosynthesis, was administered (200 mg/kg, ip) once a day for 2 days. After a day of the second administration, BB was administered (0.03 nmol/rat, icv). (2) WAY-100635 (WAY, a 5-HT_{1A} receptor antagonist, 0.3 µg/rat, icv), ritanserin (Ri, a 5-HT_2 receptor antagonist, 0.3 or 1 µg/rat, icv) or SB269970 (SB, a 5-HT_7 receptor antagonist, 0.3 or 1 µg/rat, icv) was pretreated 30 min before BB administration (0.01 or 0.03 nmol/rat, icv).

Results

(1) In PCPA untreated rats, icv administered BB (0.03 nmol/rat) significantly reduced ICI without affecting MVP compared to the pre-treatment of BB (-10~0 min) (Fig. 1). On the other hand, in PCPA treated rats, the BB-induced reduction in ICI was significantly suppressed compared to PCPA untreated rats (Fig. 1). (2) BB at a lower dose (0.01 nmol/rat, icv) showed no significant effect on ICI compared to the pre-treatment of BB (-10~0 min), while the BB significantly reduced ICI compared to the pre-treatment of BB (-10~0 min) after central pretreatment with WAY (Fig. 2), indicating that WAY significantly potentiated the BB-induced effect on ICI (Fig. 2). Central pretreatment with SB significantly suppressed the BB (0.03 nmol/rat, icv)-induced reduction in ICI; however, Ri had no significant effect on the BB-induced response (Fig. 3).

Interpretation of results

(1) It has been reported that the administration condition of PCPA described above resulted in about 95% depletion of 5-HT in the brain. Our results therefore suggest that centrally administered BB induces frequent urination through the brain serotoninergic nervous system as evidenced by the attenuation of the BB-induced ICI shortening in PCPA treated rats. (2) 5-HT_{1A} receptors are well known as autoreceptors, involved in negative feedback control of 5-HT release [3]. In rats centrally pretreated WAY, which can cause an increase in 5-HT release by blockage of these autoreceptors, centrally administered BB induced ICI shortening even at a dose which alone had no effect on ICI. These lines of evidence further suggest that the brain serotoninergic nervous system is involved in the BB-induced frequent urination. In addition, our results of experiments using Ri and SB suggest that at least brain 5-HT_7, but not 5-HT_2, receptors are involved in the BB-induced frequent urination. 5-HT_7 receptors are highly expressed in the brain regions including the hypothalamus, amygdala and cerebral cortex, where BB-like peptides are released by stress exposure. Because these brain regions innervate the midbrain periaqueductal gray matter (PAG), a site coordinating activity of the pontine micturition center, brain BB-like peptides might stimulate these pathways to the PAG through 5-HT_7 receptors, thereby inducing frequent urination.

Concluding message

The brain BB system is involved in facilitation of the rat micturition reflex to induce bladder overactivity through the brain serotoninergic nervous system, and the frequent urination is mediated at least by brain 5-HT_7 receptors. Thus, brain BB and 5-HT_7 receptors might be useful targets for alleviation of stress-induced exacerbation of urinary bladder dysfunction such as overactive bladder and bladder pain syndrome.

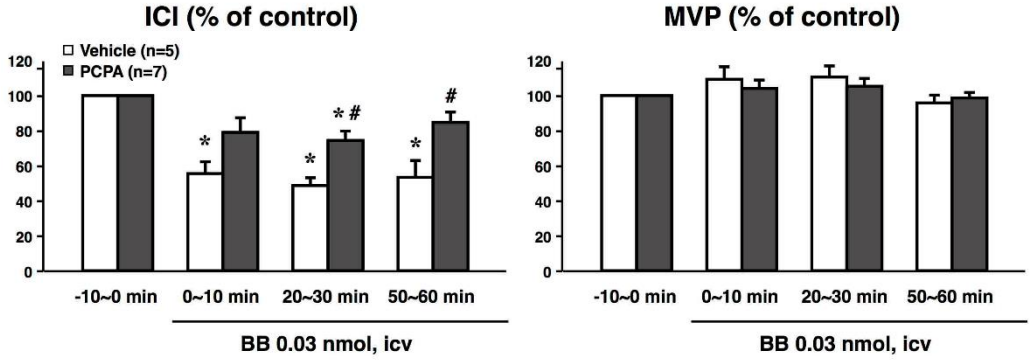

Fig. 1. Effects of centrally administered BB on ICI and MVP in rats with or without PCPA (a serotonin synthesis inhibitor) pretreatment. **P*<0.05, when compared with the Bonferroni method to the pre-treatment of BB (-10~0 min). #*P*<0.05, when compared with an unpaired Student's *t*-test to the Vehicle group. Data calculated as the ratio to the pre-treatment of BB (-10~0 min) values present means ± SEM. BB: bombesin; ICI: intercontraction intervals; MVP: maximal voiding pressure; PCPA; *p*-chlorophenylalanine.

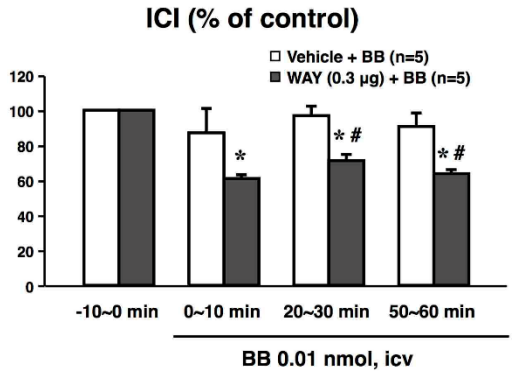

Fig. 2. Effect of central pretreatment with WAY-100635 (WAY, a 5-HT$_{1A}$ receptor antagonist) on the centrally administered BB-induced effect on ICI. **P*<0.05, when compared with the Bonferroni method to the pre-treatment of BB (-10~0 min). #*P*<0.05, when compared with an unpaired Student's *t*-test to the Vehicle + BB group. Data calculated as the ratio to the pre-treatment of BB (-10~0 min) values present means ± SEM. BB: bombesin; ICI: intercontraction intervals.

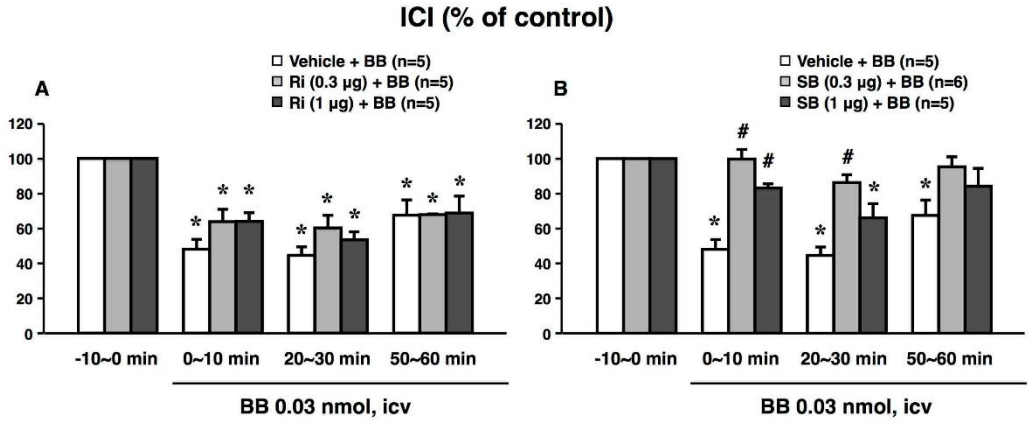

Fig. 3. Effects of central pretreatment with ritanserin (Ri, a 5-HT$_2$ receptor antagonist, A) or SB269970 (SB, a 5-HT$_7$ receptor antagonist, B) on the centrally administered BB-induced reduction in ICI. **P*<0.05, when compared with the Bonferroni method to the pre-treatment of BB (-10~0 min). #*P*<0.05, when compared with the Bonferroni method to the Vehicle + BB group. Data calculated as the ratio to the pre-treatment of BB (-10~0 min) values present means ± SEM. BB: bombesin; ICI: intercontraction intervals.

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Disclosures

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