

BOMBESIN, A STRESS-RELATED NEUROPEPTIDE, CENTRALLY INDUCES FREQUENT URINATION IN THE RAT

Hypothesis/aims of study

Psychological stress exacerbates symptoms of urinary bladder dysfunction including overactive bladder and painful bladder syndrome, but the underlying mechanisms are unknown. Bombesin (BB)-related peptides have been implicated in the mediation/integration of stress responses through brain BB receptors [1]. Actually, in rodent models, acute immobilization stress increases immunoreactivity of the peptides in the brain, and BB receptor antagonists show anxiolytic effects in the elevated plus maze test [1, 2]. These findings indicate a possibility that psychological stress can enhance expression of BB-related peptides in the brain, thereby inducing not only psychological disorders such as anxiety and depression but also exacerbation of symptoms of urinary bladder dysfunction. However, central effects of BB-related peptides on bladder function are not clarified. We previously reported that intracerebroventricularly (i.c.v.) administered BB induced secretion of noradrenaline (NA) and adrenaline (Ad) from the rat adrenal medulla [3]. Thus, we examined effects of centrally administered BB on micturition concerning with their dependence on (1) the sympatho-adrenomedullary (SA) system and (2) brain BB receptors in rats.

Study design, materials and methods

(1) In urethane anesthetized (1.0-1.2 g/kg, ip) male Wistar rats (350-400 g), catheters were inserted into the bladder from the dome, the femoral artery and vein in order to perform cystometry (CMG, 12 ml/h saline infusion), collect blood samples, and infuse saline (1.2 ml/h), respectively. BB (0.1 and 1 nmol/rat, i.c.v.) was serially administered at an interval of 60 min. Plasma levels of NA and Ad were measured at just before the initial administration and at 30 min after each BB injection. In some experiments, acute bilateral adrenalectomy (ADX) was performed before catheter insertion. (2) In urethane anesthetized (1.0-1.2 g/kg, ip) male Sprague-Dawley rats (300-400 g), a catheter was inserted into the bladder from the dome in order to perform CMG (12 ml/h saline infusion). BB (0.01 and 0.03 nmol/rat, i.c.v.) was serially administered at an interval of 60 min. Effects of pretreatment with each BB receptor (BB₁ and BB₂) antagonist (3 nmol/rat, i.c.v.) on the BB- (0.03 nmol/rat, i.c.v.) induced responses were also evaluated. In addition, effects of BB₁ or BB₂ receptor antagonist alone (1 and 3 nmol/rat, i.c.v.) on bladder function were examined.

Results

(1) Both doses of BB (0.1 and 1 nmol) rapidly shortened intercontraction intervals (ICI) without affecting maximal voiding pressure (MVP) (Figs. 1-2). The BB-induced shortening effect was not affected by ADX (Fig. 2). BB at 1 nmol induced significant increments of plasma NA and Ad levels, which were both abolished by ADX (Fig. 3). (2) BB dose-dependently shortened ICI without affecting MVP (Fig. 4) while BB at 0.03 nmol had no significant effects on voiding efficiency (VE), bladder capacity, single voided volume or post-void residual volume (data not shown). Pretreatment with either BB₁ or BB₂ receptor antagonist suppressed the BB-induced shortening of ICI (Fig. 5). Each antagonist alone had no effects on ICI or MVP (data not shown).

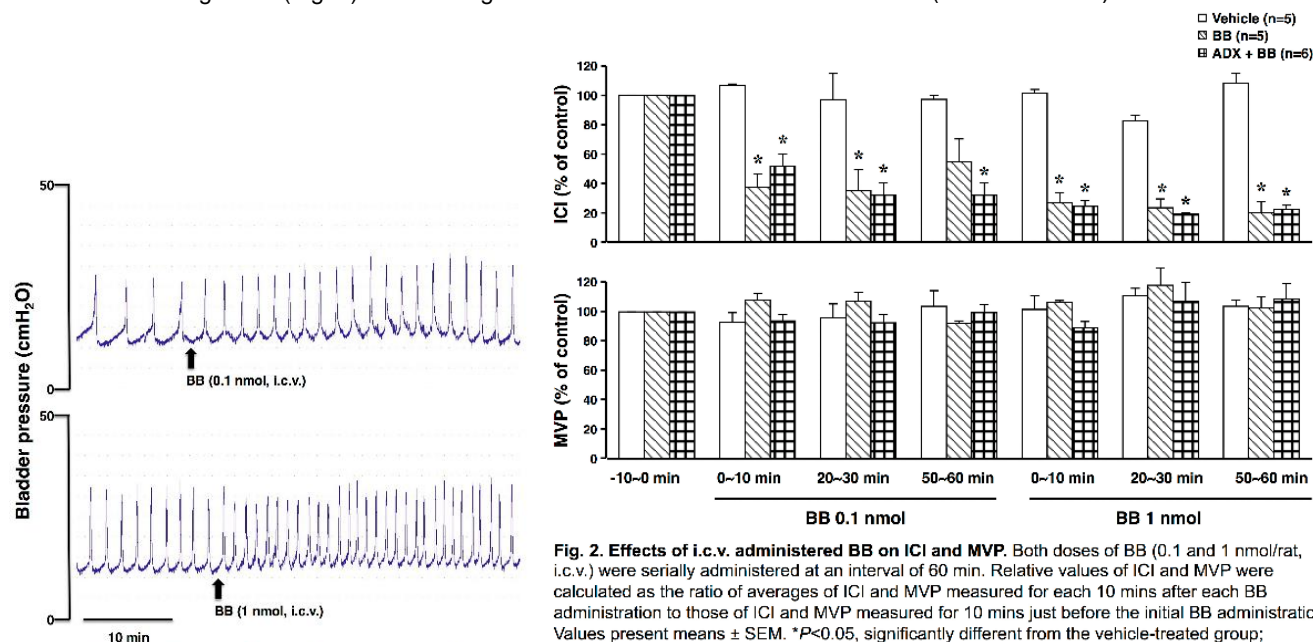


Fig. 2. Effects of i.c.v. administered BB on ICI and MVP. Both doses of BB (0.1 and 1 nmol/rat, i.c.v.) were serially administered at an interval of 60 min. Relative values of ICI and MVP were calculated as the ratio of averages of ICI and MVP measured for each 10 mins after each BB administration to those of ICI and MVP measured for 10 mins just before the initial BB administration. Values present means \pm SEM. * $P < 0.05$, significantly different from the vehicle-treated group; ANOVA, with Bonferroni *post hoc* test. ADX: acute bilateral adrenalectomy; BB: bombesin; ICI: intercontraction intervals; MVP: maximal voiding pressure.

Fig. 1. Representative CMG showing the effects of i.c.v. administered bombesin (BB, 0.1 and 1 nmol) on bladder activity in rats.

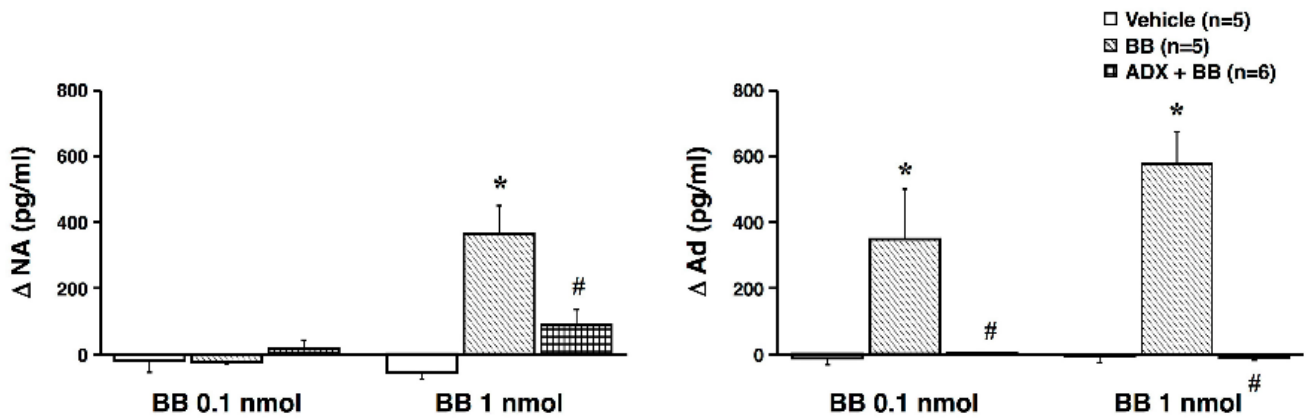


Fig. 3. Effects of i.c.v. administered BB on plasma levels of NA and Ad. Δ NA and Δ Ad: increments of NA and Ad at 30 min after each BB administration in comparison with NA and Ad measured just before the initial BB administration. Values present means \pm SEM. * P <0.05, # P <0.05, significantly different from the vehicle-treated group or the BB-treated group, respectively; ANOVA, with Bonferroni *post hoc* test. ADX: acute bilateral adrenalectomy; Ad: adrenaline; BB: bombesin; NA: noradrenaline.

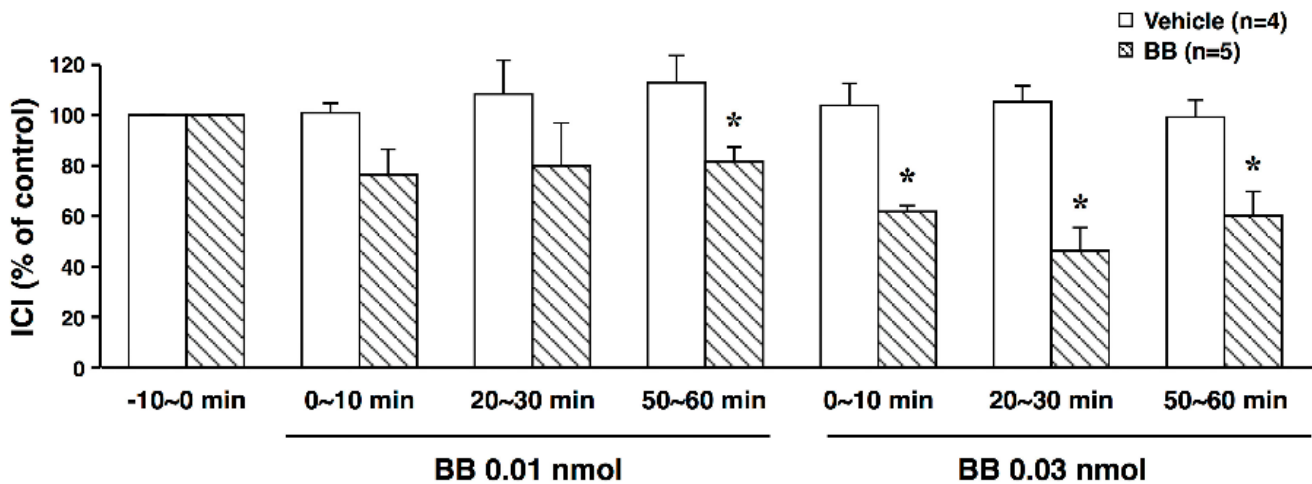


Fig. 4. Effect of i.c.v. administered BB on ICI. Both doses of BB (0.01 and 0.03 nmol/rat, i.c.v.) were serially administered at an interval of 60 min. * P <0.05, significantly different from the vehicle-treated group; Student's *t*-test. Other conditions were same as those in Fig. 2. BB: bombesin; ICI: intercontraction intervals.

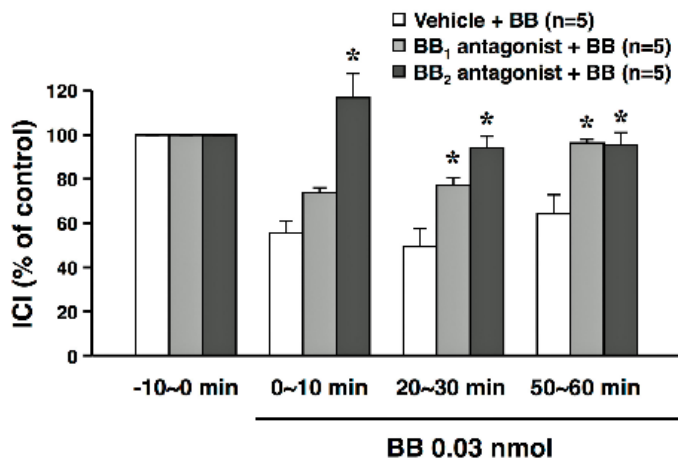


Fig. 5. Effects of BB receptor antagonists on the i.c.v. administered BB-induced shortening of ICI. BB₁ or BB₂ receptor antagonist (3 nmol/rat, i.c.v.) was administered 30 min before the administration of BB (0.03 nmol/rat, i.c.v.). * P <0.05, significantly different from the vehicle- and BB-treated group; ANOVA, with Bonferroni *post hoc* test. Other conditions were same as those in Figs. 2 and 4. BB: bombesin; ICI: intercontraction intervals.

Interpretation of results

(1) These results indicate that BB centrally stimulates bladder activity as shown by shortened ICI. The BB-induced stimulation seems to be independent of the BB-induced activation of central SA outflow because activation of the outflow generally induces urinary storage and ADX had no effect on the BB-induced shortening of ICI. (2) The present study also shows that BB centrally induces bladder overactivity as evidenced by frequent urination via brain BB₁ and BB₂ receptors. Central BB might facilitate

afferent inputs to the micturition center, thereby inducing frequent urination because BB did not affect bladder efferent function as shown by the negative effects on VE. Considering that each BB receptor antagonist had no effects on bladder function, endogenous BB-related peptides do not seem to affect bladder function at least in the normal condition.

Concluding message

The central bombesin system, which is implicated in psychological stress responses, is involved in facilitation of the micturition reflex to induce bladder overactivity, which is independent of sympathetic outflow modulation. Thus, BB receptor antagonists might be useful candidates for alleviation of psychological stress-induced exacerbation of urinary bladder dysfunction such as overactive bladder and painful bladder syndrome.

References

1. Merali Z, Kent P, Anisman H. Role of bombesin-related peptides in the mediation or integration of the stress response. *Cell Mol Life Sci.* 2002;59:272-287.
2. Bédard T, Mountney C, Kent P, et al. Role of gastrin-releasing peptide and neuromedin B in anxiety and fear-related behavior. *Behav Brain Res.* 2007;179:133-140.
3. Yokotani K, Okada S, Nakamura K, et al. Brain prostanoid TP receptor-mediated adrenal noradrenaline secretion and EP3 receptor-mediated sympathetic noradrenaline release in rats. *Eur J Pharmacol.* 2005;512:29-35.

Disclosures

Funding: Funding: JSPS KAKENHI Grant (#26460909), National Institutes of Health (DK088836), Department of Defense (W81XWH-12-1-0565). **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** The Animal Ethics Committee of the Kochi University and the Institutional Animal Care and Use Committee at the University of Pittsburgh