

Bombesin stimulates dorsal raphe nucleus serotonergic neurons *via* a mechanism involving BB1 receptors

Eliyahu Dremencov^{1,2} , Daniil Grinchii¹ , Xia Zhang^{2,3} and Zul Merali^{2,4} 

¹ Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia

² University of Ottawa, Institute of Mental Health Research, Ottawa, Ontario, Canada

³ Neuropsychiatry Research Institute, The Affiliated Hospital of Qingdao University, Qingdao, China

⁴ Brain and Mind Institute, Aga Khan University, Karachi, Pakistan

Abstract. The involvement of serotonin (5-HT) in mood and appetite regulation is well established. This neurotransmitter is released from the neurons located in brainstem raphe nuclei. The excitability of the raphe 5-HT neurons, determining 5-HT neurotransmission, is regulated by various biomolecules, among them gastroenteric hormones, such as gastrin-releasing peptide (GRP). We aimed to examine the effects of the GRP homolog bombesin, and antagonist of bombesin BB1 receptor PD 176252, on the excitability of 5-HT neurons in *in vivo* conditions. In order to achieve its permeability through the blood-brain barrier, bombesin was fused to the cell-membrane transduction domain of the human immunodeficiency virus-type-1 Tat protein. We found that Tat-bombesin complex increased the firing activity of 5-HT neurons; PD 176252 had an opposite effect. GRP might thus regulate 5-HT neurotransmission *via* a mechanism involving BB1 receptors. BB1 ligands may therefore be used for the treatment of mood and eating disorders.

Key words: Dorsal raphe nucleus (DRN) — Electrophysiology *in vivo* — Gastrin-releasing peptide (GRP) — Neuromedin B receptor (BB1) — Serotonin (5-HT)

Introduction

Bombesin is a 14-amino acid peptide originally isolated from the skin of the European fire-bellied toad (*Bombina orientalis*). In mammals, there are two known homologs of bombesin: neuromedin B (NB) and gastrin-releasing peptide (GRP). NB and GRP have been both detected in the central nervous system (CNS) and gastroenteric tract (GT) (Weber 2009). GRP is known to be released from the neurons of the enteric neural system located in the stomach and to trigger gastrin release. GRP secretion itself is triggered by acetylcholine released from the terminals of the vagus efferent fibers (Schubert et al. 1991).

There are three known receptors for bombesin, BB1, BB2, and BB3. All bombesin receptors are G protein-coupled and they are expressed in the CNS and GT. PD 176252 [3-(1H-indol-3-yl)-N-[1-(5-methoxy-pyridin-2-yl)-cyclohexylmethyl]-2-methyl-2-[3 (nitro -phenyl) ureido] propionamide], is a nonpeptide bombesin BB1/BB2 receptor antagonist (Ashwood et al. 1998). It was reported in our previous study that the intra-dorsal raphe nucleus (DRN), as well as systemic (*intraperitoneal: i.p.*) administration of PD 176252 had an anxiolytic effect in rats. The former also increased extracellular serotonin (5-HT) levels in the lateral hippocampus, as measured by *in vivo* microdialysis. It was suggested that BB1 ligands might have an antidepressant and anxiolytic effect, and this effect is mediated, at least in part, *via* 5-HT neurotransmission (Merali et al. 2006; Bédard et al. 2007).

Hippocampal 5-HT transmission is determined by the firing activity of 5-HT neurons of the DRN (Dremencov

Correspondence to: Eliyahu Dremencov, Institute of Molecular Physiology and Genetics, Center of Biosciences, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovakia
E-mail: eliyahu.dremencov@savba.sk

et al. 2003). It is thus likely that the anxiolytic effects of BBI ligands involve modulation of the firing activity of the DRN 5-HT neurons. The present study aims to test this hypothesis.

Materials and Methods

Animals

Adult male Sprague-Dawley rats, ageing 2–3 month and weighting 300–350 g, were ordered from Charles Rivers Laboratories International, Inc., (St. Constant, QC), and kept under standard laboratory conditions (12:12 light–dark cycle with access to food and water *ad libitum*). All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care as approved by Animal Care Committees of the University of Ottawa Institute of Mental Health Research and the Chinese Academy of Sciences.

Chemicals

All chemicals were purchased from Sigma-Aldrich Canada Ltd. (St. Louis, MO). In order to make bombesin permeable through the blood-brain barrier (BBB), it was fused to the cell-membrane transduction domain of the human immunodeficiency virus-type 1 Tat protein (Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg), as explained in our previous studies (Li et al. 2008; Maillet et al. 2008; Wang and Zhang 2017).

In vivo electrophysiology

Rats were anesthetized by chloral hydrate (400 mg/kg, *i.p.*) and mounted in the stereotaxic frame (David Kopf Instruments, Tujunga, CA). Rat body temperature was maintained between 36 and 37°C with a heating pad (Gaymor Instruments, Orchard Park, NY, USA). The scalp was opened, and a 3 mm hole was drilled in the skull for the insertion of electrodes. Glass-pipettes were pulled with a Narishige Vertical Electrode Puller (Amityville, NY, USA) to a fine tip approximately 1 μ m in diameter and filled with 2M NaCl solution. Electrode impedance ranged from 4 to 6 M Ω . The pipettes were inserted into the DRN, 7.8–8.3 mm posterior to bregma and 4.5–7.0 mm ventral to brain surface (Paxinos and Watson 2014) by hydraulic micro-positioner (David Kopf Instruments, Tujunga, CA). The action potentials generated by monoamine-secreting neurons were recorded using the CED Extracellular Recording System (Cambridge, UK). The 5-HT neurons were identified by their regular firing rate of 0.5–2.5 Hz and positive action potential of a long duration of 0.8–1.2

ms (Dremencov et al. 2024). The activity of 5-HT neurons was recorded for at least one minute. After completion of electrophysiological recordings, the animals were euthanized by an overdose of chloral hydrate.

Drugs administration

Tat-bombesin and PD 176252 were dissolved at 10% β -OH-cyclodextryle and administered intravenously, *via* a catheter inserted to the lateral tail vein. Tat-bombesin (15 mg/kg) was injected one hour before electrophysiological recordings. PD 176252 was administered during the recording from the spontaneously active 5-HT neurons, after their basal activity was recorded for at least one minute, at cumulative doses 1–10 mg/kg. A minimal one-minute interval was kept between PD 176252 administrations.

Statistical analysis

The data from the experiments testing the effect of Tat-bombesin are shown as mean $\pm$ standard error of the mean (SEM). Two-tailed Student's *t*-test, preceded by Shapiro-Wilk normal distribution test, was used to compare the 5-HT neuronal firing activity in the vehicle- and Tat-bombesin-treated rats. The data from the experiments testing the effect of Tat-bombesin are shown as percentage $\pm$ SEM of the baseline. Analysis of variance of repeated measures (RM ANOVA), preceded by Shapiro-Wilk normal distribution test and followed by Bonferroni *post-hoc* test, was used to compare the firing activity of 5-HT neurons (expressed as percentage of their basal firing activity) before and after the administration of each dose of PD 176252.

Results

Figure 1 shows the effect of Tat-bombesin (15 mg/kg, *intravenously*) on the firing activity of 5-HT neurons of the DRN. The firing rate of 5-HT neurons of the DRN of Tat-bombesin-treated rats (67 recordings from 5 rats) was significantly ($p < 0.001$, two-tailed Student's *t*-test) higher than that in the vehicle (10% β -OH-cyclodextryle in distilled water)-treated animals (41 recordings from 5 rats).

Figure 2 shows the effect of PD 176252 (1–10 mg/kg, *intravenously*) on the firing activity of 5-HT neurons of the DRN ($n = 5$ neurons from 5 rats). PD17652 significantly ($F_{10,54} = 2.49$, $p = 0.02$, RM ANOVA) and dose-dependently decreased the firing rate of 5-HT neurons. Bonferroni *post-hoc* test confirmed a significant difference between the basal activity of 5-HT neurons and their activity after the administration of 10 ng/kg of PD 176252 ($p = 0.01$).

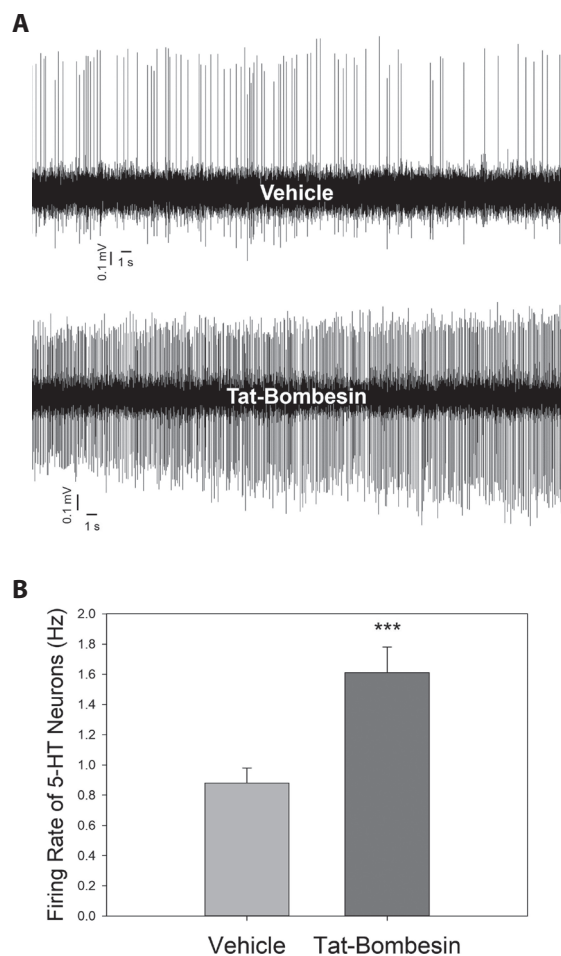


Figure 1. Effect of Tat-bombesin on the excitability of 5-HT neurons. **A.** Representative recordings from the DRN of a vehicle- and Tat-bombesin-treated rats. **B.** Summary effect of 67 neurons from 5 vehicle-treated rats and of 41 neurons from 5 Tat-bombesin-treated rats (mean $\pm$ SEM). *** $p < 0.001$ (two-tailed Student's t -test).

Discussion

We found that the acute administration of Tat-bombesin stimulated 5-HT neurons in the rat DRN. The acute administration of the nonpeptide BB1 receptor antagonist PD 176252 had the opposite effect. Since PD 176252 affinity to the BB1 receptors is five times greater than that to BB2 receptors (Ashwood et al. 1998), it is likely that the stimulatory effect of bombesin on 5-HT neurons is modulated *via* BB1 receptors.

It was reported in our previous study that the acute administration of PD 176252 led to the increased brain 5-HT levels, as measured by *in vivo* microdialysis. It is thus possible that the PD 176252-induced inhibition of 5-HT neuronal firing activity, observed in the present study, might be explained, at least in part, by increase in extracel-

lular 5-HT levels, leading to the activation of presynaptic 5-HT_{1A} autoreceptors negatively regulating the firing activity of 5-HT neurons (Fitzgerald and Watson 2019). Postsynaptic 5-HT_{1A} heteroreceptor-mediated inhibition on the cortical pyramidal neurons projecting to the DRN and tonically activating 5-HT neurons can be involved as well (Hajós et al. 1999).

To the author's best knowledge, our study was first to examine the effect of bombesin, after its permeability through the BBB was achieved using its fusion to the Tat protein, on the excitability of central 5-HT neurons in *in vivo* conditions. A stimulatory effect was observed. Similarly to our findings, it was previously shown that bombesin activates 5-HT neurons in brain slices (Pinnock and Woodruff 1991). A later study from the same laboratory confirmed that the activation of BB receptors led to depolarization of 5-HT neurons in brain slices, *via* a mechanism involving reduction in the conductivity of the potassium channels (Woodruff et al. 1996). A study from another team of researchers showed that the intra-cerebral ventriculi (ICV) administration of the GRP increased hypothalamic 5-HT neurotransmission, as measured by increased levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA). This effect was reversed by a peptide antagonist of BB receptors Leu(13)-psi-CH(2)-NH-Leu(14))-Bombesin (Garrido et al. 2002). Finally, Shimizu and colleagues reported that the ICV administration of bombesin increased frequency of urination in urethane-anesthetized male Sprague-Dawley rats. This effect was blocked by 5-HT_{1A} receptor antagonist WAY 100635 and 5-HT₇ receptor antagonist SB269970, suggesting an involvement of the central 5-HT in bombesin effect on urinary bladder functioning (Shimizu et al. 2017).

While the inhibitory effect of PD 176252 is likely to be explained by an increase in extracellular 5-HT levels, the inhibitory effect of Tat-bombesin might be explained, *vice versa*, by a decrease in extracellular 5-HT. It was indeed reported that bombesin increased hypothalamic 5-HT release in *in vitro* conditions (Saporito and Warwick 1989).

Since 5-HT is fundamental in anxiety, anxiolytic effects of BB1 ligands, observed in our previous studies, can be explained by modulation of the central 5-HT transmission in general and excitability of 5-HT neurons particularly (Merali et al. 2006; Bédard et al. 2007). BB1 ligands might thus be beneficial as antidepressant drugs by their own and/or as adjuncts to the existing antidepressants. Considering the role of the bombesin/GRP-5-HT interaction in the appetite control (Halford 2001), ligands of BB1 receptors can be also beneficial in the treatment of eating disorders. This pilot study might motivate future research on the interactions between gastric peptides and brain monoamines.

The main limitation of this study is non-inclusion of female rats. Since the excitability of 5-HT neurons in male

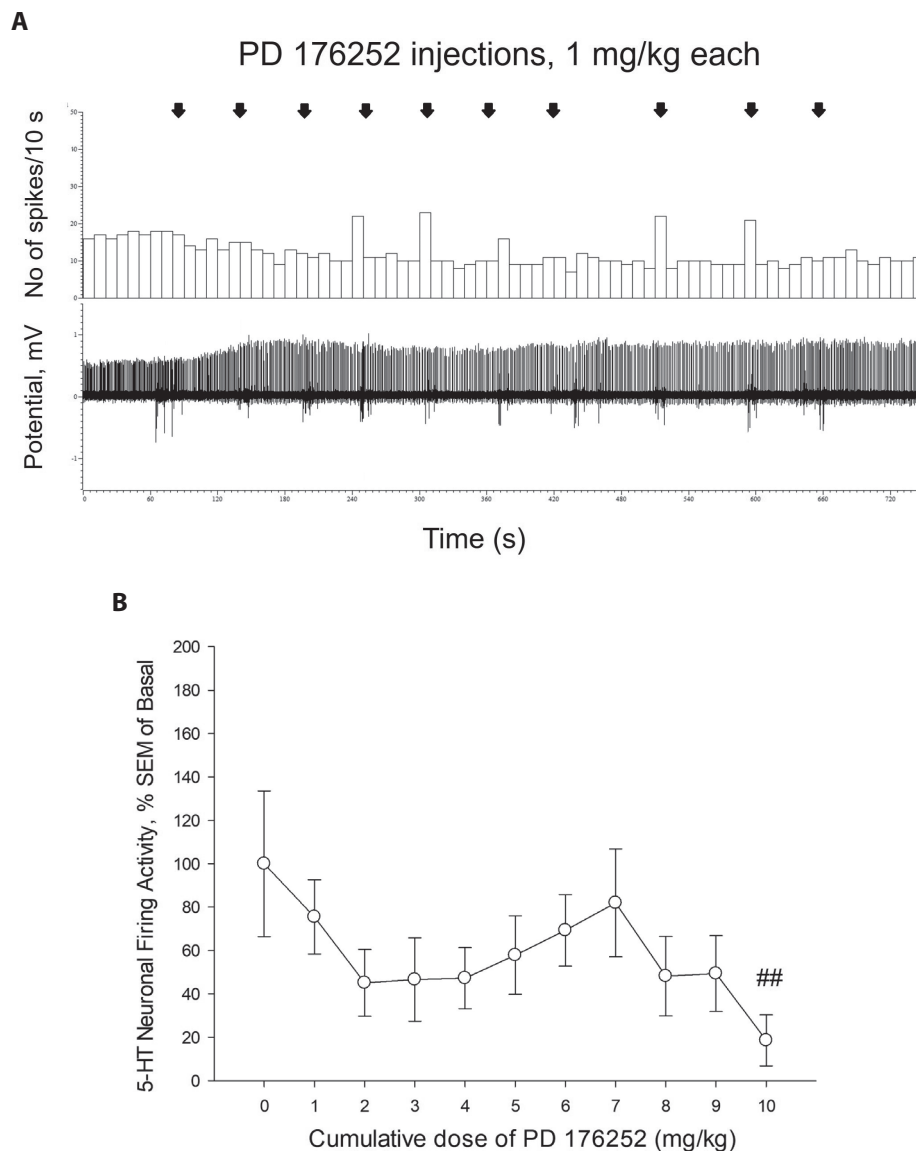


Figure 2. Effect of PD 176252 on the excitability of 5-HT neurons. **A.** Representative recordings from a 5-HT neuron during PD 176252 administration. **B.** Summary effect of 5 neurons from 5 rats (mean $\pm$ SEM). ## $p < 0.05$ (Bonferroni *post-hoc* test).

and female rats is different, bombesin-mediated modulation of 5-HT neuronal firing activity might have sex-related differences as well. In future studies, GRP-5-HT interactions in both sexes should be assessed.

Acknowledgements. The work of ED and DG was supported by the grants APVV-20-0202, VEGA- 2/0057/22, and VEGA-2/0045/24.

Conflict of interest. The authors have no conflicts of interest to declare.

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Received: October 9, 2024

Final version accepted: February 27, 2025

First published online: March 21, 2025