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ORIGINAL ARTICLE

The role of bombesin in the mechanism of pituitary hormones release

Bogusława Baranowska, Ewa Wolińska-Witort, Magdalena Chmielowska, Lidia Martyńska, Agnieszka Baranowska-Bik & Wojciech Bik

Neuroendocrinology Dept., Medical Centre of Postgraduate Education,
Fieldorfa 40, 04-158 Warsaw, Poland

Correspondence to: Professor Bogusława Baranowska MD, PhD
Neuroendocrinology Dpt.
Marymoncka 99, Warsaw, POLAND
TEL/FAX: ++48 22 834 47 12;
EMAIL: zncmkp@free.polbox.pl
zncmkp@polbox.com

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Abstract

OBJECTIVES: Functional studies indicate that bombesin may be involved in many physiological functions, including sensory transmission, the regulation of central autonomic pathways, thermoregulation, secretion of pituitary hormones, gastric and pancreatic secretion, food intake and satiety.
MATERIAL AND METHODS: In order to evaluate the role of bombesin in the mechanism of pituitary hormones release the effects of bombesin on rGH, rTSH, rPRL, rFSH and rLH release were investigated in female Wistar-Kyoto rats. In studies "in vitro" bombesin in a dose of 1nM, 10nM, 100nM was administered to pituitary cell culture. After 60, 120, 240 min. of incubation pituitary hormones were measured. In studies "in vivo" bombesin was injected intraventricularly (icv) in a dose of 0.5 μg/5 μl aCSF (artificial cerebrospinal fluid) for 5 min. (experiment I). In experiment II bombesin was administered intravenously (iv) in a dose of 10 μg. After 60 min, 120 mins the animals were decapitated and serum rGH, rPRL, rTSH, rLH, rFSH concentrations were measured with RIA methods.
RESULTS: Bombesin stimulated rLH, rPRL, rTSH release from cultured pituitary cells. A slight increase of GH was also observed. After intravenous (iv) injection of bombesin an increase in serum rPRL and rGH levels was found. However, the intraventricular (icv) administration of bombesin leads to decrease of serum rGH, rPRL and rTSH concentrations.
CONCLUSION: Our studies "in vitro" and "in vivo" indicate that bombesin may be involved in the modulation of pituitary hormones release.

Introduction

Bombesin – 14 amino acid peptide, originally extracted from the skin of the frog bombina – bombina, was discovered in the gastrointestinal tract, lungs and central nervous system (CNS) with its highest concentration in the hypothalamus. Westendorf et al. [1] characterised for the first time specific, high affinity pituitary bombesin receptors.

Bombesin was reported to have a stimulatory effect upon gastric and pancreatic secretion and upon the release of gastrin, motiline, enteroglucagon, pancreatic polypeptide, cholecystokinine and neurotensin [2, 3, 4, 5, 6].

Bombesin, like peptides, modulates biological and behavioural functions in animals [7].

Bombesin is a brain-gut peptide found in several neuronal groups in the CNS and in peripheral intrinsic gut neurons and sensory neurons. Among various functions of bombesin, the most important may be its role in feeding-related behaviour [7, 8].

Functional studies indicate that bombesin may be involved in many physiological functions, including sensory transmission, the regulation of central autonomic pathways, thermoregulation, secretion of pituitary hormones, gastric and pancreatic secretion, food intake and satiety [9, 10, 11]. Bombesin-like peptides and receptors for these peptides are widely distributed in mammalian peripheral tissues and the central nervous system.

Despite the intensive investigations, the physiological functions of endogenous bombesin-like peptides remain unclear [7].

Since there are controversial opinions about the effect of bombesin on hormones secretion [12, 13, 14], the aim of this work was to evaluate the effect of bombesin on pituitary hormones release in studies *"in vivo"* and *"in vitro"*.

Material and methods

Female Wistar-Kyoto rats (240–260g) were kept under controlled light schedule of 12-h light, 12-h dark (lights on at 0600h) in a temperature-controlled environment (22–24 °C) with free access to food and water. All experimental procedures were approved by the First Warsaw Ethic Committee for Experiments on Animals (the M. Nencki Institute of Experimental Biology, the Polish Academy of Science).

Studies "in vitro"

The method of pituitary cell culture was based on principles according to Artz et al. [15], Renner et al. [16] and Soto et al. [17] and it was published in details previously by Baranowska et al. [18].

Bombesin in a dose of 1nM, 10 nM, 100nM was administered to pituitary cell culture. After 60, 120, 240 min. of incubation, pituitary hormones: rLH, rFSH, rPRL, rGH, rTSH were measured with RIA methods. The experiments were repeated 3 times, 2x 105 /ml cells were presented in each culture.

Studies "in vivo"

Experiment I. Intracerebroventricular administration of bombesin.

Surgical procedure. Three weeks after ovariectomy (OVX) rats were anesthetized with ketamine and implanted with permanent stainless steel cannulas into the third ventricle of the brain according to the rat stereotaxic atlas. The guide cannula location was confirmed with the flow of cerebrospinal fluid and a removable stylet was inserted so that its tip was flush with the tip of guide cannula. During a 7-day period of recovery, rats were transferred to individual cages and handled daily to minimize stress-related effects of the injection procedures. On the day of experiment, freely moving rats were connected to an automatic pump (CMA/100, Sweden) and received an intracerebroventricular (icv) microinjections for 5 min. of 5 µl artificial cerebrospinal fluid (aCSF), or 0,5 µg bombesin dissolved in 5 µl aCSF. Intracereboventricular injections of bombesin or vehicle were performed in the morning (between 09.00–11.00).

At 60, 120 min. after the microinjection of bombesin or vehicle, animals were decapitated and trunk blood was collected in plastic tubes containing 1000 I.U. Trascolane (inhibitor of proteases) per each ml of blood. Blood samples were centrifuged (3000rpm for 20 min.) and serum was frozen until hormonal analyses were performed.

Experiment II – Intravenous injection of bombesin

Bombesin in a dose of 10 µg in 300 µl of saline was injected intravenously (i.v) by tail vein in rats. At 60 mins, 120 mins the animals were decapitated and the trunk blood was collected in plastic tubes containing inhibitor of proteases (Trascolan). The serum samples were stored at – 20 °C until assayed by RIA methods for rLH, rFSH, rPRL, rTSH, rGH.

Results

Effects of bombesin on rLH, rFSH, rPRL, rTSH and rGH in pituitary cell culture were presented in *Figures 1, 2, 3, 4, 5, 6, 7, 8*.

Bombesin stimulated in a dose dependent manner rLH release from cultured pituitary cells after 60 min. incubation (*Fig 1*). Bombesin increased rPRL release in a dose related manner after 60 and 120 min incubation (*Fig. 2, 3*). An increase in GH release was observed at 120 and 240 min incubation after administration of all doses of bombesin (1, 10, 100 nM) (*Fig 4, 5*). Bombesin strongly stimulated TSH release at 60, 120, 240 min. of incubation after administration of all doses (1, 10, 100 nM) (*Fig 6, 7, 8*).

Effects of bombesin injected intracerebroventricularly (icv) and intravenously i.v were demonstrated in *Tables 1, 2*.

The central administration of bombesin leads to a significant decrease of rPRL after 60 and 120 min ($p<0.05$, $p<0.05$, respectively) as well as to a decrease of rTSH ($p<0.05$, $p<0.01$, respectively).

Bombesin injected icv. strongly inhibited GH release after 60 min ($p<0.001$).

However, after 60, 120 min post intravenous (i.v.) injection bombesin significantly increased rPRL ($p<0.01$, $p<0.05$, respectively) as well markedly increased rGH release ($p<0.001$, $p<0.01$, respectively) (*Table 2*).

We did not observe any significant changes in LH, FSH, TSH concentrations in response to bombesin i.v injection.

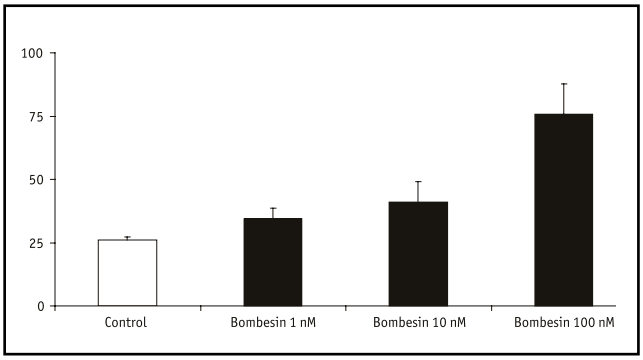

Figure 1. Effect of bombesin on LH release in pituitary cell culture (time of incubation 60 min.) rLH ng/ml

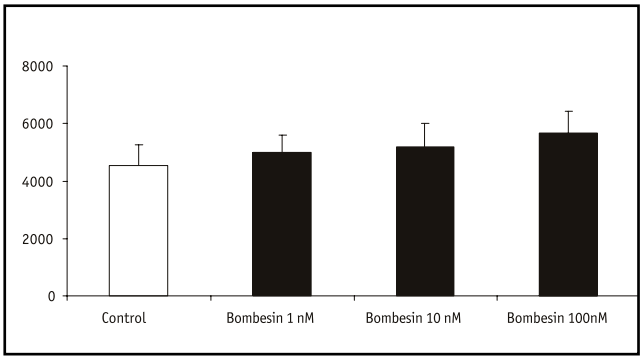

Figure 5. Effect of bombesin on GH release in pituitary cell culture (time of incubation 240 min.) rGH ng/ml

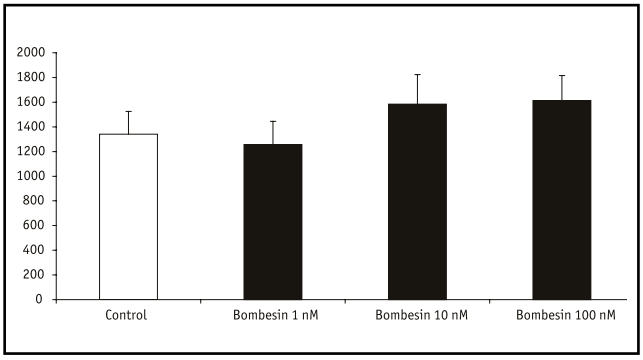

Figure 2. Effect of bombesin on PRL release in pituitary cell culture (time of incubation 60 min.) rPRL ng/ml

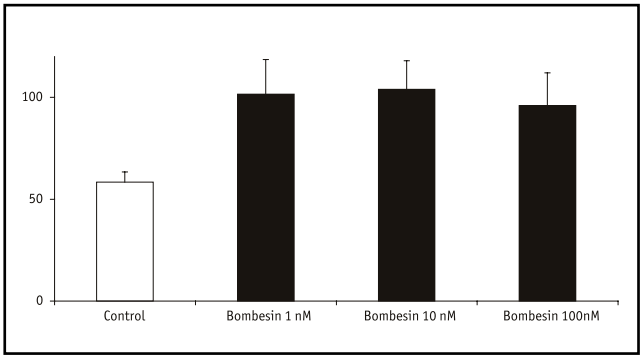

Figure 6. Effect of bombesin on TSH release in pituitary cell culture (time of incubation 60 min.) rPRL ng/ml

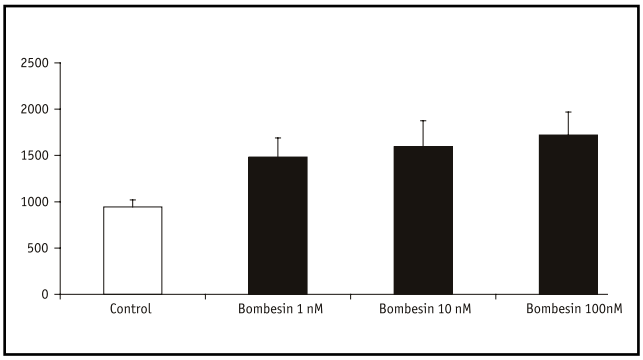

Figure 3. Effect of bombesin on PRL release in pituitary cell culture (time of incubation 120 min.) rPRL ng/ml

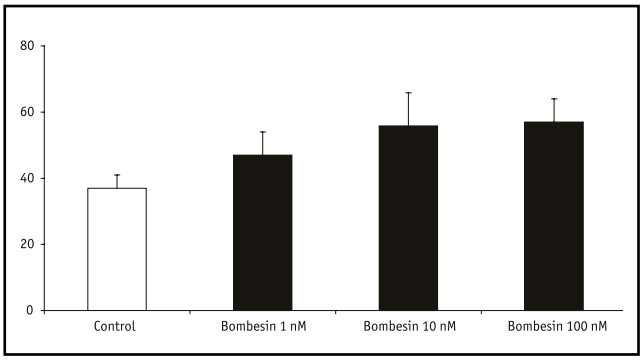

Figure 7. Effect of bombesin on TSH release in pituitary cell culture (time of incubation 120 min.) rPRL ng/ml

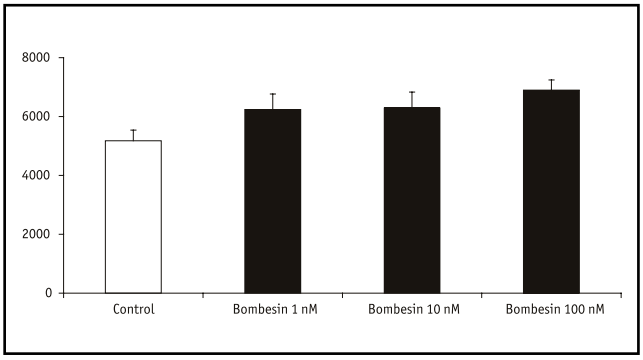

Figure 4. Effect of bombesin on GH release in pituitary cell culture (time of incubation 120 min.) rGH ng/ml

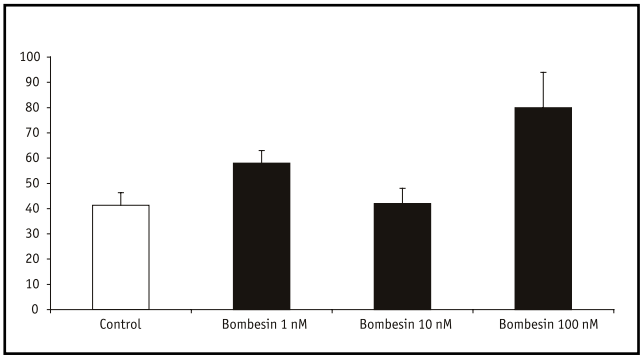

Figure 8. Effect of bombesin on TSH release in pituitary cell culture (time of incubation 240 min.) rTSH ng/ml

Table 1. Effect of bombesin injected centrally (ICV) on pituitary hormones release

Pituitary hormones	Control		Bombesin ICV	
rLH ng/ml	60′	5.3 ± 0.5	60′	4.6 ± 0.5
	120′	4.6 ± 0.4	120′	4.2 ± 0.3
rFSH ng/ml	60′	17.6 ± 1.3	60′	18.2 ± 1.6
	120′	17.7 ± 1.8	120′	15.4 ± 1.5
rPRL ng/ml	60′	1.6 ± 0.1	60′	1.0 ± 0.1 (p<0.05)
	120′	1.9 ± 0.8	120′	1.3 ± 0.1 (p<0.05)
rTSH ng/ml	60′	3.1 ± 0.3	60′	2.4 ± 0.2 (p<0.05)
	120′	2.9 ± 0.1	120′	1.3 ± 0.2 (p<0.01)
rGH ng/ml	60′	16.0 ± 0.7	60′	4.8 ± 1.2 (p<0.001)
	120′	22.7 ± 6.0	120′	24.1 ± 8.0

Table 2. Effect of bombesin injected peripherally (IV) on pituitary hormones release

Pituitary hormones	Control		Bombesin i.v.	
rLH ng/ml	60′	2.9 ± 0.2	60′	2.2 ± 0.1
	120′	2.4 ± 0.1	120′	2.2 ± 0.1
rFSH ng/ml	60′	11.0 ± 1.8	60′	10.1 ± 0.1
	120′	9.4 ± 0.7	120′	16.5 ± 1.0
rPRL ng/ml	60′	2.4 ± 0.1	60′	3.5 ± 0.3 (p<0.01)
	120′	1.6 ± 0.2	120′	2.2 ± 0.2 (p<0.05)
rGH ng/ml	60′	9.0 ± 0.3	60′	23.3 ± 5.0 (p<0.001)
	120′	5.9 ± 0.3	120′	19.6 ± 4.0 (p<0.01)
rTSH ng/ml	60′	6.0 ± 0.3	60′	6.2 ± 0.7
	120′	6.0 ± 0.6	120′	6.9 ± 0.4

Summary

1. Bombesin stimulated rLH, rPRL, rTSH and rGH release in pituitary cell culture.
2. Opposite effects of bombesin on pituitary hormones release were observed after icv and iv injection. Bombesin injected centrally (icv) significantly decreased rPRL, rTSH and rGH release. Bombesin administered peripherally (iv) significantly increased rPRL and rGH release.

Discussion

It has been reported that bombesin may be involved in the neuroendocrine control of pituitary hormone release but the data concerning the effects of bombesin on pituitary hormones secretion are controversial as well as the mechanism of bombesin action remains poorly understood.

Our studies included experiments "*in vitro*"–pituitary cell culture and experiments "*in vivo*" – after centrally (intraventricular – icv) and peripherally (intravenous – i.v) infusion of bombesin.

We demonstrated that bombesin stimulated rLH, rPRL, rTSH and rGH release from cultured pituitary cells. The results have confirmed the data of Houben et al. [19], Major et al. [20].

However, Bicnell et al. [21] observed that bombesin stimulated GH but not PRL secretion from pituitary cultures.

Moriera et al. [22] found that bombesin inhibited TSH release in pituitary explants of rats and stimulated prolactin release in male pituitary cell culture. These authors indicated that the effects of bombesin were estrogen dependent.

Bombesin–like peptide – neuromedin B in high concentration was found in rat thyrotrops and it acts as constitutive autocrine TSH–release inhibitor [23]. These effects may be under control of thyroid hormones [23].

The results achieved by Sander et al. [24] and Olsen at al. [25] showed that bombesin had stimulated ACTH and cortisol secretion. Kent and al. [26] demostrated that bombesin administered icv increased circulating levels of ACTH, corticosterone, epinephrine, norepinephrine indicating that this peptide activates the hypothalamic – pituitary – adrenal (HPA) axis and the sympathetic nervous system. The blockade of CRH receptors leads to attenuation of these effects [27]. These findings suggest that stimulation of HPA axis is mediated via activation of CRH neurons.

Our results revealed opposite effects of bombesin on pituitary hormones release after icv and i.v injection.

Bombesin administered centrally – (icv) inhibited rPRL, rTSH and rGH, however, peripheral injection of bombesin activates PRL and GH release.

Contrary to these results other authors did not observe stimulating effects of bombesin upon pituitary hormone release. Morley et al. [14], Ghatei et al. [28], Hauben et al. [12] could demonstrate no effects of bombesin on GH, PRL, TSH, LH and FSH.

Our results are in agreement with the data of Karashima et al. [29] indicating that icv administration of bombesin suppressed GH and PRL secretion.

Wakabayaski et al. [30] and Kogire et al. [31] connected the inhibiting effect of bombesin upon GH and PRL and a decrease of GH response to GHRH with its modulation of somatostatinergic and dopaminergic tone.

Mitsume et al. [32] observed the inhibitory effect of bombesin on TSH and TRH. Pontiroli et al. [33] demonstrated that PRL, TSH levels were lower in response to TRH, whereas LH levels after LH-RH were higher.

We did not observe any changes in basal LH, FSH levels after icv and i.v injection of bombesin, however, we demonstrated the stimulating effects of bombesin upon LH and FSH in pituitary cultures. These activat-

ing effects of bombesin on gonadotropins were dose dependent.

The controversial opinions concerning the action of bombesin on pituitary hormones release, as well as the opposite effects of bombesin after icv and i.v injection may be partly related and modulated by peripheral hormones such as corticosterone, thyroid hormones and gonadal hormones.

Conclusions

Our studies "*in vitro*" and "*in vivo*" indicate that bombesin may be involved in the modulation of pituitary hormone release.

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