Pharmacological Reports
2011, 63, 448–454
ISSN 1734-1140

Vasopressor and heart rate responses to systemic administration of bombesin in anesthetized rats

Katarzyna Kaczyńska, Małgorzata Szereda-Przestaszewska

Laboratory of Respiratory Reflexes, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, PL 02-106 Warszawa, Poland

Correspondence: Katarzyna Kaczyńska, e-mail: kkacz@cmdik.pan.pl

Abstract:
The aim of the present study was to investigate the effects of aortic depressor nerve (ADN) transection, supranodosal vagi denervation (NG vagi cut) and adrenergic receptor blocker treatment on the cardiovascular responses evoked by systemic injection of bombesin.
The cardiovascular effects were studied in spontaneously breathing rats that were (i) bilaterally, midcervically vagotomized (MC vagi cut) and subjected to section of the aortic depressor nerves, (ii) midcervically vagotomized and subsequently vagotomized at the supranodosal level or (iii) midcervically vagotomized before and after pharmacological blockade of α- or β-adrenergic receptors with phentolamine and propranolol, respectively.
An intravenous bolus of bombesin (10 μg/kg) in midcervically vagotomized and ADN denervated animals increased mean arterial blood pressure (MAP) and heart rate (HR). An approximate 20% increase in blood pressure occurred immediately following bombesin injection and lasted for 2–3 min. Augmentation of the heart rate occurred 30–60 s after the bombesin challenge and persisted for more than 10 min. After section of the supranodosal vagi, bombesin failed to induce an increase in heart rate. Blockade of α-adrenergic receptors with an intravenous dose of phentolamine significantly reduced post-bombesin hypertension.
These results indicate that bombesin-evoked increases in blood pressure do not require aortic depressor nerves and supranodosal vagi and are presumably mediated by the activation of peripheral α-adrenergic receptors. Bombesin-induced tachycardia was dependent on an intact supranodose pathway and was amplified by activation of β-adrenoceptors.

Key words:
bombesin, α- or β-adrenergic receptor, aortic depressor nerve, nodosal vagal afferents

Abbreviations: ADN – aortic depressor nerve, HR – heart rate, MAP – mean arterial blood pressure, MC vagi cut – midcervically vagotomized, NG vagi cut – supranodosally vagotomized, NTS – nucleus tractus solitarii

Introduction

Bombesin is a neuropeptide isolated from the skin of the frog *Bombina bombina* and has a higher affinity than its mammalian counterpart, gastrin releasing peptide (GRP), for both bombesin BB_1 and BB_2 receptors [14]. Increasing evidence suggests that endogenous bombesin and its receptors are involved in central and peripheral regulation of various physiological functions and the pathogenesis of several diseases [10]. There is very little information concerning the effects of exogenous bombesin on the cardiovascular system.

Bombesin has been shown to exert cardiovascular action. In an experimental model of bleeding, it was

shown to be very potent in restoring blood pressure in rats and rabbits [11, 17]. Bombesin applied to the cerebral ventricles or administered systemically raised mean arterial blood pressure and altered heart rate in conscious and anesthetized rats [2, 4, 5, 8, 12]. In these studies, the cardiovascular effects of bombesin were evaluated in animals with an intact neuraxis. Most of the authors ascribed these responses to activation of the adrenergic system. However, the type of adrenergic receptor responsible for these effects remains controversial. Some studies have demonstrated that α-adrenergic blockade eliminated bombesin-elicited hypertension and tachycardia [5, 8], whereas others showed the involvement of the -adrenergic pathway [2, 9].

Our recent results have shown that midcervical vagotomy effectively abrogated all respiratory effects of bombesin, but did not affect hypertension, which was similar in magnitude as in vagally intact rats [15]. Therefore, bombesin's effects on cardiovascular responses remain unclear. The present experiments were conducted to determine the potential neural pathways that mediate the cardiovascular effects of bombesin, which occur independently of lung vagal afferents, and to verify whether hypertension and tachycardia are mediated *via* excitation of either α- or β-adrenoceptors. Therefore, we investigated the contributions of aortic depressor nerves, the supranodosal vagi and the adrenergic nervous system on cardiovascular changes evoked by bombesin.

Materials and Methods

Animals and surgical procedures

All animal procedures complied with the NIH Guide for the Care and Use of Laboratory Animals. Ethical approval for the experimental procedures used in this study was obtained from the local institutional review committee. Twenty eight adult male Wistar rats, weighing 200–250 g, were anesthetized with an intraperitoneal (*ip*) injection of 600 mg/kg urethane (Sigma) and 120 mg/kg α-chloralose (Fluka AG). Supplementary doses were administered intravenously as needed based on responses to nociceptive stimuli. Anesthetized rats were placed in the supine position and were allowed to breathe room air spontaneously. The trachea was exposed, sectioned below the larynx and cannulated. A catheter was inserted into the femoral vein for drug administration and supplemental doses of anesthetic. A second catheter was inserted into the femoral artery to monitor blood pressure. Core body temperature was maintained close to 37–38°C with a heating pad.

In both series of experiments, rats were initially midcervically vagotomized. The midcervical vagi were bluntly dissected and sectioned. In the first series of experiment, the aortic depressor nerves (ADNs) were exposed at the area where they join the superior laryngeal nerves. The ADNs were identified as myelinated nerves running parallel and medial to the vagus nerve low in the neck and joining the superior laryngeal nerve close to the nodose ganglia [1, 18]. The ADNs and the superior laryngeal nerves were cut later in the experiment. In the second series of experiment, the nodose ganglia were bluntly dissected from the surrounding tissue, and their blood supply was preserved. The supranodose vagi were sectioned 2 mm distal to the rostral poles of the ganglia before measuring the cardiovascular variables in the midcervically vagotomized rats.

Apparatus and measurements

Tidal volume signals were monitored with a pneumotachograph head attached to the tracheal cannula linked to a Research Pneumotach System (RSS 100 HR, Hans Rudolph Inc., Kansas City, USA) and a computerized recording system (Windows software version 3.07.02, KORR Medical Technologies Inc., Salt Lake City, USA) for measuring and controlling the respiratory parameters.

Arterial blood pressure and heart rate were measured with a BP-2 blood pressure monitor (Columbus Instruments, Columbus, OH, USA). The recordings were registered on an Omnilight 8 M 36 apparatus (Honeywell, Tokyo, Japan).

Drugs

Each drug was prepared fresh from a powder before each experiment, dissolved in physiological saline and injected as a bolus into the femoral vein. The compounds tested included the following: bombesin (Tocris, UK), injected at a dose of 10 μg/kg; propranolol hydrochloride (1 mg/kg; Tocris, UK); and phentolamine hydrochloride (1 mg/kg; Sigma Aldrich,

USA). All drugs were administered intravenously (*iv*) in a volume of 0.2 µl.

Each drug bolus was immediately flushed with 0.2 ml of normal saline.

The bombesin dose used in these experiments was derived from our previous study [15]. The doses of propranolol and phentolamine were chosen based on previous studies that showed that they blocked adrenergic receptors [8, 13].

Treatment schedule and groups

An intravenous bolus of bombesin was injected into midcervically vagotomized rats in the following experimental protocols: (i) before and after section of the aortic depressor nerves (n = 7); (ii) before and after bilateral supranodosal vagotomy (n = 7); or (iii) in two separate groups of rats 10 min after pharmacological blockade of α- or β-adrenergic receptors with phentolamine hydrochloride or propranolol hydrochloride, respectively (n = 7 for each group).

Each individual value of mean arterial pressure and heart rate was determined by averaging the measured variables for five respiratory cycles before and after injection. The cardiovascular parameters were analyzed just before bombesin injection, at the early post-bombesin phase and 15, 30 s, 1, 2, 3, 5 and 10 min after the challenge.

The maximum post-bombesin increases in mean arterial blood pressure (MAP) and HR were used to compare the effects of the two adrenergic antagonists. The mean value was chosen from the computed time points ranging from the early post-bombesin phase to 30 s for MAP and from 30 s to 5 min for HR comparisons.

Data analysis

All experimental data were analyzed by repeated measures two-way ANOVA with time (pre-challenge and defined time points after challenge) and either innervation status (midcervically vagotomized and ADN-sectioned rats or supranodosally vagotomized rats) or antagonist pretreatment (yes or no) as between condition factors. Differences between individual time points and experimental protocol were evaluated using the Newman-Keuls *post-hoc* test. The confidence limit of $p < 0.05$ was considered to be statistically significant. The results are expressed as the means ± SEM.

Results

In the current study, we did not present or analyze respiratory data. We have previously shown that the respiratory effects of bombesin depend on preserved lung vagal afferentation [15]. Therefore, to avoid any respiratory influences on the cardiovascular responses, the animals used in all experimental groups were initially midcervically vagotomized.

Blood pressure and heart rate changes evoked by bombesin challenge in midcervically vagotomized and ADN-sectioned rats

To determine whether the aortic depressor nerve contributes to the responses to an intravenous bombesin injection, the drug was administered in 7 vagotomized rats before and after section of the ADNs. In midcervically vagotomized rats, the MAP increased immediately after bombesin challenge ($p < 10^{-6}$, two-way ANOVA), with a peak response at 15 s post-challenge; the MAP increased from a pre-drug level of 127.9 ± 5.7 mmHg to 154.2 ± 4.5 mmHg following bombesin injection ($p < 0.001$). Following section of ADNs, bombesin raised blood pressure from 122.5 ± 4.1 to 150.4 ± 3.2 mmHg ($p < 0.01$, n = 7). As shown in Figure 1A, the significant increase in blood pressure lasted until 1 min after bombesin injection in the control group (midcervically vagotomized) and in aortic depressor nerve deprived rats. Bombesin injection increased cardiac rhythm both before and after section of ADN nerves ($p < 10^{-6}$, two-way ANOVA). Increases in cardiac rhythm began earlier in midcervically vagotomized rats (1 min) compared with rats with sectioned ADNs (2 min); this effect was maintained for 10 min in both experimental conditions. As displayed in Table 1 and Figure 1A, the increases in heart rate and blood pressure caused by bombesin were similar in midcervically vagotomized rats and rats treated by both midcervical vagotomy and section of ADNs.

Cardiovascular effects of bombesin challenge in midcervically vagotomized rats before and after supranodosal vagotomy

Intravenous administration of bombesin produced hypertension in all 7 midcervically and supranodose vagotomized rats. A two-way ANOVA showed significant effects of bombesin challenge on blood pressure

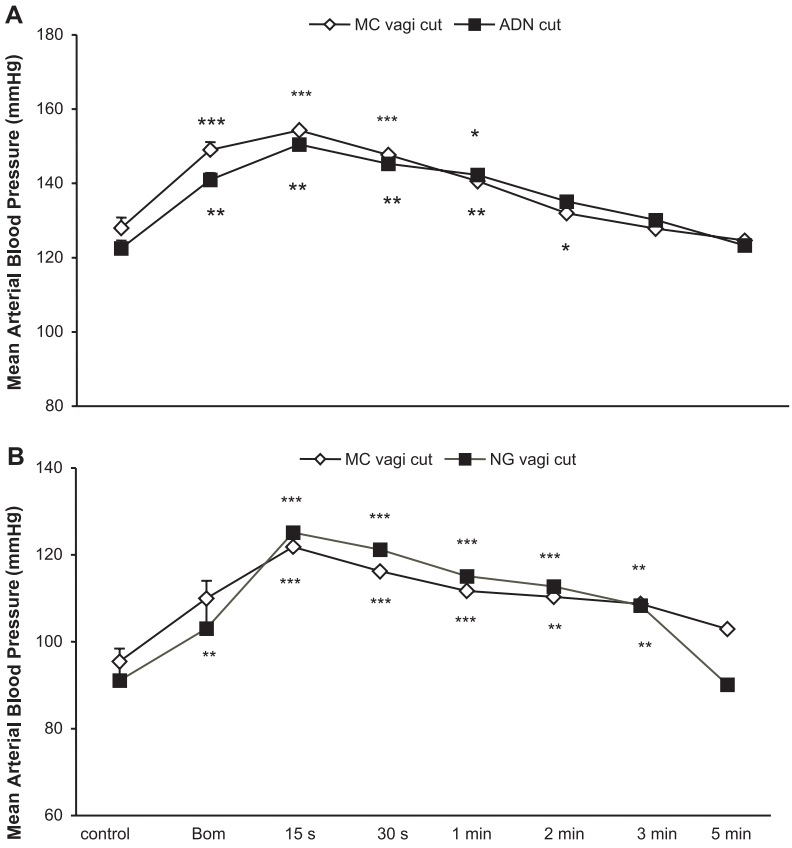

Fig. 1. Effects of aortic depressor nerve section (ADN cut) (**A**) and supranodosal vagotomy (NG vagi cut) (**B**) on post-bombesin changes in mean arterial blood pressure in midcervically vagotomized rats (MC vagi cut). Bom – bombesin; s –second; min – minute; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ *vs.* the respective pre-bombesin baseline value

Tab. 1. Effects of bombesin on heart rate in (**A**) midcervically vagotomized (MC vagi cut) and aortic depressor nerve sectioned (ADN cut) rats (n = 7), and (**B**) midcervically vagotomized rats with supranodosal vagotomy (NG vagi cut) (n = 7)

Heart rate (beats/min)	Baseline	After bombesin						
		Immediate post-bombesin phase	15 s	30 s	1 min	2 min	5 min	10 min
A								
MC vagi cut	495.1 ± 11	496.2 ± 10	502.4 ± 12	510.4 ± 12	515.5 ± 10**	522.2 ± 10***	528.0 ± 12***	529.7 ± 14***
ADN cut	523.1 ± 15$^{###}$	522.5 ± 17$^{###}$	522.0 ± 18$^{##}$	528.0 ± 18	538.7 ± 16$^{##}$	544.4 ± 17*$^{\#}$	545.4 ± 17***	541.4 ± 16*
B								
MC vagi cut	467.7 ± 16	472.7 ± 16	475.2 ± 12	484.7 ± 13**	500.2 ± 15***	509.5 ± 16***	508.5 ± 17***	501.5 ± 17***
NG vagi cut	499.4 ± 14$^{###}$	486.7 ± 16*$^{\#}$	484.1 ± 14*	491.4 ± 15	499.1 ± 13	505.9 ± 14	506.1 ± 13	509.0 ± 12

Two-way ANOVA showed: (**A**) a significant effect of time ($F_{18.65} = 7.42$, $p < 10^{-6}$) and no effect of ADN section ($F_{3.03} = 1.6$, $p = 0.13$) nor time x nerves section interaction ($F_{0.84} = 7.42$, $p = 0.55$); (**B**) a significant effect of time ($F_{30.83} = 7.42$, $p < 10^{-6}$), time x vagotomy interaction effect ($F_{5.96} = 7.42$, $p < 10^{-4}$) and no effect of supranodosal vagotomy ($F_{0.22} = 1.6$, $p = 0.64$) on heart rate. All values are presented as the means ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the respective pre-bombesin value. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ *vs.* the corresponding pre-ADN cut, pre-NG vagotomy value

($p = 10^{-6}$). Before and after supranodosal vagotomy, the MAP increased immediately after bombesin challenge, with a peak value at 15 s. The increase in MAP persisted until 3 min following injection, as compared to the baseline MAP (Fig. 1B).

Bombesin injection augmented heart rate in the control animals ($p = 10^{-6}$, two-way ANOVA). As illustrated in Table 1, a significant increase in heart rate began 30 s post- bombesin challenge and remained elevated for 10 min in the midcervically vagotomized animals. Analysis of variance demonstrated significant interaction between the bombesin challenge and supranodosal vagotomy on heart rate ($p < 10^{-4}$, two-way ANOVA). Supranodose section of the vagi prevented the tachycardia evoked by bombesin injection (Tab. 1).

Cardiovascular responses to bombesin after phentolamine and propranolol pre-treatment

To test whether the cardiovascular effects of bombesin are due to activation of adrenergic receptors, we used α- and β-adrenergic receptor antagonists in 14 rats. Under baseline conditions, administration of both propranolol and phentolamine lowered arterial blood pressure to a similar degree. Injection of propranolol reduced the MAP from 79.44 ± 4.4 mmHg to 58.1 ± 4.6 mmHg ($p < 0.01$). Maximum reduction in MAP after phentolamine reached 62.4 ± 4.5 mmHg compared to the control value of 82.8 ± 3.4 mmHg ($p < 0.001$). Bradycardia was observed following administration of propranolol hydrochloride; heart rate was reduced from the control value of 467.2 ± 4.5 beats/min to 398.7 ± 6.1 beats/min ($p < 0.001$). Phentolamine hydrochloride had no effect on heart rate (418.1 ± 24 beats/min and 413.6 ± 26 beats/min before and after injection, respectively ($p = 0.3$)).

Tab. 2. Peak cardiovascular changes following administration of bombesin in the presence of phentolamine and propranolol

	Δ MAP (mmHg)	Heart rate (beats/min)
Control (bombesin alone)	26.75 ± 4.4	25.57 ± 3.6
Bombesin after phentolamine	2.0 ± 2.4**	17.42 ± 3.7
Control (bombesin alone)	19.84 ± 3.3	23.14 ± 2.3
Bombesin after propranolol	23.95 ± 2.7	↑ 11.00 ± 2.5*

All values are the means ± SEM, (n = 7 for each group). * $p < 0.05$, ** $p < 0.01$ compared to the respective control bombesin value

Pre-treatment with the α-adrenergic receptor antagonist, phentolamine, 10 min before bombesin challenge significantly reduced hypertension induced by bombesin administration. Blockade of -adrenergic receptors with propranolol hydrochloride did not prevent the pressor response to bombesin, but significantly reduced tachycardia compared to the control injection of bombesin alone (Tab. 2).

Discussion

In our recent study, in which we examined the cardio-respiratory effects of systemic bombesin injection in rats, we reported that midcervical vagal deafferentation did not prevent bombesin-induced hypertension, and the pressor response was slightly attenuated by the blockade of BB_2 receptors [15]. Therefore, in this study, we analyzed the mechanisms underlying post-bombesin cardiovascular changes occurring besides lung vagal afferents.

The present results showed that in anesthetized, spontaneously breathing rats subjected to midcervical vagotomy, an *iv* injection of bombesin caused a prompt increase in mean arterial blood pressure and long-lived augmentation of the heart rate (see Fig. 1 and Tab. 1).

Intracerebroventricular (*icv*) administration of bombesin in conscious and anesthetized rats [4, 8] has been shown to produce a pressor response. Hedner et al. [12] attributed the insignificant increase in blood pressure to a direct effect of *icv* injection *per se*, whereas Fisher et al. [8] implicated activation of α-adrenergic receptors within the central nervous system.

Both hypertension induced by an intravenous bombesin challenge in anesthetized [5] and conscious rats [8, 13] and the increases in the heart rate described by these authors are consistent with our present data obtained in midcervically vagotomized animals. The pressor effect reported after central injection of bombesin has been shown to be of extravagal origin because it was not blocked by atropine [4, 8]. This provides further support for our hypothesis that the increases in heart rate and blood pressure recorded in our experiments had a peripheral origin. Further evidence is provided by the experiments by Bayorth and Feuerstein [2], which were performed on pithed rats (a treatment that eliminates CNS control mechanisms), which showed hypertensive and tachycardic responses to an intravenous injection of bombesin.

We presumed that the aortic depressor nerves might set a possible reflexogenic mechanism mediating bombesin effects. The sole function of the aortic bodies is reflex control of vascular resistance [6]. It has been demonstrated that stimulation of the aortic bodies increases blood pressure and has variable effects on heart rate, thus promoting additional reflex control of circulation [3, 6]. In the current experiments, section of ADNs did not appreciably change resting MAP (Fig. 1A). This is consistent with the report by Krieger [16] on isolated transection of the aortic depressor nerves in rats, which resulted in early transient hypertension but had no significant changes on heart rate. Sino-aortic denervation in rats has shown great variability in resting blood pressure, producing either acute [19] or late, sustained hypertension [16]. Our rats had preserved innervation of the carotid bodies; therefore, residual activity of the carotid bodies was present in our study. Under these conditions, bombesin still increased mean arterial pressure and heart rate (Fig. 1A, Tab. 1A). Therefore, the aortic bodies are not critical for the circulatory responses to bombesin.

In this study, the possible role of the peripheral vagal projections to the NTS was further assessed by investigating the cardiovascular consequences of bombesin following supranodose vagotomy. Our investigation provides experimental evidence showing that section of the vagal nerves above the nodose ganglia prevented post-bombesin tachycardia (Tab. 1B). Bombesin has been shown to have direct cardiac action [13], and division of the vagi rostral to the nodose ganglia, which we applied in this study, interrupts the superior cardiac branches of the vagal trunk relaying the tachycardic response. This neurotomy disturbs the passage of baroreceptor fibers from the primary sensory neurons located in the nodose ganglia to the nucleus tractus solitarii, the first site of reflex regulation of cardiovascular functions. Nodose ganglionectomy did not prevent the pressor response to bombesin in this study (Fig. 1B).

This is the first study to assess the cardiovascular effects and the involvement of the aortic depressor nerves and supranodose vagal afferents following systemic injection of bombesin in experimental animals. The contribution of bombesin to cardiovascular function appeared to be indicative of sympathoexcitation. However, the data on the action of bombesin through α- and β-adrenergic receptors are discrepant and largely inconclusive. Erspamer et al. [7] excluded the direct action of bombesin on vascular smooth muscles, showing that bombesin-evoked hypertension in dog, cat and rat occurred after blockade of - and -adrenoceptors. According to Bayorth and Feuerstein [2], pretreatment with the -adrenergic blocker – propranolol, attenuated bombesin-induced increases in blood pressure and HR. In our experiments, intravenous phentolamine, an -adrenergic antagonist, effectively eliminated post-bombesin hypertension (Tab. 2), which indicated that the response was mediated *via* stimulation of α-adrenoceptors, possibly due to adrenaline release. These results are consistent with previous studies demonstrating the abolition of bombesin-induced hypertension and reversal of the pressor response into depressor by phentolamine in rats [5, 8, 13].

The increase in heart rate evoked by bombesin in the current experiments was halved after the blockade of β-adrenoceptors with propranolol (Tab. 2). This is consistent with the results of Bayorth and Feurestein [2]. Janssen et al. [13] has reported diminished increases in heart rate after phentolamine, which were considerably greater in the presence of propranolol. However, in the latter study, the effect of propranolol alone on bombesin tachycardic action was not investigated. Our results showing the contribution of β-adrenergic receptors to post-bombesin tachycardic responses confirm those of Chahl and Walker [5], where tachycardia disappeared after treatment with propranolol in the presence of phentolamine; however, phentolamine alone did not affect the increased heart rate.

Overall, the present results have shown that the pressor and tachycardic effects triggered by bombesin do not require lung vagal afferents or aortic depressor nerves. Cardiovascular stimulation induced by bombesin is presumably relayed separately; the increase in blood pressure was mediated *via* activation of α-adrenergic receptors, and the concomitant tachycardia might occur by activation of -adrenergic receptors with the contribution of vagal cardiac nerves.

Acknowledgment:
We thank Mrs. Teresa Warnawin for her excellent technical assistance.

References:

1. Andrew BL: A laryngeal pathway for aortic baroreceptor impulses. J Physiol, 1954, 125, 352–360.

2. Bayorh MA, Feuerstein G: Bombesin and substance P modulate peripheral sympathetic and cardiovascular activity. Peptides, 1985, 1, 115–120.
3. Brophy S, Ford TW, Carey M, Jones JF: Activity of aortic chemoreceptors in the anaesthetized rat. J Physiol, 1999, 514, 821–828.
4. Brown DR, Gillespie MA: Actions of centrally administered neuropeptides on rat intestinal transport: enhancement of ileal absorption by angiotensin II. Eur J Pharmacol, 1988, 148, 411–418.
5. Chahl LA, Walker SB: Response of the rat cardiovascular system to substance P, neurotensin and bombesin. Life Sci, 1981, 29, 2009–2015.
6. Daly M de Burgh: Peripheral arterial chemoreceptors and respiratory-cardiovascular integration. In: Monographs of the Physiological Society, No. 46, Clarendon Press, Oxford, 1997, 345–366.
7. Erspamer V, Melchiorri P, Sopranzi N: The action of bombesin on the systemic arterial blood pressure of some experimental animals. Br J Pharmacol, 1972, 45, 442–450.
8. Fisher LA, Cave CR, Brown MR: Central nervous system cardiovascular effects of bombesin in conscious rats. Am J Physiol, 1985, 248, H425–H431.
9. Fregnan GB, Glasser AH: Cardiovascular pharmacology of bombesin, a new polypeptide from amphibian skin. Farmaco Sci, 1975, 30, 983–991.
10. Gonzalez N, Moody TW, Igarashi H, Ito T, Jensen RT: Bombesin-related peptides and their receptors: recent advances in their role in physiology and disease states. Curr Opin Endocrinol Diabetes Obes, 2008, 15, 58–64.
11. Guarini S, Tagliavini S, Bazzani C, Bertolini A: Bombesin reverses bleeding-induced hypovolemic shock, in rats. Life Sci, 1989, 45, 107–116.
12. Hedner J, Mueller RA, Hedner T, McCown TJ, Breese GR: A centrally elicited, respiratory stimulant effect by bombesin in the rat. Eur J Pharmacol, 1985, 115, 21–29.
13. Janssen PJ, Gardiner SM, Compton AM, Bennett T: Mechanisms contributing to the differential haemodynamic effects of bombesin and cholecystokinin in conscious, Long Evans rats. Br J Pharmacol, 1991, 102, 123–134.
14. Jensen RT, Battey JF, Spindel ER, Benya RV: International Union of Pharmacology. LXVIII. Mammalian bombesin receptors: nomenclature, distribution, pharmacology, signaling, and functions in normal and disease states. Pharmacol Rev, 2008, 60**,** 1–42.
15. Kaczyńska K, Szereda-Przestaszewska M: Peripheral cardiorespiratory effects of bombesin in anaesthetized rats. Eur J Pharmacol, 2009, 602**,** 157–162.
16. Krieger EM: Neurogenic hypertension in the rat. Circ Res, 1964, 15, 511–521.
17. Maryanovich AT, Aprelkov PA, Shipilov YI, Tchurkina SI, Kostiuchenko AL: Intracerebroventricular injection of bombesin (6–14) restores blood pressure in hemorrhaged rabbits. Resuscitation, 1994, 27, 73–76.
18. Shair HN, Myers MM: Effects of combined carotid sinus and aortic depressor nerve denervations in neonatal rat pups. Biol Neonate,1997, 71, 251–264.
19. Sved AF, Schreihofer AM, Kost CK Jr: Blood pressure regulation in baroreceptor-denervated rats. Clin Exp Pharmacol Physiol, 1997, 1, 77–82.

Received: May 20, 2010; **in the revised form:** October 7, 2010; **accepted:** October 13, 2010.