Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 514 (2005) 151 – 158

www.elsevier.com/locate/ejphar

Brain phospholipase C/diacylglycerol lipase are involved in bombesin BB_2 receptor-mediated activation of sympatho-adrenomedullary outflow in rats

Takahiro Shimizu[a], Shoshiro Okada[a], Naoko Yamaguchi[a], Junichi Arai[a,b], Hiroshi Wakiguchi[b], Kunihiko Yokotani[a,*]

[a]*Department of Neuropharmacology, Program of Neural Integration, Graduate School of Medicine, Kochi University, Nankoku, Kochi 783-8505, Japan*
[b]*Department of Pediatrics, Program of Bio-signaling and Infection Control, Graduate School of Medicine, Kochi University, Nankoku, Kochi 783-8505, Japan*

Received 15 March 2005; accepted 21 March 2005
Available online 29 April 2005

Abstract

Bombesin receptors are mainly divided into two subtypes: BB_1 receptor (neuromedin B-preferring receptor) and BB_2 receptor [gastrin-releasing peptide (GRP)-preferring receptor]. Previously, we reported that intracerebroventricularly (i.c.v.) administered bombesin elevates plasma noradrenaline and adrenaline by production of brain arachidonic acid in rats. Arachidonic acid is released mainly by phospholipase A_2 (PLA_2)-dependent pathway or phospholipase C (PLC)/diacylglycerol lipase-dependent pathway. In the present study, bombesin and GRP elevated plasma catecholamines in a dose-dependent manner (1 and 5 nmol/animal, i.c.v.), while neuromedin B (1, 5 and 10 nmol/animal, i.c.v.) had no effect in urethane-anesthetized rats (bombesin = GRP ⩾ neuromedin B). The bombesin (1 nmol/animal, i.c.v.)-induced response was dose-dependently attenuated by [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (bombesin BB_2 receptor antagonist) (15.3 and 30.6 nmol/animal, i.c.v.) and also by U-73122 (PLC inhibitor) (10 and 100 nmol/animal, i.c.v.) and RHC-80267 (diacylglycerol lipase inhibitor) (1.3 and 2.6 μmol/animal, i.c.v.). However, D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (bombesin BB_1 receptor antagonist) (30 and 100 nmol/animal, i.c.v.), mepacrine (PLA_2 inhibitor) (1.1 and 2.2 μmol/animal, i.c.v.) and U-73343 (inactive analog of U-73122) (100 nmol/animal, i.c.v.) had no effect. These results suggest the involvement of brain PLC/diacylglycerol lipase in the brain bombesin BB_2 receptor-mediated activation of sympatho-adrenomedullary outflow in rats.

Keywords: Brain; Bombesin BB_2 receptor; Phospholipase C; Diacylglycerol lipase: Sympatho-adrenomedullary outflow

1. Introduction

Bombesin is a 14-amino-acid peptide isolated from frog skin. The mammalian counterparts are neuromedin B and gastrin-releasing peptide (GRP), as well as the biologically active GRP fragment neuromedin C. Receptors for the first two ligands to be cloned are bombesin BB_1 receptor (neuromedin B-preferring receptor) and bombesin BB_2 receptor (GRP-preferring receptor) (Wada et al., 1991; Spindel et al., 1990; Benya et al., 1995). A third mammalian bombesin-like receptor subtype, bombesin BB_3 receptor, was cloned, although no endogenous ligand has been identified to date (Fathi et al., 1993).

Bombesin-like peptides have been shown to be distributed in the central nervous system (Moody et al., 1981; Steel et al., 1992) and to have a wide range of functions including learning and memory (Shumyatsky et al., 2002), hypothermia (Tsushima et al., 2003), anorexia (Ohki-Hamazaki et al., 1997) and corticotropin-releasing hormone secretion (Garrido et al., 1999). Although bombesin receptors are widely distributed in the central nervous system, the precise subtype(s) involved in these functions are ill defined. Recently, using knockout strategies, it has been shown that mice lacking either the bombesin BB_1 or

* Corresponding author. Tel./fax: +81 88 880 2328.
E-mail address: yokotani@med.kochi-u.ac.jp (K. Yokotani).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.03.033

BB_2 receptor do not display any gross phenotypic changes from the wild type (Hampton et al., 1998; Ohki-Hamazaki et al., 1999).

Previously, we reported that the centrally administered indomethacin, an inhibitor of cyclooxygenase, effectively attenuated centrally administered bombesin- and arachidonic acid-induced elevation of plasma noradrenaline and adrenaline in rats (Okuma et al., 1996; Yokotani et al., 2000). These results suggest that centrally administered bombesin evokes the release of arachidonic acid in the brain, thereby activating central sympatho-adrenomedullary outflow in rats. Arachidonic acid has been shown to be released mainly by two different pathways: (1) phospholipase A_2 (PLA_2) hydrolyzes the *sn*-2 ester bond of membrane phospholipids, thereby releasing arachidonic acid (Irvine, 1982; Axelrod, 1990); (2) diacylglycerol lipase hydrolyzes diacylglycerol to yield arachidonic acid (Bell et al., 1979; Irvine, 1982; Axelrod, 1990; Balsinde et al., 1991; Hou et al., 1996). Diacylglycerol is formed in different ways. While agonist-induced activation of phosphoinositide-specific phospholipase C (PLC) may produce rapid, transient increases in diacylglycerol along with inositol 1,4,5-trisphosphate, more sustained elevation of diacylglycerol is believed to result largely from phosphatidylcholine breakdown by PLC and phospholipase D (PLD) (Sbrissa et al., 1998). The latter leads to generation of diacylglycerol by phosphatidate phosphohydrolase (Hammond et al., 1995; Exton, 1997).

The present study was designed to characterize the mechanisms involved in the bombesin-induced activation of the central sympatho-adrenomedullary outflow in regard to the bombesin receptor subtype using anesthetized rats.

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats weighing about 350 g were maintained in an air-conditioned room at 22–24 °C under a constant day–night rhythm for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water ad libitum. Under urethane anesthesia (1.2 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.2 ml/h), and the femoral artery was cannulated for collecting blood samples, as shown in our previous papers (Yokotani et al., 1995; Shimizu et al., 2004). After the animal was placed in a stereotaxic apparatus, the skull was drilled for intracerebroventricular administration of test substances using stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of cannula were as follows (in mm): AP −0.8, L 1.5, V 4.0 (AP,

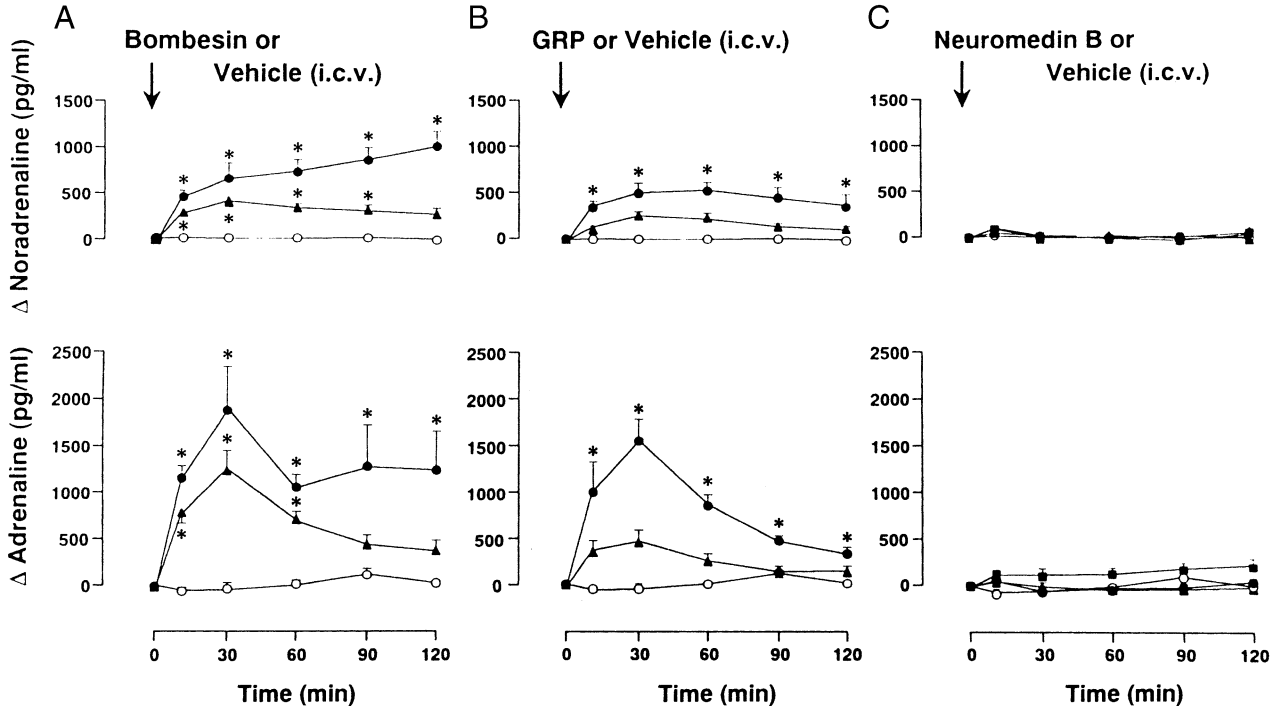

Fig. 1. Effects of bombesin, gastrin-releasing peptide (GRP), and neuromedin B on plasma levels of noradrenaline and adrenaline. Δ Noradrenaline and Δ Adrenaline: increments of noradrenaline and adrenaline above the basal. Arrow indicates the intracerebroventricular (i.c.v.) administration of vehicle (saline 10 μl/animal), bombesin (1 and 5 nmol/animal), GRP (1 and 5 nmol/animal), or neuromedin B (1, 5 and 10 nmol/animal). (A) ○, vehicle ($n=4$); ▲, bombesin (1 nmol/animal) ($n=5$); ●, bombesin (5 nmol/animal) ($n=4$). (B) ○, vehicle (cited from A); ▲, GRP (1 nmol/animal) ($n=9$); ●, GRP (5 nmol/animal) ($n=6$). (C) ○, vehicle (cited from A); ▲, neuromedin B (1 nmol/animal) ($n=6$); ●, neuromedin B (5 nmol/animal) ($n=5$); ■, neuromedin B (10 nmol/animal) ($n=4$). Each point represents the mean±S.E.M. *Significantly different ($P<0.05$) from vehicle-treated group. The actual values for noradrenaline and adrenaline at 0 min were 335.4±30.8 and 337.1±50.0 pg/ml ($n=43$), respectively.

anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas of Paxinos and Watson (1986).

Three hours were allowed to elapse before the application of peptides [bombesin, neuromedin B and gastrin-releasing peptide (GRP)] or blocking reagents. In the case of using blocking reagents, bombesin was i.c.v. administered 15 min after application of [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (BEA) and D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127), 30 min after application of 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U-73122), 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidine-dione (U-73343) and 1,6-bis(cyclohexyloximinocarbonylamino)hexane (RHC-80267), and 180 min after application of mepacrine due to their slightly elevating effects on the basal plasma levels of catecholamines. Bombesin, neuromedin B and GRP dissolved in sterile saline were slowly injected into the right lateral ventricle in a volume of 10 μl/animal using a 25-μl Hamilton syringe. BEA, BIM-23127 and mepacrine dissolved in sterile saline were intracerebroventricularly (i.c.v.) administered in a volume of 5 μl/animal, while U-73122, U-73343 and RHC-80267 dissolved in 2.5 μl of 100% *N*,*N*-dimethylformamide (DMF)/animal were i.c.v. administered using a 10-μl Hamilton syringe.

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by the Kochi University.

2.2. Measurement of plasma catecholamines

Blood samples (400 μl) were collected through an arterial catheter and were preserved on ice during experiments. Plasma was prepared immediately after the final sampling. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically with high-performance liquid chromatography (HPLC) (Shimizu et al., 2004). Briefly, after centrifugation, the plasma (100 μl) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of double deionized water, 1 ml of 1.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA and 1 ng of 3,4-dihydroxybenzylamine as an internal

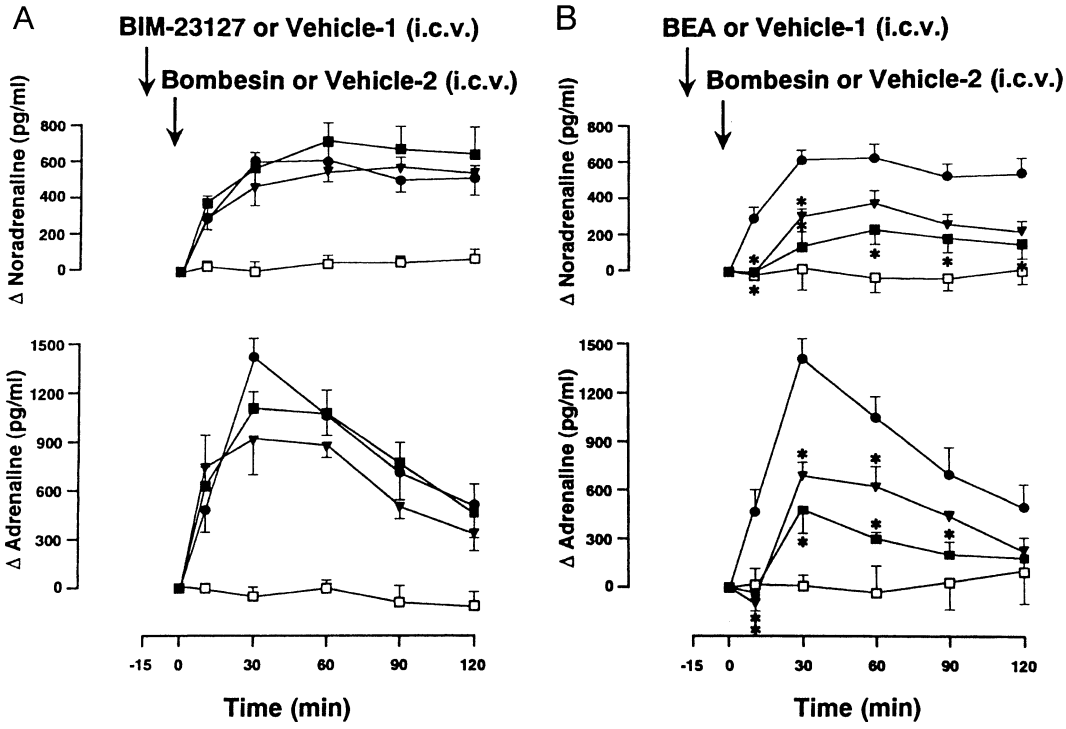

Fig. 2. Effects of D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127) and [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (BEA) on the bombesin-induced elevation of plasma noradrenaline and adrenaline. BIM-23127 (a bombesin BB_1 receptor antagonist) (30 and 100 nmol/animal), BEA (a bombesin BB_2 receptor antagonist) (15.3 and 30.6 nmol/animal) or vehicle-1 (5 μl saline/animal) was i.c.v. administered 15 min before the administration of bombesin (1 nmol/animal, i.c.v.) or vehicle-2 (10 μl saline/animal, i.c.v.). (A) ●, vehicle-1 plus bombesin ($n=6$); ▼, BIM-23127 (30 nmol/animal) plus bombesin ($n=5$); ■, BIM-23127 (100 nmol/animal) plus bombesin ($n=4$); □, BIM-23127 (100 nmol/animal) plus vehicle-2 ($n=4$). (B) ●, vehicle-1 plus bombesin (cited from A); ▼, BEA (15.3 nmol/animal) plus bombesin ($n=4$); ■, BEA (30.6 nmol/animal) plus bombesin ($n=7$); □, BEA (30.6 nmol/animal) plus vehicle-2 ($n=4$). *Significantly different ($P<0.05$) from vehicle-1 plus bombesin. Other conditions were the same as those of Fig. 1. The actual values for noradrenaline and adrenaline at 0 min were 401.0±72.6 and 330.6±57.4 pg/ml in the group pretreated with vehicle-1 ($n=6$); 468.9±96.1 and 277.5±85.0 pg/ml in the group pretreated with BIM-23127 (30 nmol/animal) ($n=5$); 408.2±73.3 and 430.3±95.2 pg/ml in the group pretreated with BIM-23127 (100 nmol/animal) ($n=8$); 393.0±97.7 and 454.2±179.7 pg/ml in the group pretreated with BEA (15.3 nmol/animal) ($n=4$); 444.5±41.4 and 469.0±85.2 pg/ml in the group pretreated with BEA (30.6 nmol/animal) ($n=11$), respectively.

standard. The tube was shaken for 10 min and the alumina was washed three times with 4 ml of ice-cold double-deionized water. Then, catecholamines adsorbed onto the alumina were eluted with 300 μl of 4% acetic acid containing 0.1 mM disodium EDTA. A pump (EP-300: Eicom, Kyoto, Japan), a sample injector (Model-231XL; Gilson, Villiers-le-Bel, France) and an electrochemical detector (ECD-300: Eicom) equipped with a graphite electrode were used with HPLC. Analytical conditions were as follows: detector, +450 mV potential against a Ag/AgCl reference electrode; column, Eicompack CA-50DS, 2.1 × 150 mm (Eicom); mobile phase, 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH 6.0) containing 50 mg/l EDTA dihydrate, 0.75 g/l sodium 1-octanesulfonate and 15% methanol at a flow of 0.18 ml/min; injection volume, 40 μl. The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine. By this assay, coefficients of variation for intra- and inter-assay were 3.0% and 3.7%, respectively, and 0.5 pg of noradrenaline and adrenaline were accurately determined.

2.3. Treatment of data and statistics

All values are expressed as the means ± S.E.M. The data were analyzed by repeated-measures analysis of variance (ANOVA), followed by post hoc analysis with the Bonferroni method for comparing a control to all other means. *P* values less than 0.05 were taken to indicate statistical significance.

2.4. Compounds

The following drugs were used: synthetic bombesin, gastrin-releasing peptide (GRP), neuromedin B (Peptide Institute, Osaka, Japan); [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (BEA), D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127) (Bachem AG, Bubendorf, Switzerland); mepacrine (quinacrine) dihydrochloride (Research Biochemicals, Natik, MA, USA); RHC-80267, U-73122, U-73343 (Biomol Research Lab., Plymouth Meeting, PA, USA). All other reagents were the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effects of bombesin, gastrin-releasing peptide and neuromedin B on plasma catecholamines

Intracerebroventricularly (i.c.v.) administered vehicle (10 μl saline/animal) and blood sampling for 6 times over 120 min had no effect on the basal plasma levels of either noradrenaline and adrenaline (Fig. 1).

Bombesin (1 and 5 nmol/animal, i.c.v.) dose-dependently elevated plasma levels of noradrenaline and adrenaline (Fig. 1A). These responses reached a maximum 30 min after administration of bombesin and then declined toward their basal levels, while a higher dose of this peptide (5 nmol/animal) persistently increased plasma noradrenaline levels throughout the experiments.

Gastrin-releasing peptide (GRP) (1 and 5 nmol/animal, i.c.v.) also elevated plasma levels of catecholamines in a dose-dependent manner (Fig. 1B). These responses reached a maximum 30 min after administration of GRP and then declined toward their basal levels. On the other hand, neuromedin B (1, 5 and 10 nmol/animal, i.c.v.) had no effect on the plasma levels of both catecholamines (Fig. 1C).

3.2. Effects of D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127) (bombesin BB_1 receptor antagonist) and [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (BEA) (bombesin BB_2 receptor antagonist) on the bombesin-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 μl saline/animal, i.c.v.) and vehicle-2 (10 μl saline/animal, i.c.v.) had no effect on the basal plasma levels of catecholamines (data not shown).

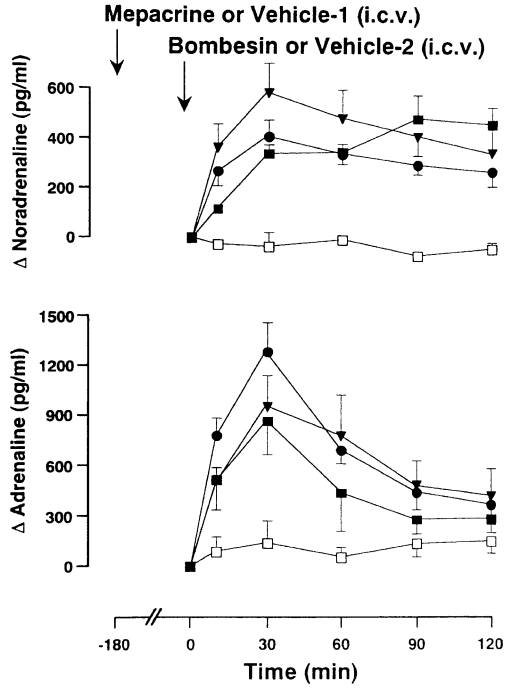

Fig. 3. Effect of mepacrine on the bombesin-induced elevation of plasma noradrenaline and adrenaline. Mepacrine [1.1 and 2.2 μmol (500 and 1000 μg)/animal] or vehicle-1 (5 μl saline/animal) was i.c.v. administered 180 min before the administration of bombesin (1 nmol/animal, i.c.v.) or vehicle-2 (10 μl saline/animal, i.c.v.). ●, vehicle-1 plus bombesin (n = 5); ▼, mepacrine (1.1 μmol/animal) plus bombesin (n = 5); ■, mepacrine (2.2 μmol/animal) plus bombesin (n = 4); □, mepacrine (2.2 μmol/animal) plus vehicle-2 (n = 4). Other conditions were the same as those of Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were 382.2 ± 86.7 and 437.3 ± 197.1 pg/ml in the group pretreated with vehicle-1 (n = 5); 271.5 ± 39.1 and 400.8 ± 108.1 pg/ml in the group pretreated with mepacrine (1.1 μmol/animal) (n = 5); 421.0 ± 59.0 and 436.2 ± 87.0 pg/ml in the group pretreated with mepacrine (2.2 μmol/animal) (n = 8), respectively.

Pretreatment with D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127) (a selective antagonist of bombesin BB_1 receptor) [30 and 100 nmol (35.4 and 118 μg)/animal, i.c.v.] and [D-Phe^6, des-Met^{14}]-bombesin(6–14) ethylamide (BEA) (a selective antagonist of bombesin BB_2 receptor) [15.3 and 30.6 nmol (15 and 30 μg)/animal, i.c.v.] also had no effect on the basal plasma levels of catecholamines (Fig. 2A and B).

The bombesin (1 nmol/animal, i.c.v.)-induced elevation of plasma noradrenaline and adrenaline was not influenced by D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127) [30 and 100 nmol (35.4 and 118 μg)/animal, i.c.v.], while [D-Phe^6, des-Met^{14}]-bombesin(6–14) ethylamide (BEA) attenuated the bombesin-induced elevation of both catecholamines in a dose-dependent manner [15.3 and 30.6 nmol (15 and 30 μg)/animal, i.c.v.] (Fig. 2A and B).

3.3. Effect of mepacrine (an inhibitor of PLA_2) on the bombesin-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 μl saline/animal, i.c.v.) and vehicle-2 (10 μl saline/animal, i.c.v.) had no effect on the basal plasma levels of catecholamines (data not shown). Pretreatment with mepacrine [1.1 and 2.2 μmol (500 and 1000 μg)/animal, i.c.v.] also had no effect on the basal plasma levels of catecholamines (Fig. 3).

The bombesin (1 nmol/animal, i.c.v.)-induced elevation of plasma catecholamines was not influenced by mepacrine [1.1 and 2.2 μmol (500 and 1000 μg)/animal, i.c.v.] (Fig. 3).

3.4. Effects of U-73122 (an inhibitor of PLC), U-73343 (an inactive analog of U-73122) and RHC-80267 (an inhibitor of diacylglycerol lipase) on the bombesin-induced elevation of plasma catecholamines

Treatment with vehicle-1 (2.5 μl of 100% DMF/animal, i.c.v.) and vehicle-2 (10 μl saline/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline (data not shown). Pretreatment with U-73122 [10 and 100 nmol (4.6 and 46 μg)/animal, i.c.v.], U-73343 [100 nmol (46 μg)/animal, i.c.v.] and RHC-80267 [1.3 and 2.6 μmol (500 and 1000 μg)/animal, i.c.v.] had no effect on the basal plasma levels of catecholamines (Fig. 4A and B).

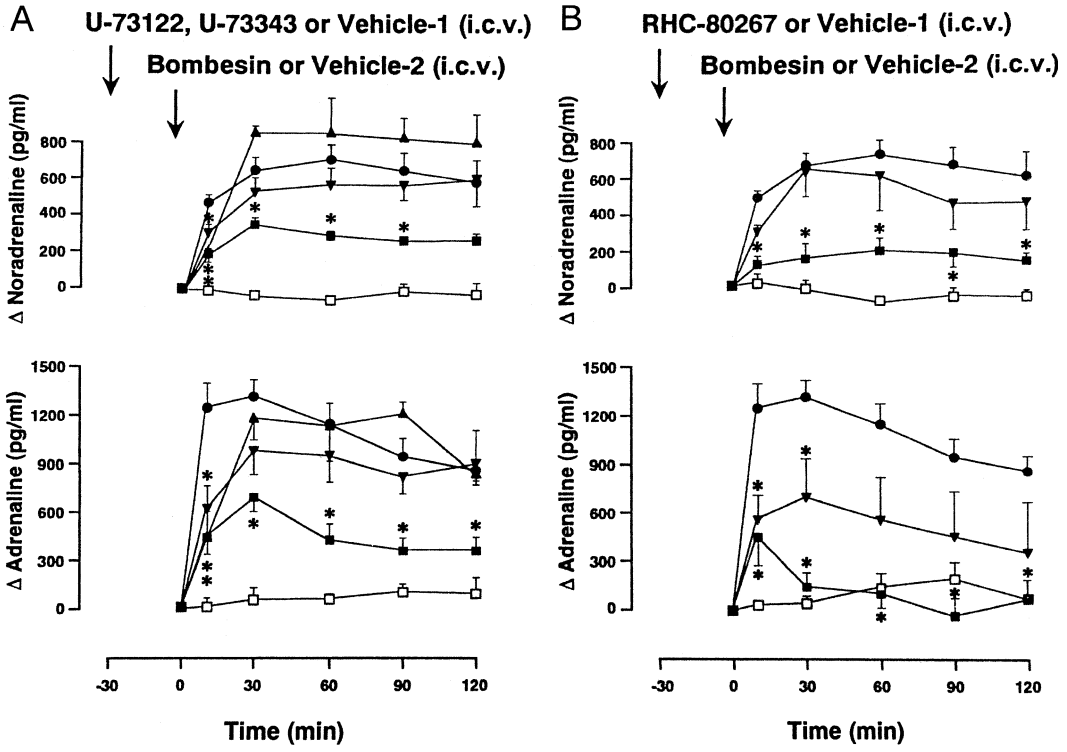

Fig. 4. Effects of U-73122, U-73343 and RHC-80267 on the bombesin-induced elevation of plasma noradrenaline and adrenaline. U-73122 (10 and 100 nmol/animal, i.c.v.), U-73343 (100 nmol/animal, i.c.v.), RHC-80267 (1.3 and 2.6 μmol/animal, i.c.v.) or vehicle-1 (2.5 μl of 100% DMF/animal, i.c.v.) was administered 30 min before the administration of bombesin (1 nmol/animal, i.c.v.) or vehicle-2 (10 μl saline/animal, i.c.v.). (A) ●, vehicle-1 plus bombesin ($n=5$); ▼, U-73122 (10 nmol/animal) plus bombesin ($n=5$); ■, U-73122 (100 nmol/animal) plus bombesin ($n=6$); ▲, U-73343 (100 nmol/animal) plus bombesin ($n=4$); □, U-73122 (100 nmol/animal) plus vehicle-2 ($n=4$). (B) ●, vehicle-1 plus bombesin (cited from (A)); ▼, RHC-80267 (1.3 μmol/animal) plus bombesin ($n=4$); ■, RHC-80267 (2.6 μmol/animal) plus bombesin ($n=5$); □, RHC-80267 (2.6 μmol/animal) plus vehicle-2 ($n=4$). *Significantly different ($P<0.05$) from vehicle-1 plus bombesin. Other conditions were the same as those in Figs. 1–3. The actual values for noradrenaline and adrenaline at 0 min were 270.2±35.1 and 320.7±106.8 pg/ml in the vehicle-1 (DMF)-pretreated group ($n=5$); 402.7±82.3 and 394.0±183.0 pg/ml in the group treated with U-73122 (10 nmol/animal) ($n=5$); 309.6±26.2 and 284.5±66.6 pg/ml in the group treated with U-73122 (100 nmol/animal) ($n=10$); 345.5±121.3 and 251.0±18.7 pg/ml in the group treated with U-73343 (100 nmol/animal) ($n=4$); 363.6±17.5 and 356.5±134.8 pg/ml in the group treated with RHC-80267 (1.3 μmol/animal) ($n=4$); 362±70.0 and 445.6±119.4 pg/ml in the group treated with RHC-80267 (2.6 μmol/animal) ($n=9$), respectively.

The bombesin (1 nmol/animal, i.c.v.)-induced elevation of both catecholamines was attenuated by U-73122 in a dose-dependent manner [10 and 100 nmol (4.6 and 46 μg)/animal, i.c.v.], while U-73343 [100 nmol (46 μg)/animal, i.c.v.] had no effect on the bombesin-induced elevation of both catecholamines (Fig. 4A). Furthermore, the bombesin-induced elevation of both catecholamines was also attenuated by RHC-80267 in a dose-dependent manner [1.3 and 2.6 μmol (500 and 1000 μg)/animal, i.c.v.] (Fig. 4B).

4. Discussion

Bombesin receptors were originally defined in terms of the rank order of potencies of bombesin, neuromedin B and gastrin-releasing peptide (GRP): neuromedin B > bombesin > GRP at bombesin BB_1 receptor; GRP > bombesin > neuromedin B at bombesin BB_2 receptor. In the present experiment, we compared the potencies of these peptides on plasma catecholamine levels. Centrally administered bombesin and GRP effectively elevated plasma catecholamines, while neuromedin B had little effect (bombesin = GRP ≫ neuromedin B), suggesting the involvement of brain bombesin BB_2 receptor (GRP-preferring receptor) in the bombesin-induced elevation of plasma catecholamines in rats.

D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127) and [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (BEA) has been shown to be a selective antagonist of bombesin BB_1 and BB_2 receptors, respectively (Orbuch et al., 1993; Jensen and Coy, 1991). D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127) has been reported to block the neuromedin B-induced contraction of cat circular esophagus (Milusheva et al., 1998) and suppression of food intake produced by neuromedin B (Ladenheim et al., 1994). [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (BEA) has been shown to block the GRP-produced depolarization of rat hippocampal interneurons (Lee et al., 1999) and the GRP-induced phase-shifts in rat and hamster circadian pacemaker (McArthur et al., 2000). In the present experiment, the bombesin-induced elevation of plasma catecholamines was not influenced by D-Nal-cyclo[Cys-Tyr-D-Trp-Orn-Val-Cys]-Nal-NH_2 (BIM-23127), while [D-Phe^6, des-Met^{14}]-bombesin (6–14) ethylamide (BEA) effectively reduced the bombesin-induced elevation of plasma catecholamines. These results further suggest the involvement of brain BB_2 receptor in the bombesin-induced elevation of plasma catecholamines in rats.

Bombesin has been shown to cause a rapid release of arachidonic acid and prostaglandin E_2 from mouse fibroblasts via bombesin BB_2 receptors (Millar and Rozengurt, 1990). GRP also potently stimulates the release of prostaglandin E_2 from rat intestinal epithelial cells (Guo et al., 2001). Previously, we suggested the involvement of active metabolites of brain arachidonic acid in the bombesin-induced elevation of plasma catecholamines in rats (Okuma et al., 1996; Yokotani et al., 2000). Arachidonic acid has originally been shown to be released by PLA_2-dependent mechanism. Hence, we attempted to clarify the involvement of brain PLA_2 in the bombesin-induced elevation of plasma catecholamines using mepacrine.

Mepacrine has been shown to inhibit the release of arachidonic acid induced by *N*-methyl-D-aspartate at concentrations that inhibit PLA_2 activity in primary cultures of cerebellar granule cells (Lazarewicz et al., 1990). Mepacrine also blocks melittin (a PLA_2 activator)-stimulated prostaglandin E_2 release from renal cortex slices (Churchill et al., 1990). We also reported that central pretreatment with mepacrine (500 μg/animal) abolished the centrally administered melittin-induced elevation of plasma catecholamines in rats (Yokotani et al., 2000). In the present experiment, however, central pretreatment with mepacrine (500 and 1000 μg/animal) was ineffective on the bombesin-induced elevation of plasma catecholamines. The result suggests the involvement of the other phospholipase than PLA_2 in the bombesin-induced elevation of plasma catecholamines in rats.

Bombesin has been shown to activate PLC in acinar pancreatic cells (Pandol and Schoeffield, 1986; Matozaki et al., 1991; Pigeon et al., 1996), in addition to the activation of PLD in mouse fibroblasts (Briscoe et al., 1994). PLC mediates the production of diacylglycerol, which is hydrolyzed by diacylglycerol lipase to yield arachidonic acid. In the next experiment, therefore, we examined the effects of U-73122, U-73343 and RHC-80267 in the bombesin-induced elevation of plasma catecholamines. U-73122 has been shown to be a selective inhibitor of PLC in human platelets and polymorphonuclear neutrophils (Bleasdale et al., 1990; Smith et al., 1990) and to block bombesin/GRP-induced Ca^{2+} spiking and depolarization of rat hippocampal neurons (Nishino et al., 1998; Lee et al., 1999). U-73343 acts as a weak inhibitor of PLC, thereby used as a negative control (Bleasdale et al., 1990; Smith et al., 1990). RHC-80267 selectively inhibits diacylglycerol lipase activity in human adrenal glomerulosa cells and rat thyroid lobes (Levasseur et al., 1984; Natarajan et al., 1988). In the present experiment, U-73122 and RHC-80267, but not U-73343, effectively reduced the bombesin-induced elevation of plasma catecholamines, respectively. These results suggest the involvement of PLC- and diacylglycerol lipase-dependent pathways in the bombesin-induced elevation of plasma catecholamines in rats. Recently, we also reported the involvement of the brain PLC- and diacylglycerol lipase-dependent pathway in the centrally administered corticotropin-releasing factor- and arginine-vasopressin-induced elevation of plasma catecholamines in rats (Okada et al., 2003; Shimizu et al., 2004).

Recently, it has been suggested that central bombesin receptors, especially bombesin BB_2 receptor, may be involved in the regulation of stress responses. Blockage of bombesin BB_2 receptor in the hippocampus or amygdala, which are involved in mediating stress responses, signifi-

cantly reduces emotionally motivated behavior in rats (Roesler et al., 2003, 2004). Moreover, GRP increases the release of corticotropin-releasing factor-like peptides from isolated hypothalamus (Garrido et al., 1999). These findings suggest the involvement of the brain bombesin BB_2 receptor in stress-induced activation of the central sympatho-adrenomedullary outflow.

In summary, we demonstrated here that the brain bombesin BB_2 receptor mediates the activation of central sympatho-adrenomedullary outflow by brain PLC- and diacylglycerol lipase-dependent mechanisms in rats.

References

Anton, A.H., Sayre, D.F., 1962. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther. 138, 360–375.

Axelrod, J., 1990. Receptor-mediated activation of phospholipase A2 and arachidonic acid release in signal transduction. Biochem. Soc. Trans. 18, 503–507.

Balsinde, J., Diez, E., Mollinedo, F., 1991. Arachidonic acid release from diacylglycerol in human neutrophils. Translocation of diacylglycerol-deacylating enzyme activities from an intracellular pool to plasma membrane upon cell activation. J. Biol. Chem. 266, 15638–15643.

Bell, R.L., Kennerly, D.A., Stanford, N., Majerus, P.W., 1979. Diglyceride lipase: a pathway for arachidonate release from human platelets. Proc. Natl. Acad. Sci. U. S. A. 76, 3238–3241.

Benya, R.V., Kusui, T., Pradhan, T.K., Battey, J.F., Jensen, R.T., 1995. Expression and characterization of cloned human bombesin receptors. Mol. Pharmacol. 47, 10–20.

Bleasdale, J.E., Thakur, N.R., Gremban, R.S., Bundy, G.L., Fitzpatrick, F.A., Smith, R.J., Bunting, S., 1990. Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. J. Pharmacol. Exp. Ther. 255, 756–768.

Briscoe, C.P., Plevin, R., Wakelam, M.J., 1994. Rapid desensitization and resensitization of bombesin-stimulated phospholipase D activity in Swiss 3T3 cells. Biochem. J. 298, 61–67.

Churchill, P.C., Rossi, N.F., Churchill, M.C., Ellis, V.R., 1990. Effect of melittin on renin and prostaglandin E2 release from rat renal cortical slices. J. Physiol. 428, 233–241.

Exton, J.H., 1997. Phospholipase D: enzymology, mechanisms of regulation, and function. Physiol. Rev. 77, 303–320.

Fathi, Z., Corjay, M.H., Shapira, H., Wada, E., Benya, R., Jensen, R., Viallet, J., Sausville, E.A., Battey, J.F., 1993. BRS-3: a novel bombesin receptor subtype selectively expressed in testis and lung carcinoma cells. J. Biol. Chem. 268, 5979–5984.

Garrido, M.M., Manzanares, J., Fuentes, J.A., 1999. Hypothalamus, anterior pituitary and adrenal gland involvement in the activation of adrenocorticotropin and corticosterone secretion by gastrin-releasing peptides. Brain Res. 828, 20–26.

Guo, Y.S., Hellmich, M.R., Wen, X.D., Townsend Jr., C.M., 2001. Activator protein-1 transcription factor mediates bombesin-stimulated cyclooxygenase-2 expression in intestinal epithelial cells. J. Biol. Chem. 276, 22941–22947.

Hammond, S.M., Altshuller, Y.M., Sung, T.C., Rudge, S.A., Rose, K., Engebrecht, J., Morris, A.J., Frohman, M.A., 1995. Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family. J. Biol. Chem. 270, 29640–29643.

Hampton, L.L., Ladenheim, E.E., Akeson, M., Way, J.M., Weber, H.C., Sutlief, V.E., Jensen, R.T., Wine, L.J., Arnheiter, H., Battey, J.E., 1998. Loss of bombesin-induced feeding suppression in gastrin-releasing peptide receptor-deficient mice. Proc. Natl. Acad. Sci. U. S. A. 95, 3188–3192.

Hou, W., Artia, Y., Morisset, J., 1996. Caerulein-stimulated arachidonic acid release in rat pancreatic acini: a diacylglycerol lipase affair. Am. J. Physiol. 271, C1735–C1742.

Irvine, R.F., 1982. How is the level of free arachidonic acid controlled in mammalian cells? Biochem. J. 204, 3–16.

Jensen, R.T., Coy, D.H., 1991. Progress in the development of potent bombesin receptor antagonists. Trends Pharmacol. Sci. 12, 13–19.

Ladenheim, E.E., Taylor, J.E., Coy, D.H., Moran, T.H., 1994. Blockade of feeding inhibition by neuromedin B using a selective receptor antagonist. Eur. J. Pharmacol. 271, R7–R9.

Lazarewicz, J.W., Wroblewski, J.T., Costa, E., 1990. *N*-methyl-D-aspartate-sensitive glutamate receptors induce calcium-mediated arachidonic acid release in primary cultures of cerebellar granule cells. J. Neurochem. 55, 1875–1881.

Lee, K., Dixon, A.K., Gonzalez, I., Stevens, E.B., McNulty, S., Oles, R., Richardson, P.J., Pinnock, R.D., Singh, L., 1999. Bombesin-like peptides depolarize rat hippocampal interneurones through interaction with subtype 2 bombesin receptors. J. Physiol. 518, 791–802.

Levasseur, S., Kostelec, M., Burke, G., 1984. RHC 80267 inhibits thyrotropin-stimulated prostaglandin release from rat thyroid lobes. Prostaglandins 27, 673–682.

Matozaki, T., Zhu, W.Y., Tsunoda, Y., Goke, B., Williams, J.A., 1991. Intracellular mediators of bombesin action on rat pancreatic acinar cells. Am. J. Physiol. 260, G858–G864.

McArthur, A.J., Coogan, A.N., Ajpru, S., Sugden, D., Biello, S.M., Piggins, H.D., 2000. Gastrin-releasing peptide phase-shifts suprachiasmatic nuclei neuronal rhythms in vitro. J. Neurosci. 20, 5496–5502.

Millar, J.B.A., Rozengurt, E., 1990. Arachidonic acid release by bombesin. A novel postreceptor target for heterologous mitogenic desensitization. J. Biol. Chem. 265, 19973–19979.

Milusheva, E.A., Kortezova, N.I., Mizhorkova, Z.N., Papasova, M., Coy, D.H., Balint, A., Vizi, E.S., Varga, G., 1998. Role of different bombesin receptor subtypes mediating contractile activity in cat upper gastrointestinal tract. Peptides 19, 549–556.

Moody, T.W., O'Donohue, T.L., Jacobowitz, D.M., 1981. Biochemical localization and characterization of bombesin-like peptides in discrete regions of rat brain. Peptides 2, 75–79.

Natarajan, R., Stern, N., Nadler, J., 1988. Diacylglycerol provides arachidonic acid for lipoxygenase products that mediate angiotensin II-induced aldosterone synthesis. Biochem. Biophys. Res. Commun. 156, 717–724.

Nishino, H., Tsunoda, Y., Owyang, C., 1998. Mammalian bombesin receptors are coupled to multiple signal transduction pathways in pancreatic acini. Am. J. Physiol. 274, G525–G534.

Ohki-Hamazaki, H., Watanabe, K., Yamamoto, K., Ogura, H., Yamano, M., Yamada, K., Maeno, H., Imaki, J., Kikuyama, S., Wada, E., Wada, K., 1997. Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. Nature 390, 165–169.

Ohki-Hamazaki, H., Sakai, Y., Kamata, K., Ogura, H., Okuyama, S., Watase, K., Yamada, K., Wada, K., 1999. Functional properties of two bombesin-like peptide receptors revealed by the analysis of mice lacking neuromedin B receptor. J. Neurosci. 19, 948–954.

Okada, S., Shimizu, T., Yokotani, K., 2003. Brain phospholipase C and diacylglycerol lipase are involved in corticotropin-releasing hormone-induced sympatho-adrenomedullary outflow in rats. Eur. J. Pharmacol. 475, 49–54.

Okuma, Y., Yokotani, K., Osumi, Y., 1996. Brain prostaglandins mediate the bombesin-induced increase in plasma levels of catecholamines. Life Sci. 59, 1217–1225.

Orbuch, M., Taylor, J.E., Coy, D.H., Mrozinski, J.E. Jr., Mantey, S.A., Battey, J.F., Moreau, J.P., Jensen, R.T., 1993. Discovery of a novel class of neuromedin B receptor antagonists, substituted somatostatin analogues. Mol. Pharmacol. 44, 841–850.

Pandol, S.J., Schoeffield, M.S., 1986. 1,2-Diacylglycerol, protein kinase C, and pancreatic enzyme secretion. J. Biol. Chem. 261, 4438–4444.

Paxinos, G., Watson, C., 1986. In: Paxinos , G., Watson, C. (Eds.), The Rat Brain in Stereotaxic Coordinates. Academic Press, Boston.

Pigeon, C., Le Romancer, M., Linard, C., Lewin, M.J., Reyl-Desmars, F., 1996. Bombesin activation of phospholipase C beta 1 in rat acinar pancreatic cells involves the pertussis toxin-sensitive G alpha i3 protein. Regul. Pept. 62, 153–159.

Roesler, R., Meller, C.A., Kopschina, M.I., Souza, D.O., Henriques, J.A.P., Schwartsmann, G., 2003. Intrahippocampal infusion of the bombesin/gastrin-releasing peptide antagonist RC-3095 impairs inhibitory avoidance retention. Peptides 24, 1069–1074.

Roesler, R., Lessa, D., Venturella, R., Vinna, M.R., Luft, T., Henriques, J.A.P., Izquierdo, I., Schwartsmann, G., 2004. Bombesin/gastrin-releasing peptide receptors in the basolateral amygdala regulate memory consolidation. Eur. J. Neurosci. 19, 1041–1045.

Sbrissa, D., Hajra, A., Bitar, K.N., 1998. Origin of molecular species of diacylglycerol induced by bombesin in smooth muscle cells from rabbit rectosigmoid. Am. J. Physiol. 275, G138–G150.

Shimizu, T., Okada, S., Yamaguchi-Shima, N., Yokotani, K., 2004. Brain phospholipase C–diacylglycerol lipase pathway is involved in vasopressin-induced release of noradrenaline and adrenaline from adrenal medulla in rats. Eur. J. Pharmacol. 499, 99–105.

Shumyatsky, G.P., Tsvetkov, E., Malleret, G., Vronskaya, S., Hatton, M., Hampton, L., Battey, J.F., Dulac, C., Kandel, E.R., Bolshakov, V.Y., 2002. Identification of a signaling network in lateral nucleus of amygdala important for inhibiting memory specifically related to learned fear. Cell 111, 905–918.

Smith, R.J., Sam, L.M., Justen, J.M., Bundy, G.L., Bala, G.A., Bleasdale, J.E., 1990. Receptor-coupled signal transduction in human polymorphonuclear neutrophils: effects of a novel inhibitor of phospholipase C-dependent processes on cell responsiveness. J. Pharmacol. Exp. Ther. 253, 688–697.

Spindel, E.R., Giladi, E., Brehm, P., Goodman, R.H., Segerson, T.P., 1990. Cloning and functional characterization of a complementary DNA encoding the murine fibroblast bombesin/gastrin-releasing peptide receptor. Mol. Endocrinol. 4, 1956–1963.

Steel, J.H., O'Halloran, D.J., Emson, M.A., Van Noorden, S., Bloom, S.R., Polak, J.M., 1992. Identification of bombesin-immunoreactive cells in rat, human, and other mammalian pituitaries, their ontogeny and the effect of endocrine manipulations in the rat. Endocrinology 130, 2587–2596.

Tsushima, H., Mori, M., Fujiwara, N., Moriyama, A., 2003. Pharmacological characteristics of bombesin receptor mediating hypothermia in the central nervous system of rats. Brain Res. 969, 88–94.

Wada, E., Way, J., Shapira, H., Kusano, K., Lebacq-Verheyden, A.M., Coy, D., Jensen, R., Battery, J., 1991. cDNA cloning, characterization, and brain region-specific expression of a neuromedin-B-preferring bombesin receptor. Neuron 6, 421–430.

Yokotani, K., Nishihara, M., Murakami, Y., Hasegawa, T., Okuma, Y., Osumi, Y., 1995. Elevation of plasma noradrenaline levels in urethane-anaesthetized rats by activation of central prostanoid EP3 receptors. Br. J. Pharmacol. 115, 672–676.

Yokotani, K., Wang, M., Murakami, Y., Okada, S., Hirata, M., 2000. Brain phospholipase A2–arachidonic acid cascade is involved in the activation of central sympatho-adrenomedullary outflow in rats. Eur. J. Pharmacol. 398, 341–347.