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Brain opioid and nociceptin receptors are involved in regulation of bombesin-induced activation of central sympatho-adrenomedullary outflow in the rat

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Abstract Previously, we reported that central administration of bombesin, a stress-related peptide, elevated plasma levels of catecholamines (noradrenaline and adrenaline) in the rat. The sympatho-adrenomedullary system, which is an important component of stress responses, can be regulated by the central opioid system. In the present study, therefore, we examined the roles of brain opioid receptor subtypes (μ, δ, and κ) and nociceptin receptors, originally identified as opioid-like orphan receptors, in the bombesin-induced activation of central sympatho-adrenomedullary outflow using anesthetized male Wistar rats. Intracerebroventricularly (i.c.v.) administered bombesin- (1 nmol/animal) induced elevation of plasma catecholamines was significantly potentiated by pretreatment with naloxone (300 and 1000 μg/animal, i.c.v.), a non-selective antagonist for μ-, δ-, and κ-opioid receptors. Pretreatment with cyprodime (100 μg/animal, i.c.v.), a selective antagonist for μ-opioid receptors, also potentiated the bombesin-induced responses. In contrast, pretreatment with naltrindole (100 μg/animal, i.c.v.) or *nor*-binaltorphimine (100 μg/animal, i.c.v.), a selective antagonist for δ- or κ-opioid receptors, significantly reduced the elevation of bombesin-induced catecholamines. In addition, pretreatment with JTC-801 (30 and 100 μg/animal, i.c.v.) or J-113397 (100 μg/animal, i.c.v.), which are selective antagonists for nociceptin receptors, also reduced the bombesin-induced responses. These results suggest that brain μ-opioid receptors play a suppressive role and that brain δ-, κ-opioid, and nociceptin receptors play a facilitative role in the bombesin-induced elevation of plasma catecholamines in the rat. Thus, in the brain, these receptors could play differential roles in regulating the activation of central sympatho-adrenomedullary outflow.

Keywords Opioid receptor · Nociceptin receptor · Bombesin · Brain · Sympatho-adrenomedullary system

Abbreviations

ANOVA	Analysis of variance
AUC	Area under the curve
DMF	*N*,*N*-dimethylformamide
GRP	Gastrin-releasing peptide
HPLC	High performance liquid chromatography
i.c.v.	Intracerebroventricularly
NMB	Neuromedin B

Introduction

All organisms are equipped with adaptation mechanisms to deal with stress. During exposure to stress, the sympatho-adrenomedullary system is one of the components of the primary systems for maintaining or reinstating homeostasis [1–3]. Activation of the sympatho-adrenomedullary system induces the elevation of plasma catecholamines, noradrenaline and adrenaline, thereby rapidly increasing the

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heart rate, blood pressure, respiration, and basal metabolic rate in order to quickly adapt to stressful conditions [4]. On the other hand, prolonged or excessive activation of the stress responses can contribute to the development of various disorders, including hypertension and arrhythmia [5–7]. The sympatho-adrenomedullary outflow is controlled by the central nervous system. Therefore, it is necessary to clarify the central regulatory mechanisms that control this outflow.

Neuromodulators are thought to play an important role in the regulation of stress responses. There is evidence indicating that the endogenous opioid system, a well-known innate pain-relieving system [8], modulates responses to stress exposure [9–11]. The opioid system consists of peptidergic modulators (opioid peptides) such as β-endorphin, enkephalin and dynorphin, and opioid receptors (μ, δ and κ subtypes) [12, 13]. Nociceptin receptors share high sequence homology with μ-, δ-, and κ-opioid receptors, but nociceptin, a peptidergic ligand for nociceptin receptors, does not show any significant binding to any of these opioid receptors [14]. These peptides and receptors are expressed throughout the peripheral and central nervous system [15] and seem to be involved in the attenuation or termination of stress responses in order to avoid prolonged or excessive activation of these responses [16, 17]. For example, μ-opioid receptors in the rat hypothalamic paraventricular nucleus are involved in decreases of mean arterial pressure and sympathetic nerve activity induced by stimulation of the hypothalamic arcuate nucleus [18], and nociceptin receptors in the rat hypothalamic paraventricular nucleus inhibit the central sympathetic outflow [19]. On the other hand, pressure responses induced by brain opioid and nociceptin receptors have also been reported [20, 21]. Therefore, the roles of these receptors in the regulation of stress responses, especially the sympatho-adrenomedullary outflow, remain controversial.

Bombesin itself is a tetradecapeptide isolated from the skin of the European frog *Bombina bombina* [22], and is not expressed in mammals. On the other hand, the mammalian counterparts of bombesin are neuromedin B (NMB) and gastrin-releasing peptide (GRP), and the receptors for these two peptides are bombesin receptor type 1 (BB_1, NMB-preferring receptor) and type 2 (BB_2, GRP-preferring receptor) [23]. Bombesin shows high affinity to both these receptor subtypes [23]. These receptors and their counterparts are widely distributed in the mammalian brain [23]. Bombesin-related peptides have been implicated in the mediation/integration of stress responses through brain BB receptors [24]. In order to mimic the stress-induced activation of a brain bombesinergic nervous system, we have been using bombesin as a "non-selective" agonist for BB receptors to examine the central regulatory mechanisms of sympatho-adrenomedullary outflow. Previously, we reported that intracerebroventricularly (i.c.v.) administered bombesin centrally elevated plasma levels of catecholamines in the rat [25, 26]. In the present study, we attempted to clarify the roles of brain opioid and nociceptin receptors in the regulation of bombesin-induced activation of central sympatho-adrenomedullary outflow in the rat.

Materials and methods

Animals

Animal care and all experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by Kochi University, which are in accordance with the "Guidelines for proper conduct of animal experiments" devised by the Science Council of Japan. All efforts were made to minimize suffering in animals and the number of animals needed to obtain reliable results. A total of 57 animals were used in the experiments described next. Eight-week-old male Wistar rats (Japan SLC Inc., Hamamatsu, Japan) weighing 200–250 g were housed at two per cage and were maintained in an air-conditioned room at 22–24 °C under a constant day-night rhythm (14/10 h light–dark cycle, lights on at 05:00) for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water ad libitum. After reaching 300–350 g, the rats were used for experiments.

Experimental procedures for intracerebroventricular administration

The femoral vein was cannulated for the infusion of saline (1.2 mL/h), and the femoral artery was cannulated in order to collect blood samples, under urethane anesthesia (1.0–1.2 g/kg, i.p.) at 9 to 10 am. Subsequently, every animal was placed in a stereotaxic apparatus (SR-6R, Narishige, Tokyo, Japan) until the end of each experiment, as shown previously in a published study from our laboratory [27]. The skull was drilled for intracerebroventricular administration of drugs using a stainless-steel cannula (outer diameter of 0.3 mm). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP -0.8, L 1.5, V 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas [28]. Three hours were allowed to elapse before the administration of drugs.

Drug administration

Naloxone (a non-selective antagonist for opioid receptors) was dissolved in sterile saline. Cyprodime (a selective

antagonist for μ-opioid receptors), naltrindole (a selective antagonist for δ-opioid receptors), *nor*-binaltorphimine (a selective antagonist for κ-opioid receptors), as well as JTC-801 and J-113397 (selective antagonists for nociceptin receptors) were dissolved using 100 % *N*,*N*-dimethylformamide (DMF). Each of these drugs was slowly administered into the right lateral ventricle in a volume of 5 μL saline/animal or 3 μL DMF/animal using the cannula connected to a 10-μL Hamilton syringe at a rate of 10 μL/min. The cannula was retained for 5 min using naloxone or for 15 min using other drugs to avoid the leakage of these reagents and then removed from the ventricle. Subsequently, bombesin dissolved in sterile saline in a volume of 10 μL/animal was then i.c.v. administered into the ventricle using the cannula connected to a 50-μL Hamilton syringe at a rate of 10 μL/min, 15 min after the application of naloxone and 30 min after the application of cyprodime, naltrindole, *nor*-binaltorphimine, JTC-801 or J-113397. Since we previously reported that bombesin (0.1, 1, and 10 nmol/animal, i.c.v.) dose-dependently elevated plasma levels of noradrenaline and adrenaline [26], we used a submaximum dose of 1 nmol/animal in the present study. After the administration of bombesin, the cannula was retained until the end of the experiment. The exact location of the cannula was confirmed at the end of each experiment by verifying that a Cresyl Violet solution, injected through the cannula, had spread throughout the entire ventricular system.

Experimental groups for i.c.v. administrations

The 57 rats placed in a stereotaxic apparatus were divided into 10 groups: vehicle- (5 μL saline/animal) and bombesin- (1 nmol/animal) administered group ($n = 6$); naloxone- [300 μg (0.75 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 9$); naloxone- [1000 μg (2.5 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 5$); vehicle- (3 μL DMF/animal) and bombesin- (1 nmol/animal) administered group ($n = 5$); cyprodime- [100 μg (0.25 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 5$); naltrindole- [100 μg (0.21 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 7$); *nor*-binaltorphimine- [100 μg (0.13 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 4$); JTC-801- [30 μg (0.07 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 6$); JTC-801- [100 μg (0.22 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 5$); J-113397 [100 μg (0.25 μmol)/animal] and bombesin- (1 nmol/animal) administered group ($n = 5$).

Measurement of plasma catecholamines

Blood samples (250 μL) were collected through the cannulated femoral artery and were preserved on ice during experiments. Plasma was prepared immediately after the final sampling. Catecholamines (noradrenaline and adrenaline) in the plasma were extracted by the method of Anton and Sayre [29] with a slight modification and were assayed electrochemically with high performance liquid chromatography (HPLC) [27]. Briefly, after centrifugation (1500×*g* for 10 min, at 4 °C), the plasma (100 μL) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 mL of water deionized in a MilliQ water purification system (Millipore, Billerica, MA, USA), 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 standard. The tube was shaken for 10 min and the alumina was washed three times with 4 mL of ice-cold 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 an 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 disodium EDTA, 0.75 g/L sodium 1-octanesulfonate and 15 % methanol at a flow rate 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. Using this assay, coefficients of variation for the intra- and inter-assay were 3.0 and 3.7 %, respectively, and 0.5 pg of catecholamines was accurately determined.

Treatment of data and statistics

Increments of plasma catecholamines above the basal level at each time period are expressed as pg/mL (Figs. 1, 2, 3, 4, 5 and 6). The area under the curve is also expressed as pg/2 h (Figs. 1, 2, 3, 4, 5 and 6). The number of animals in each group is shown in these figures (Figs. 1, 2, 3, 4, 5 and 6). All values are expressed as mean ± S.E.M. Statistical differences were determined using repeated-measure (treatment × time) or one-way analysis of variance, followed by post hoc analysis with the Bonferroni method. When only two means were compared, an unpaired Student's *t* test was used. *P* values less than 0.05 were taken to indicate statistical significance.

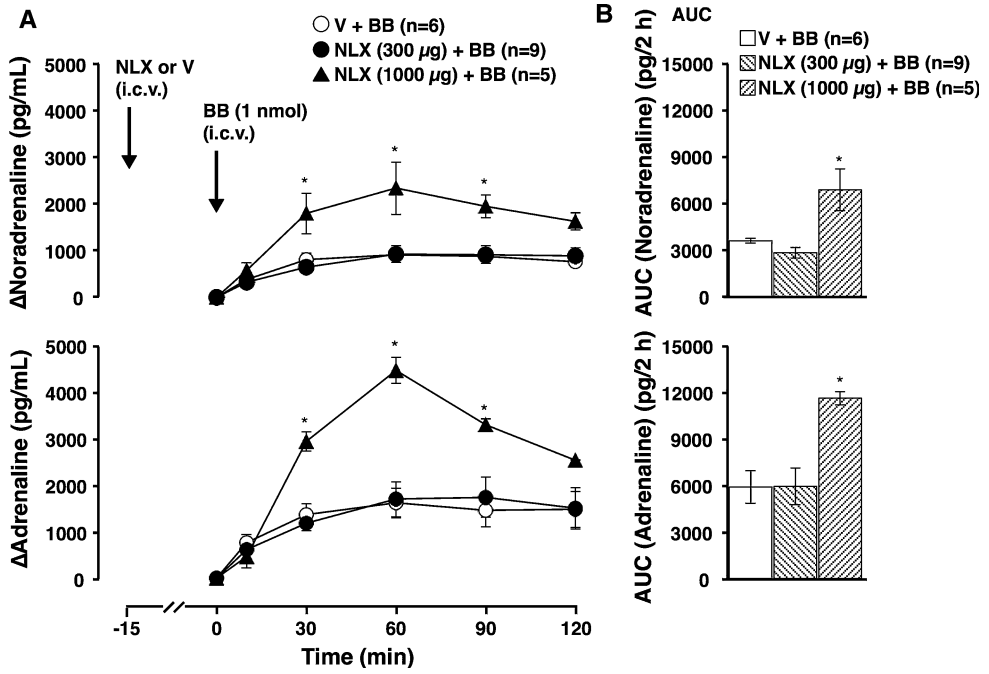

Fig. 1 Effect of naloxone on the bombesin-induced elevation of plasma catecholamines. Naloxone (NLX) (a non-selective antagonist for opioid receptors) (300 or 1000 μg/animal) or vehicle (V) (5 μL saline/animal) was administered i.c.v. 15 min before the administration of bombesin (BB) (1 nmol/animal, i.c.v.). **a** Increments of plasma catecholamines (noradrenaline and adrenaline) above the basal level. ΔNoradrenaline and ΔAdrenaline: increments of noradrenaline and adrenaline above the basal level. *Arrows* indicate the administration of NLX/V and BB. **b** The area under the curve (AUC) of the elevation of plasma catecholamines above the basal level for each group is expressed as pg/2 h. Each point represents the mean ± S.E.M. $*P < 0.05$, when compared with the Bonferroni method to the V- and BB-treated group

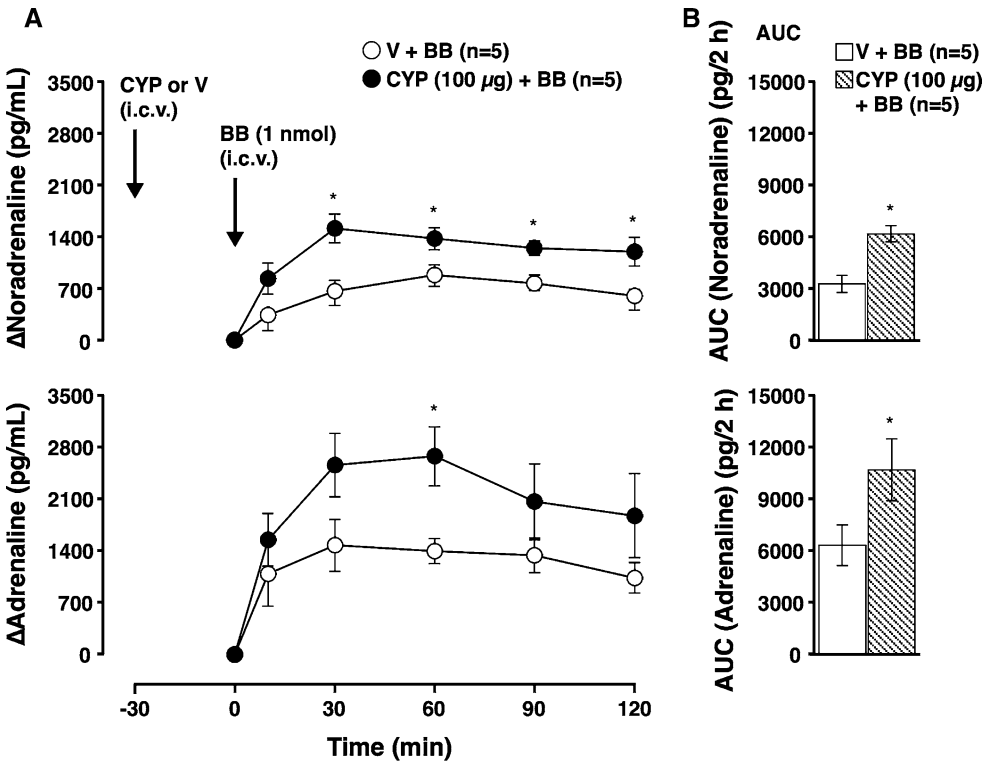

Fig. 2 Effect of cyprodime on the bombesin-induced elevation of plasma catecholamines. Cyprodime (CYP) (a selective antagonist for μ-opioid receptors) (100 μg/animal) or vehicle (V) (3 μL DMF/animal) was given i.c.v. 30 min before the administration of bombesin (BB) (1 nmol/animal, i.c.v.). **a** Increments of plasma catecholamines above the basal level. *Arrows* indicate the administration of CYP/V and BB. **b** The area under the curve (AUC) of the elevation of catecholamines above the basal level for each group. $*P < 0.05$, when compared with an unpaired Student's *t* test to the V- and BB-treated group. The other conditions are the same as those of Fig. 1

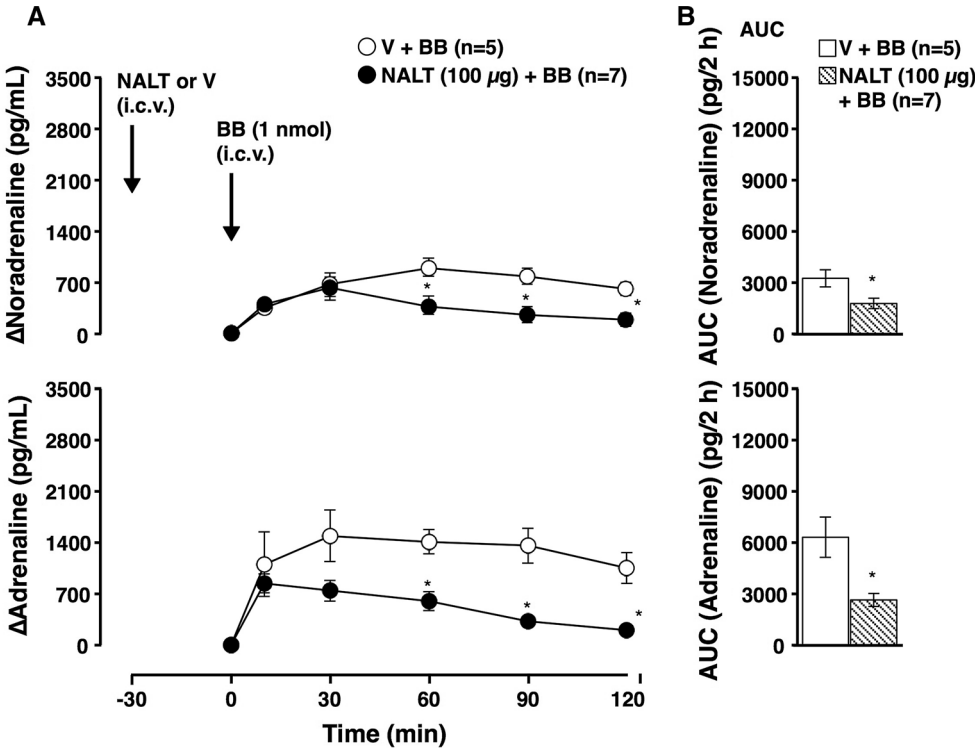

Fig. 3 Effect of naltrindole on the bombesin-induced elevation of plasma catecholamines. Naltrindole (NALT) (a selective antagonist for δ-opioid receptors) (100 μg/animal) or vehicle (V) (3 μL DMF/animal) was given i.c.v. 30 min before the administration of bombesin (BB) (1 nmol/animal, i.c.v.). **a** Increments of plasma catecholamines above the basal level. *Arrows* indicate the administration of NALT/V and BB. **b** The area under the curve (AUC) of the elevation of catecholamines above the basal level for each group. $*P < 0.05$, when compared with an unpaired Student's *t* test to the V- and BB-treated group. The other conditions are the same as those of Figs. 1 and 2

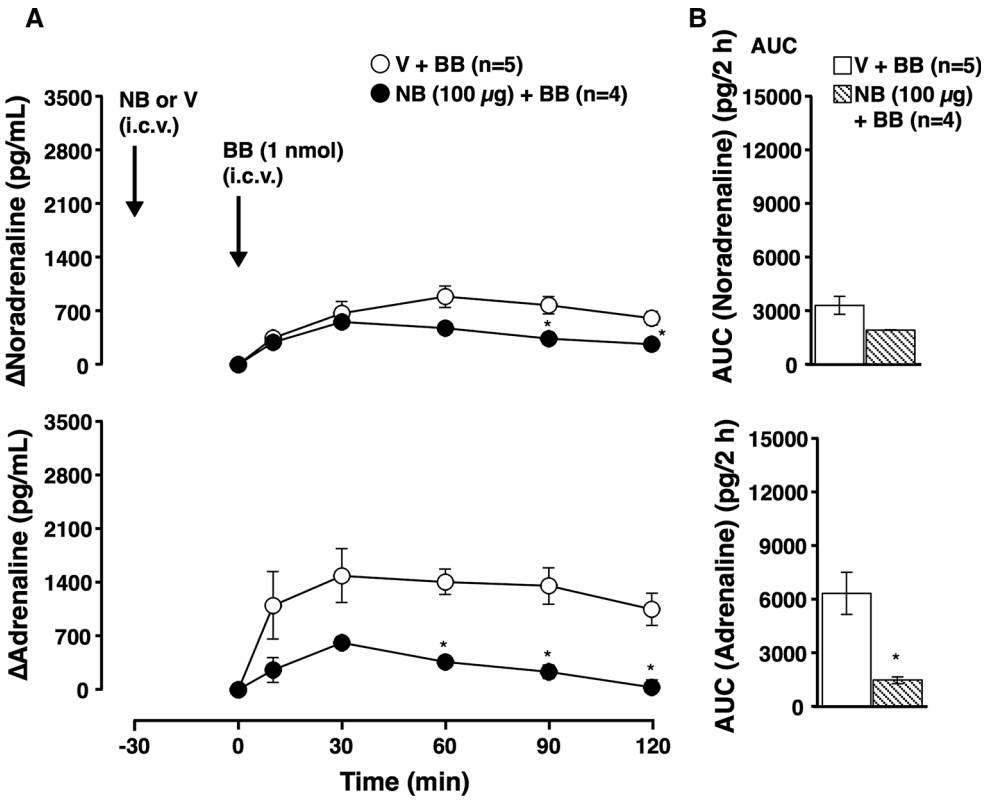

Fig. 4 Effect of *nor*-binaltorphimine on the bombesin-induced elevation of plasma catecholamines. *nor*-Binaltorphimine (NB) (a selective antagonist for κ-opioid receptors) (100 μg/animal) or vehicle (V) (3 μL DMF/animal) was given i.c.v. 30 min before the administration of bombesin (BB) (1 nmol/animal, i.c.v.). **a** Increments of plasma catecholamines above the basal level. *Arrows* indicate the administration of NB/V and BB. **b** The area under the curve (AUC) of the elevation of catecholamines above the basal level for each group. $*P < 0.05$, when compared with an unpaired Student's *t* test to the V- and BB-treated group. The other conditions are the same as those of Figs. 1, 2, and 3

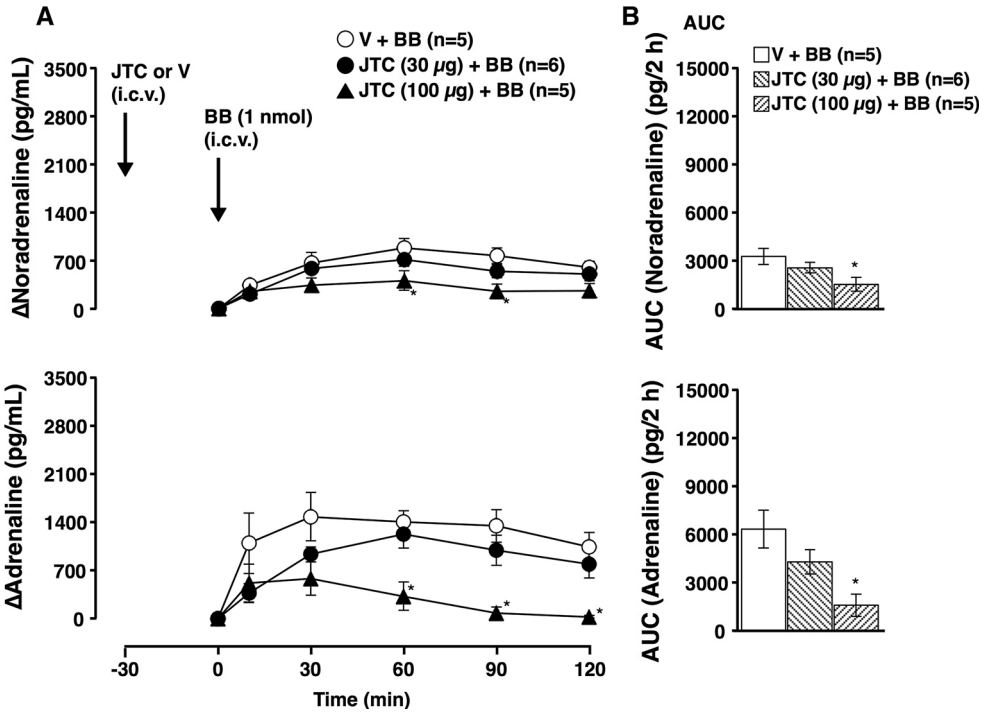

Fig. 5 Effect of JTC-801 on the bombesin-induced elevation of plasma catecholamines. JTC-801 (JTC) (a selective antagonist for nociceptin receptors) (30 or 100 μg/animal) or vehicle (V) (3 μL DMF/animal) was given i.c.v. 30 min before the administration of bombesin (BB) (1 nmol/animal). **a** Increments of plasma catecholamines above the basal level. *Arrows* indicate the administration of JTC/V and BB. **b** The area under the curve (AUC) of the elevation of catecholamines above the basal level for each group. $*P < 0.05$, when compared with the Bonferroni method to the V- and BB-treated group. The other conditions are the same as those of Figs. 1, 2, 3 and 4

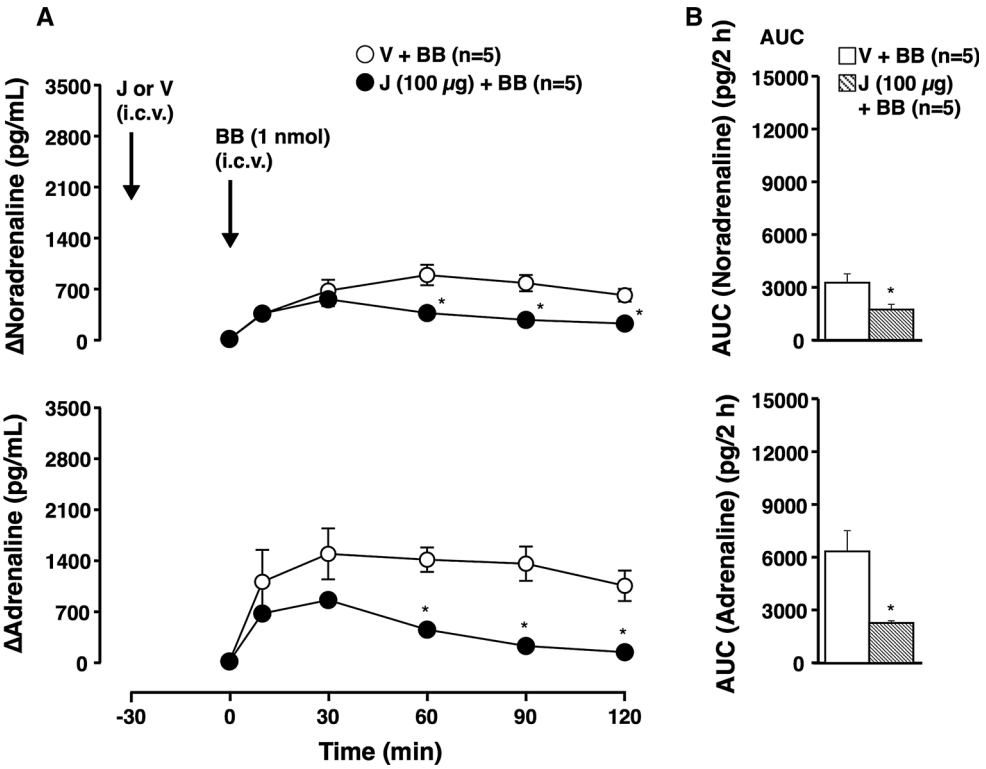

Fig. 6 Effect of J-113397 on the bombesin-induced elevation of plasma catecholamines. J-113397 (J) (a selective antagonist for nociceptin receptors) (100 μg/animal) or vehicle (V) (3 μL DMF/animal) was given i.c.v. 30 min before the administration of bombesin (BB) (1 nmol/animal). **a** Increments of plasma catecholamines above the basal level. *Arrows* indicate the administration of J/V and BB. **b** The area under the curve (AUC) of the elevation of catecholamines above the basal level for each group. $*P < 0.05$, when compared with an unpaired Student's *t* test to the V- and BB-treated group. The other conditions are the same as those of Figs. 1, 2, 3, 4 and 5

Drugs and chemicals

The following materials were used: synthetic bombesin (Peptide Institute, Osaka, Japan); naloxone hydrochloride (naloxone) [(5α)-4,5-epoxy-3,14-dihydro-17-(2-propenyl)morphinan-6-one hydrochloride] (MP Biochemicals, Santa Ana, CA, USA); cyprodime hydrochloride (cyprodime) [17-(cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one hydrochloride] and (±)-J-113397 (J-113397) [(±)-1-[(3*R*,4*R*)-1-(cyclooctylmethyl)-3-(hydroxymethyl)-4-piperidinyl]-3-ethyl-

1,3-dihydro-2*H*-benzimidazol-2-one] (Tocris Bioscience, Bristol, UK); naltrindole hydrochloride (naltrindole) [17-(cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2′,3′-indolomorphinan hydrochloride] and *nor*-binaltorphimine dihydrochloride (*nor*-binaltorphimine) [17,17′-(dicyclopropylmethyl)-6,6′,7,7′-6,6′-imino-7,7′-binorphinan-3,4′,14,14′-tetrol dihydrochloride] (Sigma Aldrich Fine Chemicals, St. Louis, MO, USA); JTC-801 [*N*-(4-amino-2-methyl-6-quinolinyl)-2-[(4-ethylphenoxy)methyl]benzamide hydrochloride] (Adooq Bioscience, Irvine, CA, USA). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

Results

Effect of naloxone on the centrally administered bombesin-induced elevation of plasma catecholamines

We initially checked that treatment with vehicle corresponding to naloxone (5 μL saline/animal, i.c.v.) and vehicle corresponding to bombesin (10 μL saline/animal, i.c.v.) had no effect on the plasma levels of catecholamines (data not shown). We also conducted a preliminary check to confirm that treatments with naloxone (1000 μg/animal, i.c.v.) and vehicle corresponding to bombesin had no obvious effect on plasma levels of catecholamines (data not shown). Administration of bombesin (1 nmol/animal, i.c.v.) elevated the level of plasma catecholamines (adrenaline > noradrenaline) (Fig. 1a, b). The responses of catecholamines peaked at 60 min after the administration of bombesin and then slowly declined (Fig. 1a). Pretreatment with naloxone at a smaller dose (300 μg/animal, i.c.v.) had no effect on the bombesin-induced elevation of plasma catecholamines, while the bombesin-induced responses were significantly potentiated by a larger dose of naloxone (1000 μg/animal, i.c.v.) (Fig. 1a, 1b). The actual values for noradrenaline and adrenaline at 0 min were 377 ± 53 and 502 ± 170 pg/mL in the vehicle-pretreated group ($n = 6$), 414 ± 83 and 436 ± 109 pg/mL in the naloxone- (300 μg/animal) pretreated group ($n = 9$), and 302 ± 58 and 525 ± 228 pg/mL in the naloxone- (1000 μg/animal) pretreated group ($n = 5$), respectively.

Effect of cyprodime on the centrally administered bombesin-induced elevation of plasma catecholamines

In a preliminary step, we checked that treatment with vehicle corresponding to cyprodime (3 μL DMF/animal, i.c.v.) and vehicle corresponding to bombesin (10 μL saline/animal, i.c.v.) had no effect on the plasma levels of catecholamines (data not shown). We also conducted a preliminary check to ensure that treatments with cyprodime (100 μg/animal, i.c.v.) and vehicle corresponding to bombesin had no obvious effect on plasma levels of catecholamines (data not shown). Pretreatment with cyprodime (100 μg/animal, i.c.v.) significantly potentiated the bombesin- (1 nmol/animal, i.c.v.) induced elevation of plasma catecholamines (Fig. 2a, b). The actual values for noradrenaline and adrenaline at 0 min were 507 ± 84 and 422 ± 119 pg/mL in the vehicle-pretreated group ($n = 5$), and 552 ± 84 and 734 ± 506 pg/mL in the cyprodime-pretreated group ($n = 5$), respectively.

Effect of naltrindole on the centrally administered bombesin-induced elevation of plasma catecholamines

We initially checked that treatments with naltrindole (100 μg/animal, i.c.v.) and vehicle corresponding to bombesin (10 μL saline/animal, i.c.v.) had no obvious effect on plasma levels of catecholamines (data not shown). Pretreatment with naltrindole (100 μg/animal, i.c.v.) significantly reduced the bombesin- (1 nmol/animal, i.c.v.) induced elevation of plasma catecholamines (Fig. 3a, b). The vehicle- (3 μL DMF/animal, i.c.v.) and bombesin-treated group was the same as that used in Fig. 2. The actual values for noradrenaline and adrenaline at 0 min were 417 ± 36 and 181 ± 42 pg/mL in the naltrindole-pretreated group ($n = 7$), respectively.

Effect of *nor*-binaltorphimine on the centrally administered bombesin-induced elevation of plasma catecholamines

In a preliminary check, we verified that treatments with *nor*-binaltorphimine (100 μg/animal, i.c.v.) and vehicle corresponding to bombesin (10 μL saline/animal, i.c.v.) had no obvious effect on plasma levels of catecholamines (data not shown). Pretreatment with *nor*-binaltorphimine (100 μg/animal, i.c.v.) significantly reduced the bombesin- (1 nmol/animal, i.c.v.) induced elevation of plasma catecholamines (Fig. 4a, b). Regarding the area under the curve of noradrenaline, there was no significance ($P = 0.052$) but *nor*-binaltorphimine had a tendency to reduce the bombesin-induced response (Fig. 4b). The vehicle- (3 μL DMF/animal, i.c.v.) and bombesin-treated group was the same as that used in Fig. 2. The actual values for noradrenaline and adrenaline at 0 min were 467 ± 76 and 432 ± 111 pg/mL in the *nor*-binaltorphimine-pretreated group ($n = 4$), respectively.

Effect of JTC-801 on the centrally administered bombesin-induced elevation of plasma catecholamines

We conducted a preliminary check to verify that treatments with JTC-801 (100 μg/animal, i.c.v.) and vehicle corresponding to bombesin (10 μL saline/animal, i.c.v.) had no obvious effect on plasma levels of catecholamines (data not shown). Pretreatment with JTC-801 at a smaller dose (30 μg/animal, i.c.v.) reduced, but not significantly, the bombesin- (1 nmol/animal, i.c.v.) induced elevation of plasma catecholamines, while the bombesin-induced responses were significantly reduced by a larger dose of JTC-801 (100 μg/animal, i.c.v.) (Fig. 5a, b). The vehicle- (3 μL DMF/animal, i.c.v.) and bombesin-treated group was the same as that used in Fig. 2. The actual values for noradrenaline and adrenaline at 0 min were 462 ± 43 and 172 ± 35 pg/mL in the JTC-801- (30 μg/animal) pretreated group ($n = 6$), and 295 ± 18 and 325 ± 124 pg/mL in the JTC-801- (100 μg/animal) pretreated group ($n = 5$), respectively.

Effect of J-113397 on the centrally administered bombesin-induced elevation of plasma catecholamines

We conducted a preliminary check to ensure that treatments with J-113397 (100 μg/animal, i.c.v.) and vehicle corresponding to bombesin (10 μL saline/animal, i.c.v.) had no obvious effect on plasma levels of catecholamines (data not shown). Pretreatment with J-113397 (100 μg/animal, i.c.v.) significantly reduced the bombesin- (1 nmol/animal, i.c.v.) induced elevation of plasma catecholamines (Fig. 6a, b). The vehicle- (3 μL DMF/animal, i.c.v.) and bombesin-treated group was the same as that used in Fig. 2. The actual values for noradrenaline and adrenaline at 0 min were 332 ± 24 and 93 ± 38 pg/mL in the J-113397-pretreated group ($n = 5$), respectively.

Discussion

In this study, we demonstrated that i.c.v. administered bombesin-induced elevation of plasma catecholamines was potentiated by central pretreatment with naloxone or cyprodime. On the other hand, central pretreatment with naltrindole, *nor*-binaltorphimine, JTC-801 or J-113397 reduced the bombesin-induced response. In addition, pretreatment with each antagonist alone had no effect on the basal level of plasma catecholamines. These results suggest that brain μ-opioid receptors play a suppressive role and that brain δ-, κ-opioid, and nociceptin receptors play a facilitative role in the centrally administered bombesin-induced activation of central sympatho-adrenomedullary outflow in the rat. In addition, endogenous opioid peptides/nociceptin in the brain do not seem to affect the outflow, at least in the rat.

Naloxone is a traditional and non-selective antagonist for μ-, δ-, and κ-opioid receptors. When systemically administered, this antagonist augmented hypoxia-induced plasma adrenaline elevation in sheep [30]. In the present study, central pretreatment with naloxone effectively potentiated the centrally administered bombesin-induced elevation of plasma catecholamines, indicating that brain opioid receptors are suppressively involved in the bombesin-induced activation of central sympatho-adrenomedullary outflow. Subsequently, we attempted to clarify which opioid receptor subtype (μ, δ, or κ) plays the suppressive role.

Central activation of μ-opioid receptors by selective agonists induced hypertension and elevation of plasma catecholamine in conscious rats [31] and rabbits [20, 32]. On the other hand, stimulation of μ-opioid receptors in the hypothalamic paraventricular nucleus is involved in the decrease of arterial blood pressure and sympathetic nerve activity in anesthetized rats [18, 33]. These findings suggest that a role of brain μ-opioid receptors in the regulation of sympatho-adrenomedullary outflow might differ on the conscious or anesthetized state of the subject. Additionally, using conscious rats, Kiritsy-Roy et al. reported that μ-opioid receptors in the hypothalamic paraventricular nucleus are involved in the induction of hypertension, tachycardia, and elevation of plasma catecholamines, while these receptors suppressively modulated resistant stress-induced tachycardia and plasma adrenaline elevation [34]. These results indicate that brain μ-opioid receptors can positively regulate the central sympatho-adrenomedullary outflow in normal conditions but negatively regulate the activation of the outflow in response to stress exposure. In the present study, we used anesthetized rats and cyprodime, which is a selective antagonist for μ-opioid receptors. This antagonist exhibits K_i values of 5.4, 244.6, and 2187 nM for μ-, δ-, and κ-opioid receptors, respectively [35]. Central pretreatment with cyprodime effectively potentiated the centrally administered bombesin-induced elevation of plasma catecholamines. These results indicate that, at least in the anesthetized rats, brain μ-opioid receptors are suppressively involved in the bombesin-induced activation of central sympatho-adrenomedullary outflow. Enkephalin, an opioid peptide showing high affinity for μ-opioid receptors [12], has been recognized as an anti-stress neuromodulator [36], supporting our findings showing the suppressive role of these receptors.

Activation of δ-opioid receptors by selective agonists in the rat hypothalamic paraventricular nucleus induced hypotension [33], while activation in the rabbit solitary

tract nucleus induced hypertension [32]. Considering these findings, the role of brain δ-opioid receptors in the regulation of sympatho-adrenomedullary outflow seems to be controversial. Kraft et al. reported that chronic and systemic antagonism of δ-opioid receptors retard the development of hypertension in young spontaneously hypertensive rats [37], suggesting a possibility that δ-opioid receptors could enhance sympatho-adrenomedullary outflow. In the present study, we used naltrindole, a selective antagonist for δ-opioid receptors. This antagonist exhibits K_i values of 3.72, 0.04, and 5.78 nM for μ-, δ-, and κ-opioid receptors, respectively [38]. Central pretreatment with naltrindole effectively reduced the centrally administered bombesin-induced elevation of plasma catecholamines. These results indicate that brain δ-opioid receptors play a facilitative role in the bombesin-induced activation of central sympatho-adrenomedullary outflow.

Shen and Ingenito reported that activation of hippocampal κ-opioid receptors induced hypotension in spontaneously hypertensive rats [39, 40]. However, investigating the relationship between brain κ-opioid receptors and central regulation of sympatho-adrenomedullary outflow is limited. In the present study, we used *nor*-binaltorphimine, which is a selective antagonist for κ-opioid receptors. This antagonist exhibits K_i values of 8.02, 12.1, and 0.06 nM for μ-, δ-, and κ-opioid receptors, respectively [38]. Central pretreatment with *nor*-binaltorphimine effectively reduced the centrally administered bombesin-induced elevation of plasma catecholamines. These results indicate that brain κ-opioid receptors play a facilitative role in the bombesin-induced activation of central sympatho-adrenomedullary outflow. Interestingly, activation of κ-opioid receptors can antagonize various μ-opioid receptor-mediated actions in the brain such as analgesia [41]. Taken together, brain κ-opioid receptors might induce activation of central sympatho-adrenomedullary outflow by inhibiting the suppressive role of brain μ-opioid receptors in the outflow.

Nociceptin is an endogenous ligand of the nociceptin receptor, which was originally identified as an opioid-like orphan receptor [14]. Although nociceptin and nociceptin receptors are structurally similar to the traditional opioid peptides and receptors [42], nociceptin receptor activity is insensitive to naloxone [43]. Centrally administered nociceptin produced hypotension, bradycardia, and inhibition of renal sympathetic nerve activity in rats [19, 44]. On the other hand, several contradictory data have been reported; i.c.v. administered nociceptin increased blood pressure and heart rate in sheep [30] and nociceptin administered into the rat solitary tract nucleus increased blood pressure and heart rate [45]. These observations suggest that the role of brain nociceptin receptors in the regulation of sympatho-adrenomedullary outflow seems to be controversial. In the present study, we used two selective antagonists for nociceptin receptors, JTC-801 and J-113397. JTC-801 exhibits IC_{50} values of 94, 325, >10,000, and >10,000 nM for nociceptin, μ-, δ-, and κ-opioid receptors, respectively [46], indicating its low selectivity over μ-opioid receptors [47]. On the other hand, J-113397 is a highly selective antagonist of nociceptin receptors, exhibiting IC_{50} values of 2.3, 2200, >10,000, and 1400 nM for nociceptin, μ-, δ-, and κ-opioid receptors, respectively [48]. Central pretreatment with JTC-801 or J-113397 effectively reduced the centrally administered bombesin-induced elevation of plasma catecholamines. These results indicate that brain nociceptin receptors play a facilitative role in the bombesin-induced activation of central sympatho-adrenomedullary outflow. Tekes et al. reported that nociceptin stimulated histamine release in the rat brain [49]. In a published study by our laboratory, we reported that centrally administered histamine induced the activation of central sympatho-adrenomedullary outflow [50], while brain histamine H_1 receptors mediated the bombesin-induced response [51]. These findings suggest a possibility that brain nociceptin receptors might facilitate the bombesin-induced activation of central sympatho-adrenomedullary outflow by augmenting the brain histaminergic nervous system.

Brain bombesin-related peptides have been implicated in the mediation/integration of stress responses [24]. In fact, in rodent models, exposure to acute stress, such as restraint and aversive stimuli, increases immunoreactivity and the in vivo release of bombesin-like peptides in the brain [52–54]. Separately, BB receptor antagonists show anxiolytic effects in the elevated plus maze test and attenuating effects on the fear-potentiated startle response [55, 56]. Considering our previous reports showing that stimulation of brain BB receptors can induce central sympatho-adrenomedullary outflow [25, 26, 57], exposure to stress could enhance the release of bombesin-like peptides in the brain, thereby inducing not only psychological disorders such as anxiety and depression but also diseases based on excessive activation of the sympatho-adrenomedullary outflow such as hypertension. From the present results, drugs that modulate brain opioid and nociceptin receptors might be useful candidates to alleviate the stress-induced diseases described above. A possible interaction between BB receptor signaling and μ-opioid receptor signaling has been reported in sensory nerves [58]; however, there are no reports showing such an interaction in the brain. Further studies are required to clarify these interaction underlying stress responses, including sympatho-adrenomedullary outflow.

In summary, brain μ-opioid receptors play a suppressive role, and brain δ-, κ-opioid, and nociceptin receptors play a facilitative role in the bombesin-induced activation of

central sympatho-adrenomedullary outflow in the rat. Thus, these brain receptors could play differential roles in regulating the activation of central sympatho-adrenomedullary outflow.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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