Brain neuronal/inducible nitric oxide synthases and cyclooxygenase-1 are involved in the bombesin-induced activation of central adrenomedullary outflow in rats

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Abstract

Brain nitric oxide (NO) is mainly generated by neuronal NO synthase (NOS) and inducible NOS. In various cells, NO has been shown to regulate cyclooxygenase (COX), which is divided into two isoforms, COX-1 and COX-2. We previously reported that bombesin injected into the right lateral ventricle evokes the secretion of noradrenaline and adrenaline from adrenal medulla by brain COX-mediated mechanisms in rats. In the present study, we examined whether NOS is involved and which types of NOS and COX are involved in the bombesin-induced activation of central adrenomedullary outflow using urethane-anesthetized rats. Intracerebroventricularly (i.c.v.) administered bombesin (1 nmol/animal)-induced elevation of plasma noradrenaline and adrenaline was attenuated by pretreatment with N^{ω}-nitro-L-arginine methyl ester (a non-selective NOS inhibitor) (0.37 and 1.11 μmol/animal, i.c.v.). 7-Nitroindazole (a neuronal NOS inhibitor) (0.03 and 0.12 μmol/animal, i.c.v.) attenuated the bombesin-induced elevation of plasma noradrenaline alone, while *S*-ethylisothiourea (an inducible NOS inhibitor) (2.7 and 27 nmol/animal, i.c.v.) and cycloheximide (an inhibitor of protein synthesis) (0.1 and 0.2 μmol/animal, i.c.v.) only attenuated the bombesin-induced elevation of plasma adrenaline. Furthermore, the bombesin-induced elevation of both catecholamines was attenuated by ketoprofen (a

selective COX-1 inhibitor) (1 and 2 μmol/animal, i.c.v.), but not influenced by NS-398 (a selective COX-2 inhibitor) (0.8 and 1.6 μmol/animal, i.c.v.). These results suggest that the brain neuronal NOS/COX-1 and inducible NOS/COX-1 are respectively involved in the bombesin-induced secretion of noradrenaline and adrenaline from the adrenal medulla in rats.

Keywords: Brain; Bombesin; Nitric oxide synthase; Cyclooxygenase; Plasma catecholamine

1. Introduction

Nitric oxide (NO) has been recognized as a neurotransmitter or a neuromodulator in the central nervous system (Moncada et al., 1991; Pacher et al., 2007). NO is generated from the amino acid L-arginine by NO synthase (NOS) (Palmer et al., 1988; Moncada et al., 1991; Jaffrey and Snyder, 1995). Brain NOS mainly consists of neuronal NOS and inducible NOS, although NOS has been divided into three isoforms: neuronal, endothelial and inducible NOS (Costa et al., 1996; Pacher et al., 2007). Constitutively expressed neuronal NOS is the most abundant isoform in the brain including the hypothalamus, especially the paraventricular nucleus (Vincent and Kimura, 1992). Since the hypothalamic paraventricular nucleus has been recognized as a regulatory center of the sympatho-adrenomedullary system (Swanson and Sawchenko, 1983; Jansen et al., 1995; Kenney et al., 2003), NO may play a role in regulating the central sympatho-adrenomedullary outflow (Zhang et al., 1997; Stern, 2004). Inducible NOS is formed in response to various cytokines and endotoxin in various cells including neurons and glial cells in the brain (Galea et al., 1992; Romero et al., 1996; Rothwell et al., 1996; Heneka and Feinstein, 2001). We previously reported the involvement of the brain NOS in centrally

administered interleukin-1β- and corticotropin-releasing factor (CRF)-induced activation of the sympatho-adrenomedullary outflow using N^{ω}-nitro-L-arginine methyl ester (L-NAME) (a non-selective NOS inhibitor) in rats (Murakami et al., 1996; Okada et al., 2003b).

Cyclooxygenase (COX) is divided into two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed in the majority of tissues, while COX-2 is generally undetectable or present at low levels in the resting state but is induced by various stimuli such as inflammatory cytokines (Smith et al., 1996). However, COX-2 has also been shown to be constitutively expressed in some tissues including the brain (Breder et al., 1995; Hetu and Riendeau, 2005). We have already reported the involvement of brain COX in the bombesin-, interleukin-1β- and CRF-induced activation of sympatho-adrenomedullary outflow using indomethacin (a non-selective COX inhibitor) in rats (Okuma et al., 1996; Murakami et al., 1996; Yokotani et al., 2001).

Centrally administrated bombesin has been shown to evoke the secretion of both noradrenaline and adrenaline from the adrenal medulla by brain COX-mediated mechanisms in rats, since the peptide-induced responses were abolished by central pretreatment with indomethacin and bilateral adrenalectomy in rats (Okuma et al., 1996; Yokotani et al., 2005). The adrenal secretion of both catecholamines was

also demonstrated in the centrally administered arginine-vasopressin- and histamine-induced responses in rats (Okada et al., 2003a; Shimizu et al., 2006; Yamaguchi-Shima et al., 2007). In the present study, therefore, we examined whether NOS is involved and which types of NOS and COX are involved in the bombesin-induced activation of central adrenomedullary outflow with regard to the isoforms of NOS and COX, using urethane-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 (1.0 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.5 ml/h), and the femoral artery was cannulated for collecting blood samples. After these procedures, animals were placed in a stereotaxic apparatus, as explained in our previous papers (Yokotani et al., 1995; Shimizu et al., 2004). The skull was drilled for administration of test substances into the right lateral ventricle using a stainless-steel cannula (0.3 mm outer diameter). 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 (Paxinos and Watson, 1986). Four hours were allowed to elapse before the start of each experiment with the application of blocking reagent or vehicle-1.

Bombesin dissolved in filtered saline was slowly administered intracerebroventricularly (i.c.v.) in a volume of 10 μl/animal using a 25-μl Hamilton syringe. 7-

Nitroindazole, ketoprofen, and *N*-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS-398) dissolved in 100% *N*,*N*-dimethylformamide (DMF) were i.c.v. administered in a volume of 3 μl/animal using a 10-μl Hamilton syringe. L-NAME, *S*-ethylisothiourea and cycloheximide dissolved in filtered saline were i.c.v. administered in a volume of 5 μl/animal using a 10-μl Hamilton syringe. Where blocking reagents were used, bombesin was i.c.v. administered 30 min after the application of L-NAME, *S*-ethylisothiourea, 7-nitroindazole, ketoprofen or NS-398, but 120 min after the application of cycloheximide due to its slightly elevating effect on the basal plasma levels of catecholamines. Each animal received only one dose of blocking reagents or vehicles. Correct placement of the cannula was confirmed at the end of each experiment by verifying that a blue dye, injected through the cannula, had spread throughout the entire ventricular system.

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

2.2. Measurement of plasma catecholamines

Blood samples (250 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 twice 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 standard. The tube was shaken for 10 min and the alumina was washed three times with 4 ml of ice-cold twice 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 x 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 1-octanesulfate sodium and 15% methanol at a flow of 0.18 ml/min; injection volume, 40 μl. The amount of catecholamine 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 was accurately determined.

2.3. Treatment of data and statistics

The area under the curve (AUC) was used to compare the effects of several kinds of inhibitors on the bombesin-induced elevation of plasma catecholamines. All values are expressed as the means±S.E.M. of the net changes above the respective basal values. The data were analyzed by repeated-measure analysis of variance (ANOVA), followed by post-hoc analysis with the Bonferroni method. *P* values less than 0.05 were taken to indicate statistical significance.

2.4. Compounds

The following drugs were used: synthetic bombesin (Peptide Institute, Osaka, Japan); cycloheximide (MP Biomedicals, Solon, OH, U.S.A.); L-NAME hydrochloride (Sigma Aldrich Fine Chemicals, St. Louis, MO, U.S.A.); *S*-ethylisothiourea hydrobromide, 7-nitroindazole and NS-398 (Cayman Chemical, Ann Arbor, MI, U.S.A.); ketoprofen (Wako, Osaka, Japan). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effects of L-NAME, 7-nitroindazole and S-ethylisothiourea on the centrally administered 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.) followed by blood sampling six times over a 120-min period had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 1).

Since we previously reported that bombesin (0.1, 1 and 10 nmol/animal, i.c.v.) elevated plasma levels of both catecholamines in a dose-dependent manner (Okuma et al., 1996), we used the dose of 1 nmol/animal in the present experiment. The administered bombesin rapidly increased plasma levels of noradrenaline and adrenaline (adrenaline > noradrenaline) (Fig. 1). These responses reached a maximum 30 min after administration of bombesin and then declined to near basal levels. However, intravenous administration of bombesin (1 nmol/animal) had no effect on the plasma levels of both catecholamines (data not shown).

Pretreatment with L-NAME (a non-selective NOS inhibitor) [1.11 μmol (300 μg)/animal, i.c.v.] had no effect on the basal plasma levels of both catecholamines (Fig. 1). L-NAME effectively attenuated the bombesin-

induced elevation of both catecholamines in a dose-dependent way [0.37 and 1.11 μmol (100 and 300 μg)/animal, i.c.v.] (Fig. 1).

Pretreatment with vehicle-1 (3 μl DMF/animal, i.c.v.) or 7-nitroindazole (a neuronal NOS inhibitor) [0.12 μmol (20 μg)/animal, i.c.v.] had no effect on the basal plasma levels of both catecholamines (Fig. 2). 7-Nitroindazole [0.03 and 0.12 μmol (5 and 20 μg)/animal, i.c.v.] effectively attenuated the bombesin-induced noradrenaline response, but had no effect on the adrenaline response induced by the peptide (Fig. 2).

Pretreatment with *S*-ethylisothiourea (an inducible NOS inhibitor) [27 nmol (5 μg)/animal, i.c.v.] had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 3). *S*-Ethylisothiourea had no effect on the bombesin-induced noradrenaline response, but effectively attenuated the bombesin-induced adrenaline response in a dose-dependent manner [2.7 and 27 nmol (0.5 and 5 μg)/animal, i.c.v.] (Fig. 3).

3.2. Effect of cycloheximide on the centrally administered bombesin-induced elevation of plasma catecholamines

Pretreatment with vehicle-1 (5 μl saline/animal, i.c.v.) or cycloheximide [0.2 μmol (60 μg)/animal, i.c.v.]

had slightly, but not significantly, elevating effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 4).

Cycloheximide [0.1 and 0.2 μmol (30 and 60 μg)/animal, i.c.v.] had no effect on the bombesin-induced noradrenaline response, while the reagent attenuated the bombesin-induced adrenaline response at a high dose of cycloheximide (0.2 μmol/animal, i.c.v) (Fig. 4).

3.3. Effects of ketoprofen and NS-398 on the centrally administered bombesin-induced elevation of plasma catecholamines

Pretreatment with vehicle-1 (3 μl DMF/animal, i.c.v), ketoprofen (a inhibitor of COX-1) [2 μmol (500 μg)/animal, i.c.v.] or NS-398 (a selective inhibitor of COX-2) [1.6 μmol (500 μg)/animal, i.c.v.] had no effect on the basal plasma levels of noradrenaline and adrenaline (Figs. 5 and 6).

Ketoprofen [1 and 2 μmol (250 and 500 μg)/animal, i.c.v.] attenuated the bombesin-induced elevation of both catecholamines (Fig. 5), while NS-398 [0.8 and 1.6 μmol (250 and 500 μg)/animal, i.c.v.] left the bombesin-induced elevation of both catecholamines largely unaffected (Fig. 6).

4. Discussion

In the present experiments, we examined whether brain NOS is involved in the centrally administered bombesin-induced elevation of plasma noradrenaline and adrenaline using L-NAME, a non-selective NOS inhibitor (Gross et al., 1990). L-NAME effectively attenuated the bombesin-induced elevation of both catecholamines, indicating the involvement of brain NOS in the bombesin-induced secretion of noradrenaline and adrenaline from the adrenal medulla in rats. Then, we examined which type of NOS is involved in the bombesin-induced responses using 7-nitroindazole and *S*-ethylisothiourea.

7-Nitroindazole is a potent and selective inhibitor of neuronal NOS with a *K*i value of 0.09 μM in purified porcine brain (Mayer et al., 1994) and with a *K*i value of 1.6 μM in bovine brain (Wolff and Gribin, 1994). The reagent decreased NO production in the rat cerebellum and hippocampus (Babbedge et al., 1993; Bush and Pollack, 2001), and prevented neuronal NOS-derived NO-mediated brain damage after hypoxia in the developing brain of the chick embryo (Giusti et al., 2008). We previously reported that the central pretreatment with 7-nitroindazole had no effect on the centrally administered interleukin-1β-induced release

of noradrenaline from sympathetic nerves in rats (Murakami et al., 2002). In the present experiment, however, 7-nitroindazole effectively attenuated the bombesin-induced elevation of plasma noradrenaline, but not adrenaline, suggesting the involvement of brain neuronal NOS in the bombesin-induced secretion of noradrenaline from the adrenal medulla in rats.

S-Ethylisothiourea is a potent and selective inhibitor of inducible NOS (Szabó et al., 1994; Southan et al., 1995; Nakane et al., 1995). *S*-Ethylisothiourea potently inhibits inducible NOS activity with a K_i value of 14.7 nM for partially purified inducible NOS obtained from lipopolysaccharide- and interferon-γ-treated RAW 264.7 macrophages and with about 20-fold more selectivity for murine inducible NOS than rat neuronal NOS (Nakane et al., 1995). The reagent also inhibits inducible NOS-derived NO-mediated suppression of the enhancement of vasoconstriction in diabetic rats (Ishikawa et al., 2004). We previously reported that central pretreatment with *S*-ethylisothiourea effectively attenuated the release of noradrenaline from sympathetic nerves induced by centrally administered interleukin-1β in rats (Murakami et al., 2002). In the present experiment, the *S*-ethylisothiourea effectively attenuated the bombesin-induced elevation of plasma adrenaline, but not noradrenaline. The result

suggests the involvement of brain inducible NOS in the bombesin-induced secretion of adrenaline from the adrenal medulla in rats.

To further clarify the involvement of brain inducible NOS in the bombesin-induced adrenaline response, we used cycloheximide, an inhibitor of protein synthesis (Obrig et al., 1971). Cycloheximide inhibits serum albumin-induced inducible NOS expression in RAW 267.4 macrophages (Poteser and Wakabayashi, 2004), and central pretreatment with this reagent effectively attenuated the release of noradrenaline from sympathetic nerves induced by centrally administered interleukin-1β in rats (Murakami et al., 2002). In the present experiments, cycloheximide attenuated the bombesin-induced adrenaline response alone. The result further suggests the involvement of inducible NOS in the bombesin-induced secretion of adrenaline from the adrenal medulla in rats.

Since the central pretreatment with indomethacin (500 μg/animal) effectively attenuated the bombesin (1 nmol/animal)-induced elevation of plasma noradrenaline and adrenaline in rats (Okuma et al., 1996), we examined which type of COX isoform is involved in the bombesin-induced responses using ketoprofen and NS-398. Ketoprofen has IC_{50} values of 0.02 and 1.08 μM for COX-1 and COX-2 in human whole blood (Brideau et al., 1996), while NS-398 has IC_{50}

values of 75 and 1.77 μM for human recombinant COX-1 and COX-2 (Barnett et al., 1994), indicating that ketoprofen and NS-398 are selective inhibitors for COX-1 and COX-2, respectively. Recently, we reported the involvement of brain COX-1 in the centrally administered histamine-induced secretion of both catecholamines from the adrenal medulla using ketoprofen and NS-398 in rats (Shimizu et al., 2006). In the present experiments, central pretreatment with ketoprofen effectively attenuated the bombesin-induced elevation of plasma noradrenaline and adrenaline, while NS-398 had no effect on the peptide-induced responses. These results suggest the involvement of brain COX-1 in the bombesin-induced secretion of both catecholamines from the adrenal medulla in rats.

The biosynthesis of prostaglandins is regulated by NO in various cell types. This phenomenon was first reported by Salvemini and coworkers (1993) who showed that NO activated COX and enhanced prostaglandin synthesis in the mouse macrophage cell line RAW264.7. Since then, this observation was confirmed and extended by other investigators in various cellular systems and animal models (Mollace et al., 2005). NO interacts with the thiol groups of cysteine in COX, thereby directly activating COX (Kim et al., 2005; Upmacis et al., 2006). This modification of cysteine residues in proteins is called *S*-

nitrosylation, which has been recognized as an important biological reaction of NO for post-translational regulation (Hess et al., 2005). We previously reported that centrally administered 3-morpholinosydnonimine (an NO donor)-induced elevation of plasma noradrenaline and adrenaline was abolished by central pretreatment with 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (an NO scavenger) and indomethacin, suggesting that NO is upstream to COX activation in the centrally mediated elevation of plasma catecholamines (Murakami et al., 1998). The possible link between brain NOS and COX has also been reported in the centrally administered interleukin-1β-induced elevation of plasma noradrenaline in rats, due to the inhibitory effect of L-NAME and indomethacin on the cytokine-induced response (Murakami et al., 1996). The present results further suggest the functional linkage between brain NOS and COX in the bombesin-induced secretion of both catecholamines from the adrenal medulla in rats.

In summary, we demonstrated that the brain neuronal NOS/COX-1 and inducible NOS/COX-1 are respectively involved in the centrally administered bombesin-induced secretion of noradrenaline and adrenaline from the adrenal medulla in rats.

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Legends to figures

Fig. 1. Effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME) (a non-selective NOS inhibitor) on the centrally administered bombesin-induced elevation of plasma catecholamines. L-NAME [0.37 and 1.11 μmol (100 and 300 μg)/animal] or vehicle-1 (5 μl saline/animal) was intracerebroventricularly (i.c.v.) 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) Increments of plasma catecholamines above the basal at each time point are expressed as pg/ml. Arrows indicate the administrations of L-NAME/vehicle-1 and bombesin/vehicle-2. (B) The area under the curve (AUC) of the bombesin-induced elevation of plasma catecholamines above the basal is expressed as pg/2 h. Δ Noradrenaline and Δ adrenaline: increments of noradrenaline and adrenaline above the basal in (A) and (B). Each point represents the mean±S.E.M. *Significantly different (p<0.05) from the vehicle-1- and bombesin-treated group. The actual values for noradrenaline and adrenaline at 0 min were 117±15 and 127±27 pg/ml in the group pretreated with vehicle-1 (n=10); 84±13 and 57±26 pg/ml in the group pretreated with L-NAME (0.37 μmol/animal) (n=6); 110±20 and 126±26 pg/ml in the group pretreated with L-NAME (1.11 μmol/animal) (n=12), respectively.

Fig. 2. Effect of 7-nitroindazole (a neuronal NOS inhibitor) on the centrally administered bombesin-induced elevation of plasma catecholamines. 7-Nitroindazole [0.03 and 0.12 μmol (5 and 20 μg)/animal] or vehicle-1 (3 μl DMF/animal) was i.c.v. 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) Increments of plasma catecholamines above the basal. Arrows indicate the administrations of 7-nitroindazole/vehicle-1 and bombesin/vehicle-2. (B) AUC of the bombesin-induced elevation of plasma catecholamines above the basal. *p<0.05, significantly different from vehicle-1- and bombesin-treated group. Other conditions were the same as those of Fig. 1. The actual values for noradrenaline and adrenaline at 0 min were 241±40 and 219±55 pg/ml in the group pretreated with vehicle-1 (n=11); 214±40 and 112±23 pg/ml in the group pretreated with 7-nitroindazole (0.03 μmol/animal) (n=5); 134±22 and 104±30 pg/ml in the group pretreated with 7-nitroindazole (0.12 μmol/animal) (n=10), respectively.

Fig. 3. Effect of *S*-ethylisothiourea (an inducible NOS inhibitor) on the centrally administered bombesin-induced elevation of plasma catecholamines. *S*-Ethylisothiourea [2.7 and 27 nmol (0.5 and 5 μg)/animal] or vehicle-1 (5 μl saline/animal) was i.c.v. 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) Increments of plasma catecholamines above the basal. Arrows indicate the administrations of *S*-ethylisothiourea/vehicle-1 and bombesin/vehicle-2. (B) AUC of the bombesin-induced elevation of plasma catecholamines above the basal. The vehicle-1-treated groups are those of Fig. 1. *Significantly different ($p<0.05$) from the vehicle-1- and bombesin-treated group. Other conditions were the same as those of Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were 181±37 and 84±31 pg/ml in the group pretreated with *S*-ethylisothiourea (2.7 nmol/animal) (n=6); 161±21 and 90±16 pg/ml in the group pretreated with *S*-ethylisothiourea (27 nmol/animal) (n=11), respectively.

Fig. 4. Effect of cycloheximide (an inhibitor of protein synthesis) on the centrally administered bombesin-induced elevation of plasma catecholamines. Cycloheximide [0.1 and 0.2 μmol (30 and 60 μg)/animal] or vehicle-1 (5 μl saline/animal) was i.c.v. administered 120 min before the administration of bombesin (1 nmol/animal, i.c.v.) or vehicle-2 (10 μl saline/animal, i.c.v.). (A) Increments of plasma catecholamines above the basal. Arrows indicate the administrations of cycloheximide/vehicle-1 and bombesin/vehicle-2. (B) AUC of the bombesin-induced elevation of plasma catecholamines above the basal. *$p<0.05$, significantly different from vehicle-1- and

bombesin-treated group. Other conditions were the same as those of Figs. 1-3. The actual values for noradrenaline and adrenaline at 0 min were 105±14 and 89±18 pg/ml in the group pretreated with vehicle-1 (n=13); 159±32 and 129±30 pg/ml in the group pretreated with cycloheximide (0.1 μmol/animal) (n=6); 140±29 and 360±138 pg/ml in the group pretreated with cycloheximide (0.2 μmol/animal) (n=13), respectively.

Fig. 5. Effect of ketoprofen (a selective COX-1 inhibitor) on the centrally administered bombesin-induced elevation of plasma catecholamines. Ketoprofen [1 and 2 μmol (250 and 500 μg)/animal] or vehicle-1 (3 μl DMF/animal) was i.c.v. 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) Increments of plasma catecholamines above the basal. Arrows indicate the administrations of ketoprofen/vehicle-1 and bombesin/vehicle-2. (B) AUC of the bombesin-induced elevation of plasma catecholamines above the basal. *p<0.05, significantly different from vehicle-1- and bombesin-treated group. The vehicle-1-treated groups are those of Fig. 2. Other conditions were the same as those of Figs. 1-4. The actual values for noradrenaline and adrenaline at 0 min were 219±47 and 288±60 pg/ml in the group pretreated with ketoprofen (1 μmol/animal) (n=7);

226±26 and 238±35 pg/ml in the group pretreated with ketoprofen (2 μmol/animal) (n=11), respectively.

Fig. 6. Effect of NS-398 (a selective COX-2 inhibitor) on the centrally administered bombesin-induced elevation of plasma catecholamines. NS-398 [0.8 and 1.6 μmol (250 and 500 μg)/animal] or vehicle-1 (3 μl DMF/animal) was i.c.v. 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) Increments of plasma catecholamines above the basal. Arrows indicate the administrations of NS-398/vehicle-1 and bombesin/vehicle-2. (B) AUC of bombesin-induced elevation of plasma catecholamines above the basal. *p<0.05, significantly different from vehicle-1- and bombesin-treated group. The vehicle-1-treated groups are those of Fig. 2. Other conditions were the same as those of Figs. 1-5. The actual values for noradrenaline and adrenaline at 0 min were 335±99 and 306±61 pg/ml in the group pretreated with NS-398 (0.8 μmol/animal) (n=7); 383±42 and 111±29 pg/ml in the group pretreated with NS-398 (1.6 μmol/animal) (n=9), respectively.

Figure 1

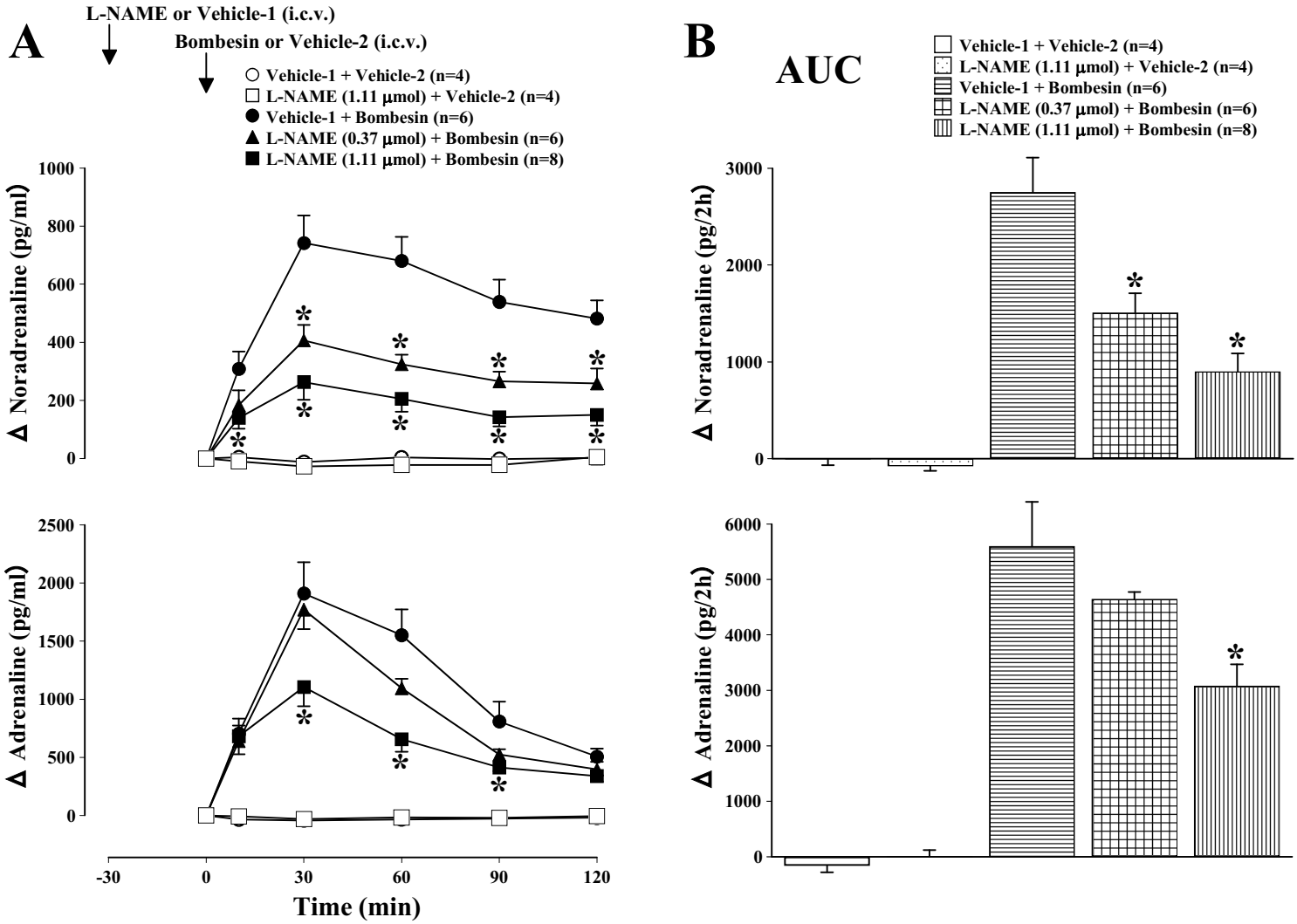

A
L-NAME or Vehicle-1 (i.c.v.)
Bombesin or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
L-NAME (1.11 µmol) + Vehicle-2 (n=4)
Vehicle-1 + Bombesin (n=6)
L-NAME (0.37 µmol) + Bombesin (n=6)
L-NAME (1.11 µmol) + Bombesin (n=8)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
Time (min)
B
AUC
Vehicle-1 + Vehicle-2 (n=4)
L-NAME (1.11 µmol) + Vehicle-2 (n=4)
Vehicle-1 + Bombesin (n=6)
L-NAME (0.37 µmol) + Bombesin (n=6)
L-NAME (1.11 µmol) + Bombesin (n=8)
Δ Noradrenaline (pg/2h)
Δ Adrenaline (pg/2h)

Figure 2

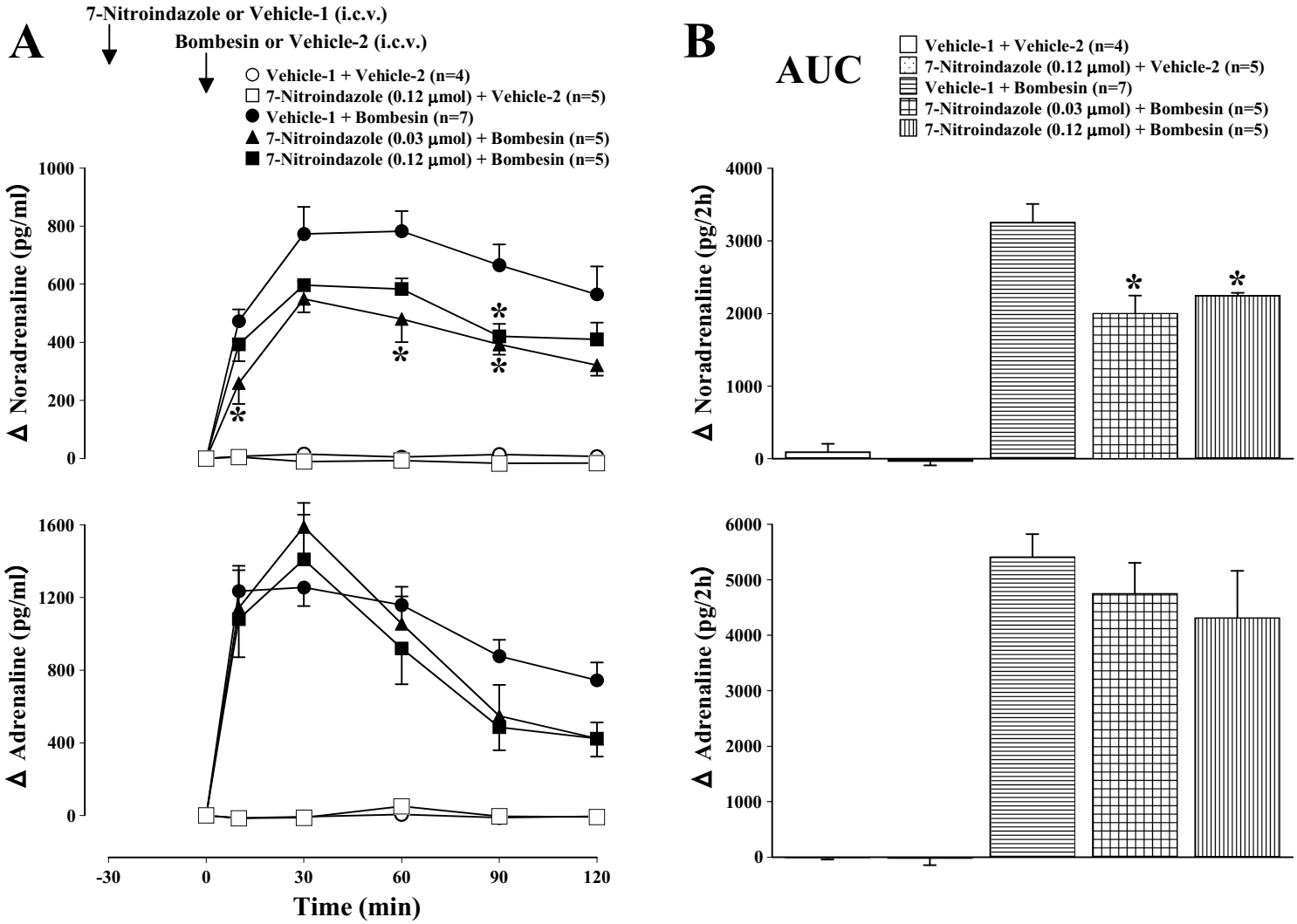

A
7-Nitroindazole or Vehicle-1 (i.c.v.)
Bombesin or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
7-Nitroindazole (0.12 µmol) + Vehicle-2 (n=5)
Vehicle-1 + Bombesin (n=7)
7-Nitroindazole (0.03 µmol) + Bombesin (n=5)
7-Nitroindazole (0.12 µmol) + Bombesin (n=5)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
Time (min)
B
AUC
Vehicle-1 + Vehicle-2 (n=4)
7-Nitroindazole (0.12 µmol) + Vehicle-2 (n=5)
Vehicle-1 + Bombesin (n=7)
7-Nitroindazole (0.03 µmol) + Bombesin (n=5)
7-Nitroindazole (0.12 µmol) + Bombesin (n=5)
Δ Noradrenaline (pg/2h)
Δ Adrenaline (pg/2h)

Figure 3

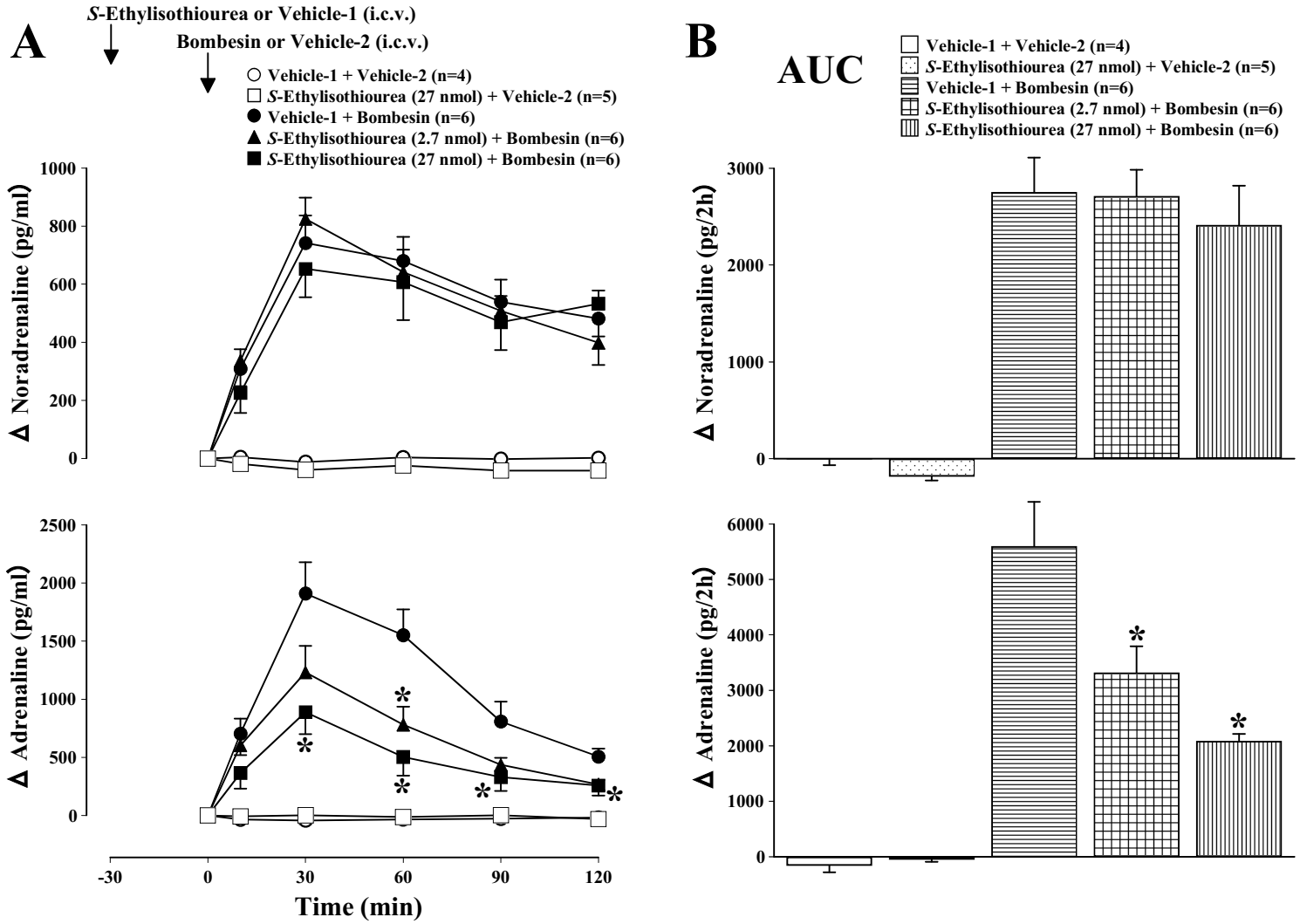
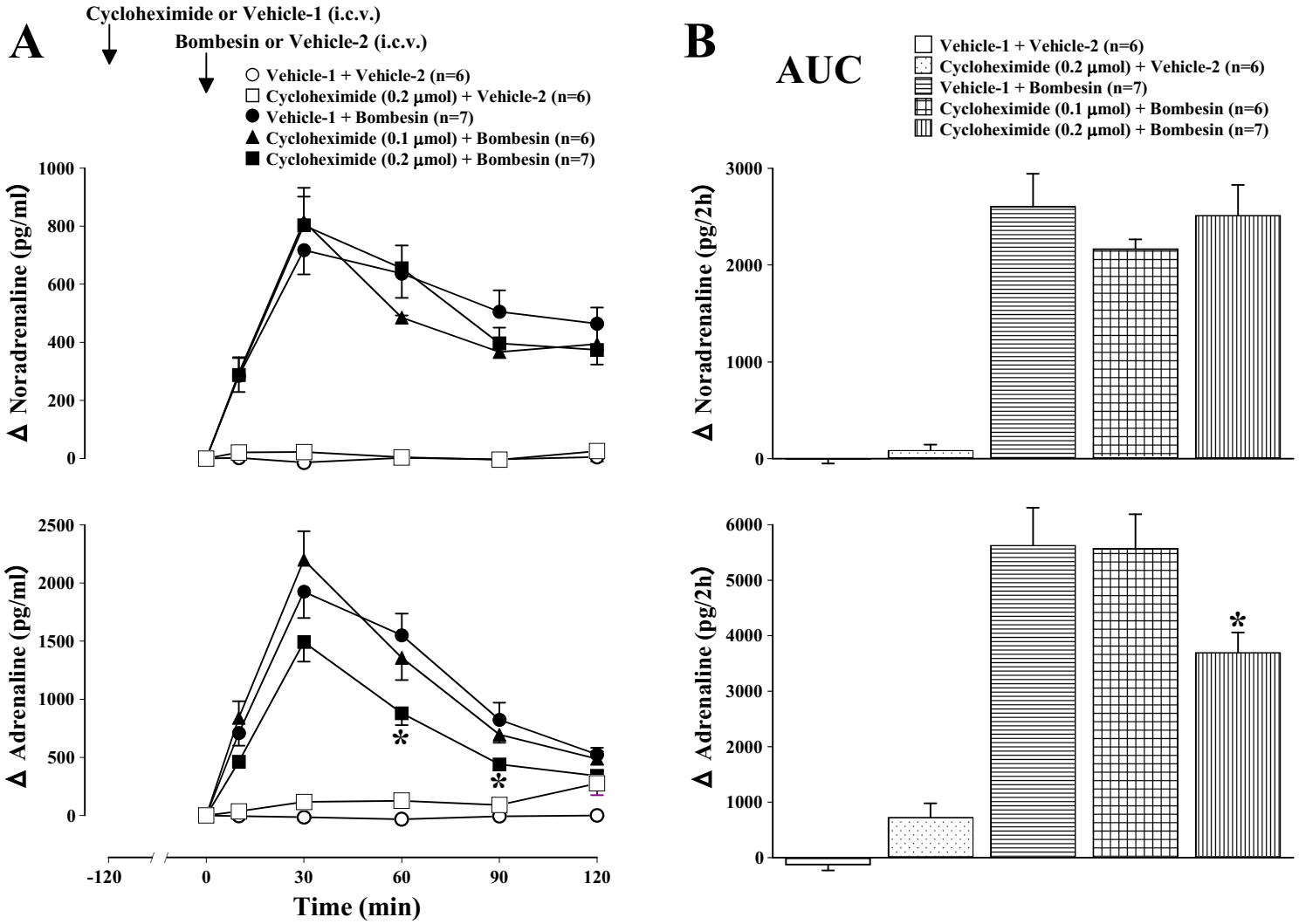

A
S-Ethylisothiourea or Vehicle-1 (i.c.v.)
Bombesin or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
S-Ethylisothiourea (27 nmol) + Vehicle-2 (n=5)
Vehicle-1 + Bombesin (n=6)
S-Ethylisothiourea (2.7 nmol) + Bombesin (n=6)
S-Ethylisothiourea (27 nmol) + Bombesin (n=6)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
Time (min)
B
AUC
Vehicle-1 + Vehicle-2 (n=4)
S-Ethylisothiourea (27 nmol) + Vehicle-2 (n=5)
Vehicle-1 + Bombesin (n=6)
S-Ethylisothiourea (2.7 nmol) + Bombesin (n=6)
S-Ethylisothiourea (27 nmol) + Bombesin (n=6)
Δ Noradrenaline (pg/2h)
Δ Adrenaline (pg/2h)

Figure 4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Cycloheximide or Vehicle-1 (i.c.v.)
Bombesin or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=6)
Cycloheximide (0.2 µmol) + Vehicle-2 (n=6)
Vehicle-1 + Bombesin (n=7)
Cycloheximide (0.1 µmol) + Bombesin (n=6)
Cycloheximide (0.2 µmol) + Bombesin (n=7)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
Time (min)
B
AUC
Δ Noradrenaline (pg/2h)
Δ Adrenaline (pg/2h)

Figure 5

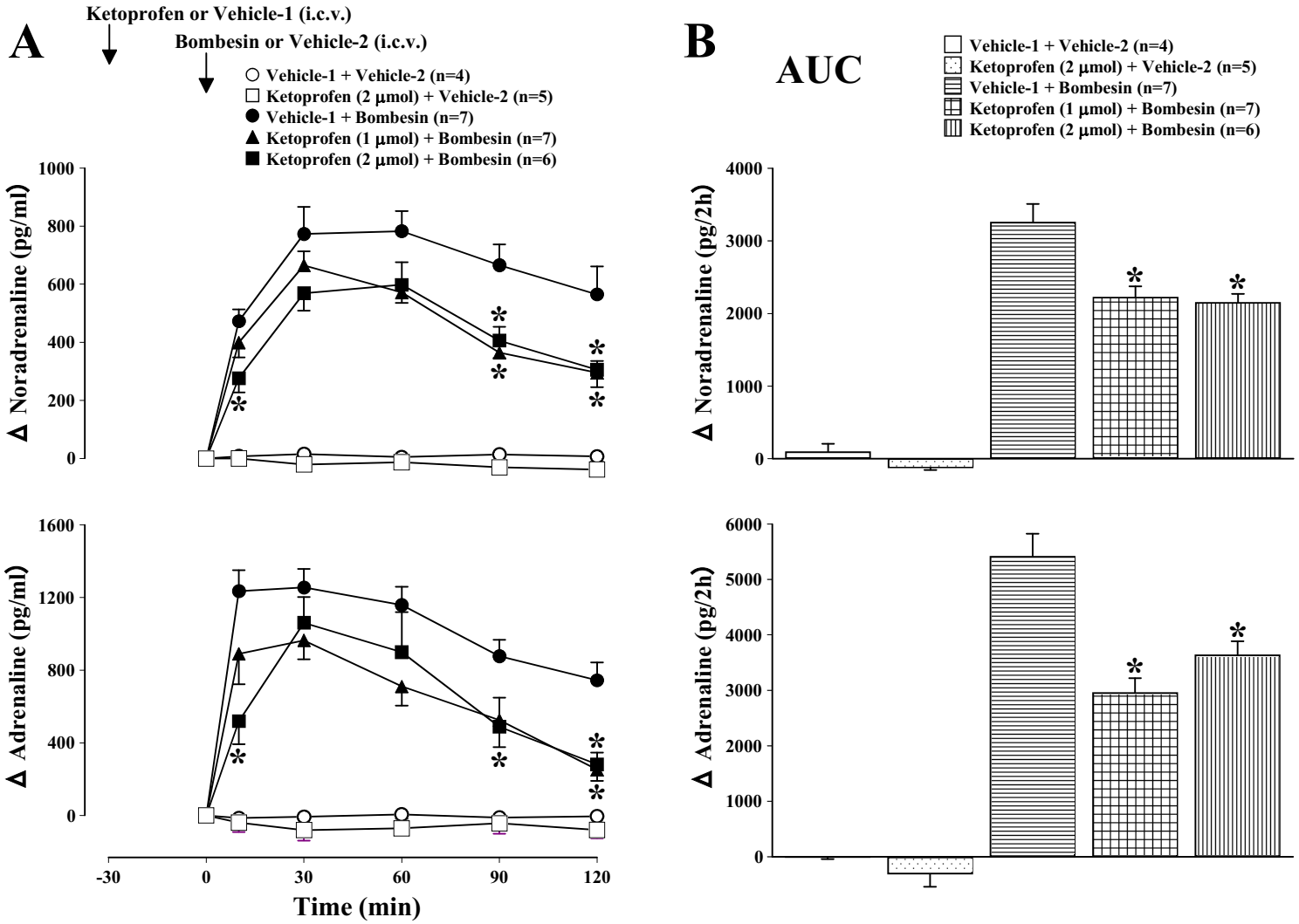
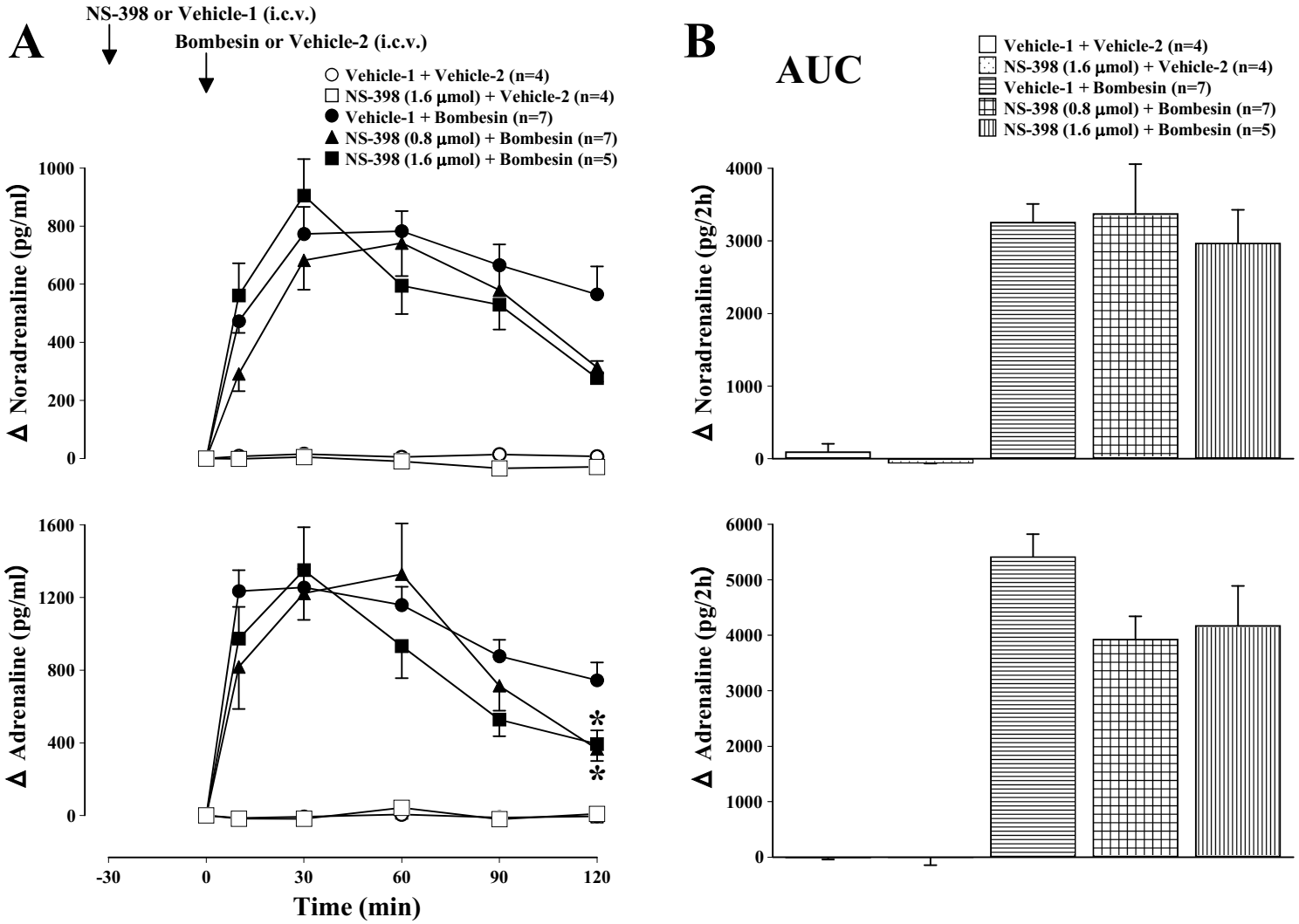

A
Ketoprofen or Vehicle-1 (i.c.v.)
Bombesin or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
Ketoprofen (2 µmol) + Vehicle-2 (n=5)
Vehicle-1 + Bombesin (n=7)
Ketoprofen (1 µmol) + Bombesin (n=7)
Ketoprofen (2 µmol) + Bombesin (n=6)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
Time (min)
B
AUC
Vehicle-1 + Vehicle-2 (n=4)
Ketoprofen (2 µmol) + Vehicle-2 (n=5)
Vehicle-1 + Bombesin (n=7)
Ketoprofen (1 µmol) + Bombesin (n=7)
Ketoprofen (2 µmol) + Bombesin (n=6)
Δ Noradrenaline (pg/2h)
Δ Adrenaline (pg/2h)

Figure 6


A
NS-398 or Vehicle-1 (i.c.v.)
Bombesin or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
NS-398 (1.6 µmol) + Vehicle-2 (n=4)
Vehicle-1 + Bombesin (n=7)
NS-398 (0.8 µmol) + Bombesin (n=7)
NS-398 (1.6 µmol) + Bombesin (n=5)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
Time (min)
B
AUC
Vehicle-1 + Vehicle-2 (n=4)
NS-398 (1.6 µmol) + Vehicle-2 (n=4)
Vehicle-1 + Bombesin (n=7)
NS-398 (0.8 µmol) + Bombesin (n=7)
NS-398 (1.6 µmol) + Bombesin (n=5)
Δ Noradrenaline (pg/2h)
Δ Adrenaline (pg/2h)