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Brain prostanoid TP receptor-mediated adrenal noradrenaline secretion and EP_3 receptor-mediated sympathetic noradrenaline release in rats

Keiko Yokotani[a], Shoshiro Okada[a], Kumiko Nakamura[a], Naoko Yamaguchi-Shima[a], Takahiro Shimizu[a], Junichi Arai[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*

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Abstract

Sympathetic nerves release noradrenaline, whereas adrenal medullary chromaffin cells secrete noradrenaline and adrenaline. Therefore, plasma noradrenaline reflects the secretion from adrenal medulla in addition to the release from sympathetic nerves, however the exact mechanisms of adrenal noradrenaline secretion remain to be elucidated. The present study was designated to characterize the source of plasma noradrenaline induced by intracerebroventricularly (i.c.v.) administered bombesin and prostaglandin E_2 in urethane-anesthetized rats. Bombesin (1.0 nmol/animal, i.c.v.) elevated plasma noradrenaline and adrenaline, while prostaglandin E_2 (0.3 nmol/animal, i.c.v.) elevated only plasma noradrenaline. The bombesin-induced elevations of both catecholamines were attenuated by pretreatments with furegrelate (an inhibitor of thromboxane A_2 synthase) [250 and 500 μg (0.9 and 1.8 μmol)/animal, i.c.v.)] and [(+)-S-145] [(+)-(1*R*,2*R*,3*S*,4*S*)-(5*Z*)-7-(3-[4-^{3}H]-phenylsulphonyl-aminobicyclo[2.2.1]hept-2-yl)hept-5-enoic acid sodium salt] (an antagonist of prostanoid TP receptors) [100 and 250 μg (250 and 625 nmol)/animal)], and abolished by acute bilateral adrenalectomy. On the other hand, the prostaglandin E_2-induced elevation of plasma noradrenaline was not influenced by acute bilateral adrenalectomy. These results suggest that adrenal noradrenaline secretion and sympathetic noradrenaline release are mediated by differential central mechanisms; brain prostanoid TP receptors activated by bombesin are involved in the adrenal noradrenaline secretion, while brain prostanoid EP (probably EP_3) receptors activated by prostaglandin E_2 are involved in the sympathetic noradrenaline release in rats. Brain prostanoid TP receptors activated by bombesin are also involved in the adrenal adrenaline secretion.

Keywords: Bombesin; Thromboxane A_2; Prostaglandin E_2; Noradrenaline; Adrenal gland; Sympathetic nerve

1. Introduction

The relative importance of sympathetic nerve activity and adrenomedullary secretion in various physiological situations has generally been inferred from measurement of plasma noradrenaline and adrenaline. Noradrenaline and adrenaline have overlapping, but essentially distinct roles: noradrenaline is the more potent vasoconstrictor, while adrenaline is responsible for metabolic actions (such as raising the blood glucose level) in addition to cardiovascular effects. Hypoglycemia causes the elevation of plasma adrenaline (Young et al., 1984; Fujino and Fujii, 1995; Vollmer et al., 1997), while hypotension elevates both catecholamines (noradrenaline>adrenaline) (Brown and Fisher, 1984; Vollmer et al., 2000). Centrally administered neuropeptides, such as bombesin, corticotropin-releasing factor (CRF), thyrotropin-releasing hormone, calcitonin gene-related peptide and vasopressin, also produce differential changes in the plasma levels of noradrenaline and adrenaline (Brown et al., 1979, 1985; Fisher et al., 1983; Feuerstein et al., 1984; Brown and Fisher, 1984; Hasegawa et al., 1993; Okuma et al., 1996; Yokotani et al., 2001; Okada et al., 2002). However, plasma noradrenaline reflects the release not only from sympathetic nerves but also from

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

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adrenal medulla (Folkow and von Euler, 1954; Vollmer et al., 1997; Yokotani et al., 2002; Okada et al., 2003).

Anatomical studies have provided histochemical differentiation of the populations of adrenal medullary chromaffin cells secreting noradrenaline and those secreting adrenaline (Goldstein et al., 1971; Verhofstad et al., 1985; Dorsey and Schmidt, 1993). Some studies emphasized the mechanisms that would lead to differential secretion of noradrenaline and adrenaline to be dependent on differences in receptors located on each chromaffin cell (Vollmer et al., 1988; Aunis and Langley, 1999) and in neurotransmitters released from adrenal branch of the splanchnic nerves (preganglionic sympathetic nerves) (Malhotra and Wakade, 1987; Wakade et al., 1991). However, several lines of evidence suggest that two populations of adrenal chromaffin cells are regulated by distinct neural pathways to adrenal medulla (Vollmer et al., 2000). Stimulation of different hypothalamic sites in cats can evoke selective secretion of adrenal noradrenaline and adrenaline (Folkow and von Euler, 1954; Robinson et al., 1983). The noradrenaline- and adrenaline-containing cells are independently innervated by separate groups of preganglionic neurons located in spinal cord (Edwards et al., 1996). Recently, we reported that centrally administered vasopressin evokes adrenal secretion of noradrenaline and adrenaline, while centrally administered CRF evokes adrenal adrenaline secretion and sympathetic noradrenaline release in rats (Okada et al., 2003). The result suggests the existence of separate neural circuits between vasopressin-induced adrenal noradrenaline secretion and CRF-induced sympathetic noradrenaline release.

In the present study, we aimed to clarify the source of plasma noradrenaline elevated by centrally administered bombesin and prostaglandin E_2 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 femoral artery was cannulated for collecting blood samples. In some experiments, acute bilateral adrenalectomy [plus hydrocortisone (5 mg/kg, i.m.)] or sham-operation (plus 200 μl saline/animal, i.m.) was done just before the experiments by an abdominal midline incision (Ikushima et al., 1982). After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous paper (Yokotani et al., 2001). The skull was drilled for intracerebroventricular administration of test substances using stainless-steel cannula (0.3 mm outer diameter) or a double lumens cannula (0.50 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, 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). Then, 3 h were allowed to elapse before the application of bombesin and prostaglandin E_2 dissolved in sterile saline. These reagents were slowly injected into the right lateral ventricle in a volume of 5 μl using a 50-μl Hamilton syringe. Prostaglandin E_2 dissolved in 99% ethanol was stored at −20 °C. The stock solution was diluted with saline whenever we used prostaglandin E_2 and the final concentration of ethanol was adjusted to 0.5%. Furegrelate and (+)-S-145 dissolved in sterile saline was also administered into the right lateral ventricle in a volume of 10 μl 60 min before the application of bombesin. 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. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically by high performance liquid chromatography (Okada et al., 2000). Briefly, after centrifugation, plasma (100 μl) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of double deionized water, 1 ng of 3,4-dihydroxybenzylamine as an internal standard and 1 ml of 1.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA. The tube was shaken for 10 min and 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 high performance liquid chromatography. 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, 750 mg/l 1-octane sulfate sodium (Nacalai Tesque, Kyoto, Japan) and 15% methanol at a flow of 0.22 ml/min. The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine. This assay could determine 0.5 pg of noradrenaline and adrenaline accurately.

2.3. Treatment of data and statistics

Results are expressed as the mean ± S.E.M. of the net changes above the respective basal values. Data were analyzed by one-way analysis of variance (ANOVA), followed by post-hoc analysis with the Bonferroni method for comparing a control to all other means (Figs. 1 and 2). When only two means were compared, the data were analyzed by ANOVA followed by unpaired Student's *t*-test or Welch's *t*-test (Figs. 3 and 4). *P* values less than 0.05 were taken to indicate significance.

2.4. Compounds

The following drugs were used: bombesin (Frog, *Bombina bombina*) (Peptide Institude, Osaka, Japan); furegrelate sodium (Biomol Research Lab., Plymouth Meeting, PA, U.S.A.); (+)-S-145 (a kind gift from Shionogi Pharmaceutical Co. Ltd., Osaka, Japan); hydrocortisone, prostaglandin E_2 (Sigma Aldrich Fine Chemicals, St. Louis, MO, U.S.A.). All other reagents were the highest grade available (Nacalai Tesque, Kyoto, Japan).

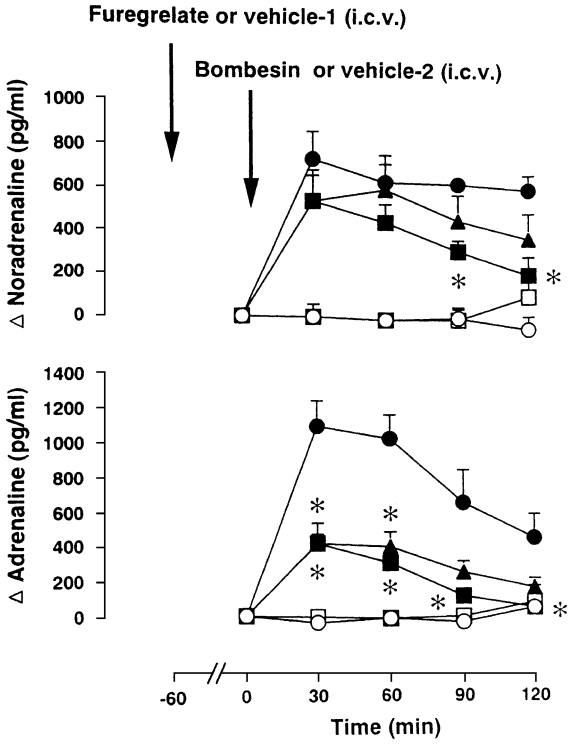

Fig. 1. Effects of furegrelate, an inhibitor of thromboxane A_2 synthase, on the bombesin-induced elevations of plasma catecholamines. Δ noradrenaline and Δ adrenaline; increase of noradrenaline and adrenaline above the basal. Furegrelate [250 and 500 μg (0.9 and 1.8 μmol/animal] or vehicle-1 (10 μl saline/animal) was intracerebroventricularly (i.c.v.) administered 60 min before administration of bombesin (1.0 nmol/animal, i.c.v.) or vehicle-2 (5 μl saline/animal, i.c.v.). Arrows indicate intracerebroventricular administration of vehicles or reagents (furegrelate and bombesin). ○, vehicle-1 plus vehicle-2 ($n=5$); □, furegrelate (500 μg/animal) plus vehicle-2 ($n=4$); ●, vehicle-1 plus bombesin ($n=6$); ▲, furegrelate (250 μg/animal) plus bombesin ($n=6$); ■, furegrelate (500 μg/animal) plus bombesin ($n=6$). Each point represents the mean ± S.E.M. *Significantly different ($P<0.05$) from those treated with vehicle-1 plus bombesin. The actual values for noradrenaline and adrenaline at 0 min were 339 ± 29 and 197 ± 36 pg/ml in vehicle-1-pretreated group ($n=11$), 431 ± 54 and 217 ± 51 pg/ml in furegrelate (250 μg/animal)-pretreated group ($n=6$) and 401 ± 24 and 188 ± 46 pg/ml in furegrelate (500 μg/animal)-pretreated group ($n=10$), respectively.

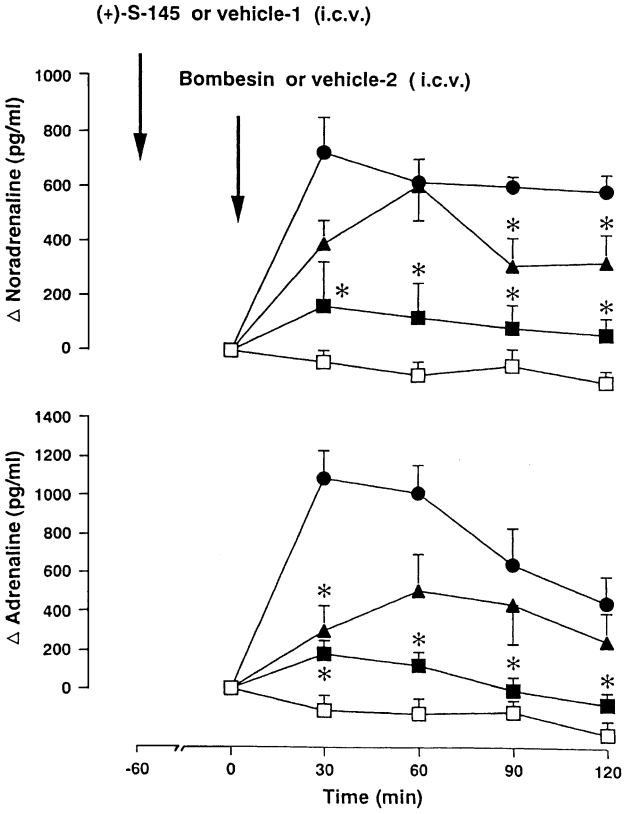

Fig. 2. Effects of (+)-S-145, a blocker of prostanoid TP receptors on the bombesin-induced elevations of plasma catecholamines. (+)-S-145 [100 and 250 μg (250 and 625 μmol)/animal, i.c.v.] or vehicle-1 (10 μl saline/animal, i.c.v.) was administered 60 min before administration of bombesin (1.0 nmol/animal, i.c.v.) or vehicle-2 (5 μl saline/animal, i.c.v.). Arrows indicate intracerebroventricular administration of vehicles or reagents [(+)-S-145 and bombesin]. ●, vehicle-1 plus bombesin ($n=6$) (cited from Fig. 1); ▲, (+)-S-145 (100 μg/animal) plus bombesin ($n=5$); ■, (+)-S-145 (250 μg/animal) plus bombesin ($n=5$); □, (+)-S-145 (250 μg/animal) plus vehicle-2 ($n=5$). *Significantly different ($P<0.05$) from the group treated with 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 311 ± 65 and 234 ± 73 pg/ml in the (+)-S-145 (100 μg/animal)-pretreated group ($n=5$), 337 ± 49 and 283 ± 52 pg/ml in the (+)-S-145 (250 μg/animal)-pretreated group ($n=10$), respectively.

3. Results

3.1. Effects of furegrelate, an inhibitor of thromboxane A_2 synthase, on the bombesin-induced elevations of plasma catecholamines

Intracerebroventricularly (i.c.v.) administered vehicle-1 (10 μl saline/animal) and vehicle-2 (5 μl saline/animal) and

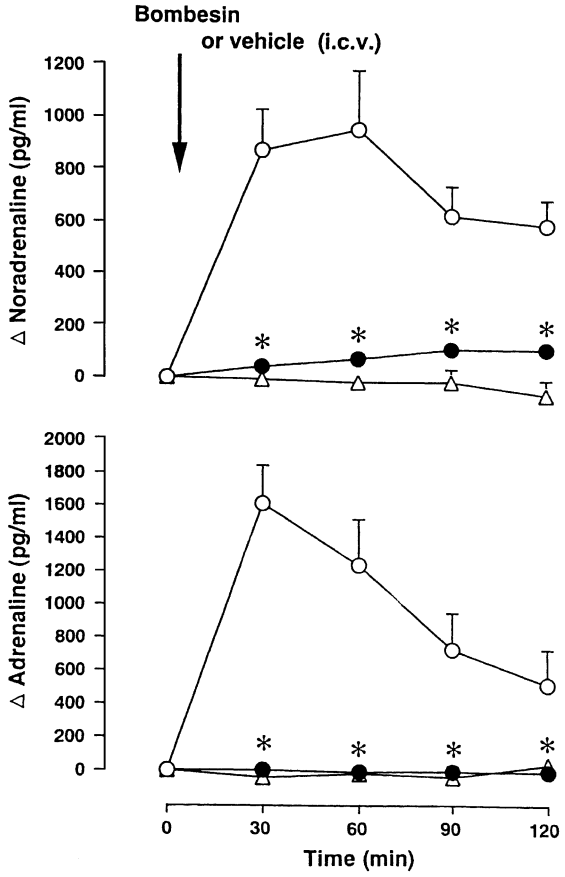

Fig. 3. Effects of acute bilateral adrenalectomy on the bombesin-induced elevations of plasma catecholamines. Hydrocortisone (5 mg/kg) or 200 μl saline was intramuscularly administered in adrenalectomized or sham-operated group, respectively. Arrow indicates the administration of bombesin (1.0 nmol/animal, i.c.v.) or vehicle (5 μl saline/animal, i.c.v.). △, Sham-operation plus vehicle ($n=4$); ○, sham-operation plus bombesin ($n=5$); ●, adrenalectomy plus bombesin ($n=5$). *Significantly different ($P<0.05$) from the group treated with sham-operation plus bombesin. Other conditions were the same as those of Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were 299 ± 44 and 232 ± 23 pg/ml in sham-operated group ($n=9$) and 135 ± 29 and 0 pg/ml in adrenalectomized group ($n=5$), respectively.

blood sampling five times during a 120-min period had no effect on the basal plasma levels of either noradrenaline or adrenaline (Fig. 1). Pretreatment with furegrelate (an inhibitor of thromboxane A_2 synthase) [500 μg (1.2 μmol)/animal, i.c.v.] had no effect on the basal plasma levels of catecholamines (Fig. 1).

Previously, we reported that bombesin (0.1, 1.0 and 10 nmol/animal, i.c.v.) dose-dependently elevated plasma levels of noradrenaline and adrenaline (Okuma et al., 1996). In the present experiments, therefore, we used the dose of 1.0 nmol/animal of bombesin. Bombesin (1.0 nmol/animal, i.c.v.) rapidly elevated plasma levels of noradrenaline and adrenaline (Fig. 1). These responses reached a maximum 30 min after the administration of this peptide and then gradually declined. The bombesin-induced elevation of plasma levels of noradrenaline and adrenaline was attenuated by furegrelate in a dose-dependent manner [250 and 500 μg (0.6 and 1.2 μmol)/animal, i.c.v.] (Fig. 1).

3.2. Effects of (+)-S-145, a selective blocker of prostanoid TP receptors, on the bombesin-induced elevations of plasma catecholamines

Previously, we reported that (+)-S-145 [100, 250 and 1000 μg (250, 625 and 2500 nmol)/animal, i.c.v.] dose-dependently reduced the centrally administered nitric oxide donor (3-morpholinosydnonimine)-induced elevation of plasma catecholamines (Murakami et al., 1998). In the present experiments, therefore, we used the doses of 100 μg/animal and 250 μg/animal of (+)-S-145.

Pretreatment with (+)-S-145 (250 μg/animal, i.c.v.) had no effect on the basal plasma levels of both catecholamines (Fig. 2). (+)-S-145 (100 and 250 μg/animal, i.c.v.) dose-

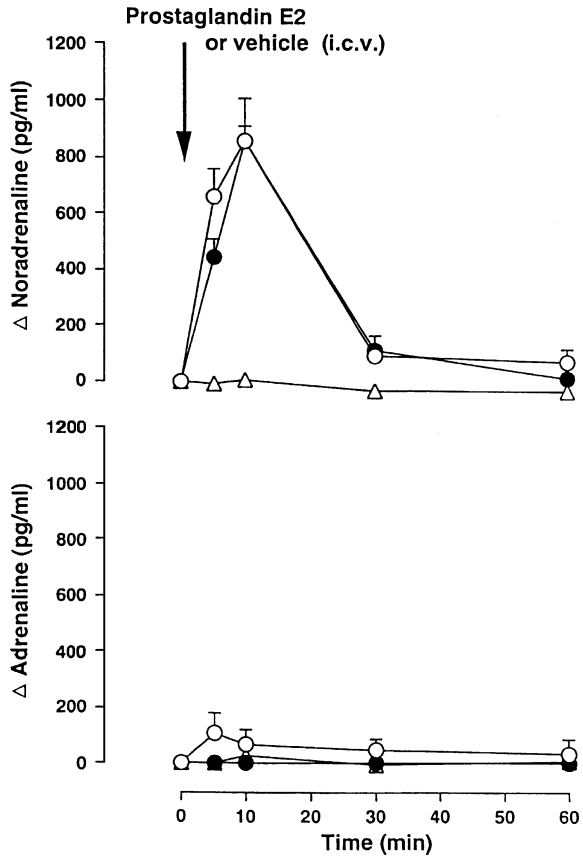

Fig. 4. Effect of acute bilateral adrenalectomy on the prostaglandin E_2-induced elevation of plasma noradrenaline. Hydrocortisone (5 mg/kg) or 200 μl saline was intramuscularly administered in adrenalectomized or sham-operated group, respectively. Arrow indicates the administration of prostaglandin E_2 (0.3 nmol/animal, i.c.v.) or vehicle (5 μl saline/animal, i.c.v.). △, sham-operation plus vehicle ($n=5$); ○, sham-operation plus prostaglandin E_2 ($n=5$); ●, adrenalectomy plus prostaglandin E_2 ($n=5$). *Significantly different ($P<0.05$) from the group treated with sham-operation plus prostaglandin E_2. Other conditions were the same as those of Fig. 3. The actual values for noradrenaline and adrenaline at 0 min were 250 ± 31 and 269 ± 30 pg/ml in sham-operated group ($n=10$) and 200 ± 34 and 0 pg/ml in adrenalectomized group ($n=5$), respectively.

dependently attenuated the bombesin (1.0 nmol/animal, i.c.v.)-induced elevations of both catecholamines (Fig. 2).

3.3. Effects of acute bilateral adrenalectomy on the bombesin-induced elevations of plasma catecholamines

Sham-operation had no effect on the basal plasma level of catecholamines. Acute bilateral adrenalectomy reduced the basal plasma level of noradrenaline, while plasma adrenaline was not detectable in bilaterally adrenalectomized rats (Fig. 3).

Bombesin (1.0 nmol/animal, i.c.v.)-induced elevation of plasma level of adrenaline was augmented in the sham-operated rats. The peptide-induced elevation of plasma levels of noradrenaline and adrenaline was abolished by bilateral adrenalectomy (Fig. 3).

3.4. Effect of acute bilateral adrenalectomy on the prostaglandin E_2-induced elevation of plasma noradrenaline

Previously, we reported that prostaglandin E_2 (0.15, 0.3 and 1.5 nmol/animal, i.c.v.) dose-dependently elevates plasma level of noradrenaline, but had no effect on the plasma level of adrenaline (Yokotani et al., 1995). In the present experiments, we used a dose of 0.3 nmol/animal of prostaglandin E_2.

Sham-operation had no effect on the basal plasma levels of both catecholamines. On the other hand, acute bilateral adrenalectomy reduced the basal plasma level of noradrenaline and abolished plasma adrenaline. Prostaglandin E_2 (0.3 nmol/animal, i.c.v.) rapidly elevated plasma level of noradrenaline, but had no effect on plasma adrenaline (Fig. 4). Acute bilateral adrenalectomy had no effect on the prostaglandin E_2-induced elevation of plasma level of noradrenaline.

4. Discussion

Arachidonic acid released from membrane phospholipids is metabolized rapidly to oxygenated products by several distinct enzymes, including cyclooxygenase, prostaglandin E synthase and thromboxane A synthase (Irvine, 1982; Axelrod, 1990). Previously, we reported that indomethacin (an inhibitor of cyclooxygenase) attenuates the centrally administered CRF-, vasopressin- and arachidonic acid-induced elevations of plasma catecholamines in rats (Okuma et al., 1996; Yokotani et al., 2000; Murakami et al., 2002; Okada et al., 2002). In addition, we reported that centrally administered prostaglandin E_2 elevates plasma noradrenaline by activation of the brain prostanoid EP_3 receptors in rats (Yokotani et al., 1995). Injection of a thromboxane A_2 mimetic into the hypothalamic paraventricular nucleus predominantly elevates plasma adrenaline (Murakami et al., 2002). The hypothalamic paraventricular nucleus has been considered to be the control center of the sympatho-adrenomedullary outflow (Swanson and Sawchenko, 1980; Jansen et al., 1995). These results suggest that brain prostaglandin E_2 and thromboxane A_2 are involved in the central activation of the sympatho-adrenomedullary outflow in rats.

We have reported that centrally administered bombesin elevates plasma levels of noradrenaline and adrenaline in rats (Okuma et al., 1996). The bombesin-induced elevations of plasma catecholamines were attenuated by central pretreatment with indomethacin (an inhibitor of cyclooxygenase), suggesting the involvement of arachidonic acid cascade in the bombesin-induced activation of the central sympatho-adrenomedullary outflow. In the present experiment, we further examined the effect of furegrelate [a selective inhibitor of thromboxane A_2 synthase (Gorman et al., 1983)] and (+)-S-145 [a selective blocker of prostanoid TP receptors (Hanasaki and Arita, 1988; Mihara et al., 1989; Murakami et al., 1998)] on the bombesin-induced elevations of plasma catecholamines. Central pretreatment with furegrelate and (+)-S-145 effectively reduced the bombesin-induced elevations of plasma noradrenaline and adrenaline. Previously we examined the effect of furegrelate on centrally administered vasopressin- and CRF-induced elevations of plasma catecholamines in rats (Okada et al., 2003). Furegrelate attenuated the vasopressin-induced elevations of plasma noradrenaline and adrenaline, while the CRF-induced elevation of plasma adrenaline, but not noradrenaline, was attenuated by this reagent (Okada et al., 2003). Recently, Yalcin and Savci (2004) reported that activation of brain prostanoid TP receptors elevates plasma levels of adrenaline and noradrenaline in addition to its pressor effect. From these evidence and results, it seems likely that bombesin evokes the release of both catecholamines by activation of the brain prostanoid TP receptors.

To explore the source of noradrenaline and adrenaline evoked by centrally administered bombesin, we examined the effect of acute bilateral adrenalectomy on the bombesin-induced elevations of plasma catecholamines. In sham-operated rats, centrally administered bombesin-induced elevation of adrenaline was augmented. This augmentation seems to be due to the laparotomy-induced increase of plasma corticosterone, which has been shown to upregulate the gene transcription of phenylethanolamine *N*-methyltransferase, which methylates noradrenaline into adrenaline in adrenaline-containing chromaffin cells (Ross et al., 1990; Hodel, 2001). The bombesin-induced elevations of plasma noradrenaline and adrenaline were abolished by acute bilateral adrenalectomy. Previously we reported that the centrally administered vasopressin-induced elevations of plasma noradrenaline and adrenaline was abolished by acute bilateral adrenalectomy, while the procedure only abolished the CRF-induced elevation of plasma adrenaline alone (Okada et al., 2003). These results suggest that bombesin evokes the secretion of noradrenaline and adrenaline from adrenal medulla, as shown in the vasopressin-induced

secretion of noradrenaline and adrenaline from adrenal medulla (Okada et al., 2003).

Previously we reported that centrally administered prostaglandin E_2 increases plasma noradrenaline by activation of the brain prostanoid EP_3 receptors in rats (Yokotani et al., 1995). A question has arisen whether prostaglandin E_2 evokes the release of noradrenaline from adrenal medulla or sympathetic nerves. In the present experiment, acute bilateral adrenalectomy had no effect on the prostaglandin E_2-induced elevation of plasma noradrenaline, indicating that activation of brain prostanoid EP (EP_3) receptors evokes the release of noradrenaline from the sympathetic nerves in rats.

In summary, we demonstrated here that centrally administered bombesin evokes the secretion of noradrenaline and adrenaline from the adrenal medulla by activation of the brain prostanoid TP receptors. On the other hand, activation of the brain prostanoid EP (probably EP_3) receptors evokes the release of noradrenaline from sympathetic nerves in rats.

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