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Effects of 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ on the release of noradrenaline from the isolated rat stomach

Kumiko Nakamura, Shoshiro Okada, Kaori Ono, Kunihiko Yokotani*

Department of Pharmacology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan

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Abstract

In the present experiment, we examined the effect of 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ on the release of noradrenaline from the isolated rat stomach. The postganglionic sympathetic nerves were electrically stimulated twice at 1 Hz for 1 min and test reagents were added during the second stimulation. 8-Iso-prostaglandin E_2 (10^{-8}–10^{-6} M) and 8-*iso*-prostaglandin $F_{2\alpha}$ (10^{-7}–10^{-5} M) dose-dependently reduced the evoked noradrenaline release, and these inhibitory potencies were as follows: 8-*iso*-prostaglandin E_2>8-*iso*-prostaglandin $F_{2\alpha}$. The inhibitory effect of 8-*iso*-prostaglandin $F_{2\alpha}$, but not 8-*iso*-prostaglandin E_2, was abolished by 10^{-6} M SQ-29548 ([1*S*-[1α,2α(*Z*),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino] methyl]-7-oxabicyclo[2,2,1]hept-2-yl]-5-heptenoic acid) (a prostanoid TP receptor antagonist). On the other hand, the inhibitory effect of 8-*iso*-prostaglandin E_2 was abolished by 10^{-5} M AH-6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) (a prostanoid EP receptor antagonist), which also attenuated the inhibitory effects of ONO-AE-248 (16*S*-9-deoxy-9β-chloro-15-deoxy-16-hyfroxy-17,17-trimethylene 19, 20-didehydro prostaglandin F_2) (a selective EP_3 receptor agonist) on the evoked release of noradrenaline. The inhibitory effect of 8-*iso*-prostaglandin $F_{2\alpha}$, but not 8-*iso*-prostaglandin E_2, was abolished by pertussis toxin. These results suggest that 8-*iso*-prostaglandin $F_{2\alpha}$ inhibits noradrenaline release through TP receptors, whereas 8-*iso*-prostaglandin E_2 seems to inhibit noradrenaline release through EP_3 receptors, located on the gastric sympathetic nerve terminals in rats.

Keywords: 8-Iso-prostaglandin E_2; 8-Iso-prostaglandin $F_{2\alpha}$; Prostanoid EP_3 receptor; TP receptor; Noradrenaline release; Stomach, rat; Pertussis toxin

1. Introduction

The isoprostanes have been shown to be produced via peroxyl radical isomers of arachidonic acid that undergo endocyclization and subsequent reduction independent of cyclooxygenase activity (Morrow et al., 1990, 1992, 1994), although they are also produced in a cyclooxygenase-dependent manner (Pratico et al., 1995; Bachi et al., 1997). They differ structurally from prostaglandins by the *cis*-orientation at the cyclopentane ring junction compared with the *trans*-orientation in the classical prostaglandins; isoprostanes of the E and F series have been reported. 8-Isoprostanes are present in substantial amounts even in normal plasma and urine (Morrow et al., 1990), and they are further elevated in many states in which oxidative stress is a prominent feature. Therefore, 8-isoprostanes have been used extensively as clinical makers of lipid peroxidation in human diseases (Montuschi et al., 1999; Pratico et al., 1998).

The biological activity of 8-isoprostanes has also been investigated in several organs. They exert contractions of the rat stomach and the guinea pig ileum (Sametz et al., 2000), the rat preglomerular vessels (Takahashi et al., 1992), the rat and rabbit lungs (Kang et al., 1993; Banerjee et al., 1992), the canine intestine (Elmhurst et al., 1997), and the human saphenous vein (Gardan et al., 2000). Besides their potent direct constrictor actions, they may modulate the release of neurotransmitters. Indeed, 8-*iso*-prostaglandin $F_{2\alpha}$ inhibits acetylcholine release from the guinea pig trachea (Spicuzza et al., 2001). On the other hand, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ potentiated the release of noradrenaline from bovine iris (Opere et al., 2001), while they have dual effects on the noradrenaline release from the human iris-ciliary body (inhibition by 8-*iso*-prostaglandin E_2; potentiation by 8-*iso*-prostaglandin $F_{2\alpha}$) (Awe et al., 2000). However, it has been debated whether the biological

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

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effects of these isoprostanes are exerted on prostanoid receptors or on a "unique" isoprostane receptor.

Recently, we reported that the prostanoid EP_3 and TP receptors located on the rat gastric sympathetic nerve terminals mediate inhibition of noradrenaline release from the rat stomach (Yokotani et al., 2003). In the present study, therefore, we examined the effects of 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ on the release of noradrenaline from the rat stomach in relation to the prostanoid EP_3 and TP receptors using the isolated, vascularly perfused rat stomach.

2. Materials and methods

2.1. Perfusion experiments

Male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing about 350 g were fasted overnight before experiments. The isolated, vascularly perfused stomach preparations were made as described previously (Yokotani et al., 1992; Nakamura et al., 2003). Briefly, under urethane anesthesia, the abdomen was opened with a midline incision. After ligation of the abdominal aorta just above the branching of celiac artery, the cannula was inserted into the celiac artery via an incision placed on the aorta and modified Krebs–Ringer solution (pH 7.4) bubbled with a mixture of 95% O_2 and 5% CO_2 was perfused with a constant flow rate of 2.5 ml/min. Modified Krebs–Ringer solution was composed of 117.5 mM NaCl, 4.7 mM KCl, 2.4 mM $CaCl_2$, 1.1 mM $MgCl_2$, 1.1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM glucose, 0.05% of bovine serum albumin, 10 μM pargyline and 1 μM phentolamine. A tube was inserted into the lumen of the stomach via pylorus ring to drain the contents of the stomach throughout the experiment. The esophagus, duodenum, spleen and pancreas were dissected after ligation of the vessels, and the vascularly perfused stomach was kept in a chamber prewarmed at 37 °C. Each 2-min effluent from the portal vein was collected in chilled tubes containing 0.5 ml of 4 M perchloric acid, 1 ng of 3,4-dihydroxybenzylamine as an internal standard, and 50 μl of 2% sodium pyrosulfite solution.

After an equilibration period of 60 min, the first electrical stimulation consisting square-wave pulses [1 Hz, 2-ms duration, 10 mA (supramaximal intensity) for 1 min] was applied to the periarterial nerves around the left gastric artery, which contain the postganglionic sympathetic nerves, using bipolar electrodes. The second electrical stimulation was carried out 26 min after the first stimulation. Perfusion medium containing test substances was changed 14 min before the second electrical stimulation.

In some experiments, rats were pretreated with pertussis toxin (10 μg per rat dissolved in 100 μl of sodium phosphate-buffered saline, pH 7.0, 4 days before experiments) or vehicle (100 μl of sodium phosphate-buffered saline) injected into the dorsal penic vein under a light ether anesthesia, as described in our previous paper (Yokotani and Osumi, 1993).

2.2. Noradrenaline assay in the medium and the stomach

At the end of each experiment, the stomach was homogenized in 20 ml of 0.4 M perchloric acid containing 18.6 mg of disodium EDTA, 200 ml of 2% sodium pyrosulfite solution and 500 ng of 3,4-dihydroxybenzylamine as an internal standard. The homogenate was centrifuged for 10 min at 14,000 × *g* at 4 °C. The supernatant was analyzed to determine the tissue level of noradrenaline.

Catecholamines in the effluent and the supernatant of tissue homogenate 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). Specifically, 2 ml of effluent or an aliquot (0.1 ml) of supernatant was transferred to a centrifuge tube containing 30 mg of activated alumina and 3 ml of 1.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA dihydrate; after which the preparations were shaken for 10 min. The supernatant was discarded and the alumina was washed three times with double-deionized water. After centrifugation, the supernatant was discarded and samples were evaporated to dryness. Then, catecholamines adsorbed onto the alumina were eluted with 300 μl of 4% of acetic acid containing 0.1 mM disodium EDTA. The recovery of catecholamines was about 85%.

The high-performance liquid chromatography-electrochemical detection system consisted of 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 accurately.

2.3. Evaluation and statistical analysis

Spontaneous and evoked release of noradrenaline is expressed as a percentage of its tissue content per 2 min. Basal release of noradrenaline was calculated by averaging the amount of noradrenaline released in two subsequent samples before electrical stimulation. The release of noradrenaline is expressed as percentage of its tissue content

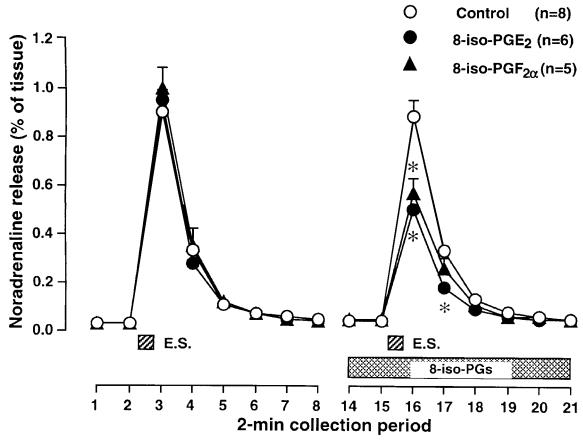

Fig. 1. Effects of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$. Periarterial nerves of the left gastric artery were electrically stimulated twice. The first stimulation was carried out in the normal medium and the second stimulation was carried out in the medium containing 8-iso-prostaglandin E_2(10^{-7} M) or 8-iso-prostaglandin $F_{2\alpha}$ (10^{-6} M). The release of noradrenaline is expressed as percentage of its tissue content per 2 min. E.S., electrical stimulation of the gastric sympathetic nerves at 1 Hz for 1 min. Values are means ± S.E.M. *Significant difference ($P<0.05$) from the values of the vehicle-treated control.

per 2 min. The amounts of the evoked noradrenaline release above the basal level during 12 min after the first and second electrical stimulation are expressed as S_1 and S_2. The effects of test substances are expressed as the ratio of S_2 to S_1. All values are expressed as the means ± S.E.M.

All data were analyzed by repeated-measures analysis of variance, followed by post-hoc analysis with Bonferroni method for comparing a control to all other means in Figs. 1–5. *P* values of less than 0.05 were taken to indicate statistic significance.

2.4. Drugs

The following drugs were used: 3,4-dihydroxybenzylamine hydrobromide, pargyline hydrochloride, pertussis toxin, phentolamine hydrochloride (Sigma, St. Louis, MO, USA); 8-*iso*-prostaglandin E_2 (9-oxo-11α,15*S*-dihydroxy-(8β)-prosta-5Z,13E-dien-l-oic acid), 8-*iso*-prostaglandin $F_{2\alpha}$ (9α,11α,15*S*-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid), AH-6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid), SQ-29548 ([1*S*-[1α,2α(*Z*),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo [2,2,1]hept-2-yl]-5-heptenoic acid) (Cayman Chemical, Ann Arbor, MI, USA); ONO-AE-248 (16*S*-9-deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-19,20-didehydro prostaglandin F_2) was a kind gift of Ono Pharmaceuticals, (Osaka, Japan); alumina activated (Wako, Osaka, Japan). All other reagents were of the highest grade available (Nacalai Tesque).

8-Iso-prostaglandin E_2, 8-*iso*-prostaglandin $F_{2\alpha}$ and related compounds were dissolved in 100% dimethyl sulfoxide (DMSO) and stored at −20 °C. More dilute aqueous solution were made daily and the final concentration of DMSO was 0.2%.

3. Results

3.1. Effects of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$

The amount of noradrenaline present in the stomach was 683 ± 12 ng ($n=133$). Spontaneous release of noradrenaline was about 0.03% of its tissue content per 2 min. Electrical stimulation of the gastric sympathetic nerves at 1 Hz for 1 min evoked an increase of noradrenaline release and this increase rapidly declined toward the basal level (Fig. 1). Repetitive stimulations evoked consistent and reproducible increases in noradrenaline release.

After the first stimulation of the gastric sympathetic nerves, the medium was changed to the next one containing 8-*iso*-prostaglandin E_2 (10^{-8}–10^{-6} M) or 8-*iso*-prostaglandin $F_{2\alpha}$ (10^{-7}–10^{-5} M). 8-Iso-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ inhibited the evoked release of noradrenaline in a concentration-dependent manner (Figs. 1 and 2). 8-Iso-prostaglandin E_2 was more potent than 8-*iso*-prostaglandin $F_{2\alpha}$. 8-Iso-prostaglandin E_2 (10^{-7} M)- and 8-*iso*-prostaglandin $F_{2\alpha}$ (10^{-6} M)-induced inhibitions

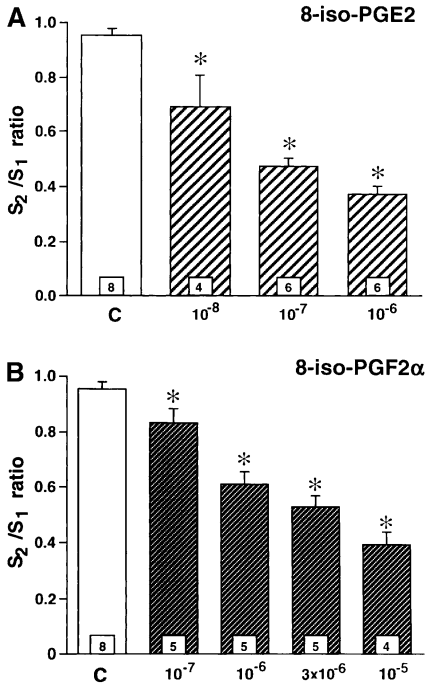

Fig. 2. Effects of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$. 8-Iso-prostaglandin E_2(10^{-8}–10^{-6} M) or 8-iso-prostaglandin $F_{2\alpha}$ (10^{-7}–10^{-5} M) were added during the second electrical stimulation. The effects of these 8-iso-prostaglandins are expressed as S_2/S_1 ratio. *Significant difference ($P<0.05$) from the control (C). Other conditions were the same as those in Fig. 1.

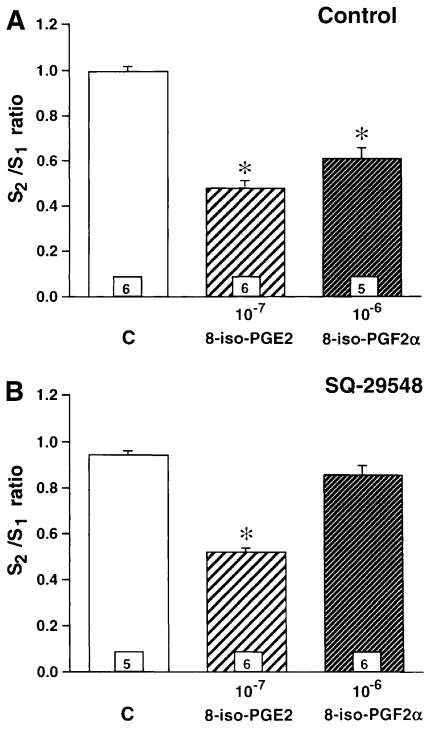

Fig. 3. Interaction of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ with SQ-29548. SQ-29548 (a prostanoid TP receptor antagonist), 8-iso-prostaglandin E_2 (10^{-7} M), and 8-Iso-prostaglandin $F_{2\alpha}$ (10^{-6} M) were added during the second electrical stimulation. (A) Control experiments (data for 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ were cited from Fig. 2); (B) SQ-29548 (10^{-6} M)-treated experiments. *Significant difference ($P<0.05$) from vehicle-treated control (C). Other conditions were the same as those in Figs. 1 and 2.

of noradrenaline release were not changed by indomethacin (3×10^{-6} M) (data not shown).

3.2. Interaction of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ with SQ-29548

We examined the effects of SQ-29548, a selective prostanoid TP receptor antagonist, on the 8-*iso*-prostaglandin E_2 (10^{-7} M)- and 8-*iso*-prostaglandin $F_{2\alpha}$ (10^{-6} M)-induced inhibitions of the evoked release of noradrenaline from the stomach. SQ-29548 (10^{-6} M) alone had no effect on the basal and evoked release of noradrenaline from the stomach (Fig. 3B). SQ-29548 (10^{-6} M) abolished the 8-*iso*-prostaglandin $F_{2\alpha}$-induced inhibition of the evoked release of noradrenaline, while the reagent had no effect on the 8-*iso*-prostaglandin E_2-induced inhibition of the evoked release of noradrenaline from the stomach (Fig. 3A and B).

3.3. Interaction of 8-iso-prostaglandin E_2 and ONO-AE-248 with AH-6809

8-Iso-prostaglandin E_2 (10^{-7} M) and ONO-AE-248 (10^{-7} M), a selective prostanoid EP_3 receptor agonist, reduced the evoked release of noradrenaline from the stomach (Fig. 4A), as shown in our recent paper (Yokotani et al., 2003). Then, we examined the effects of AH-6809, an antagonist of prostanoid EP_1/EP_2 receptors, on the 8-*iso*-prostaglandin E_2- and ONO-AE-248-induced inhibitions of the evoked release of noradrenaline (Fig. 4).

AH-6809 (10^{-6} and 10^{-5} M) had no effect on the basal and evoked release of noradrenaline from the stomach (Fig. 4B and C); however, the reagent dose-dependently changed the both inhibitory effects of 8-*iso*-prostaglandin E_2 and ONO-AE-248 on the evoked release of noradrenaline (Fig. 4B and C).

3.4. Interaction of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ with pertussis toxin

Rats were pretreated with pertussis toxin (10 μg per rat, i.v., 4 days before experiments) or vehicle. The basal

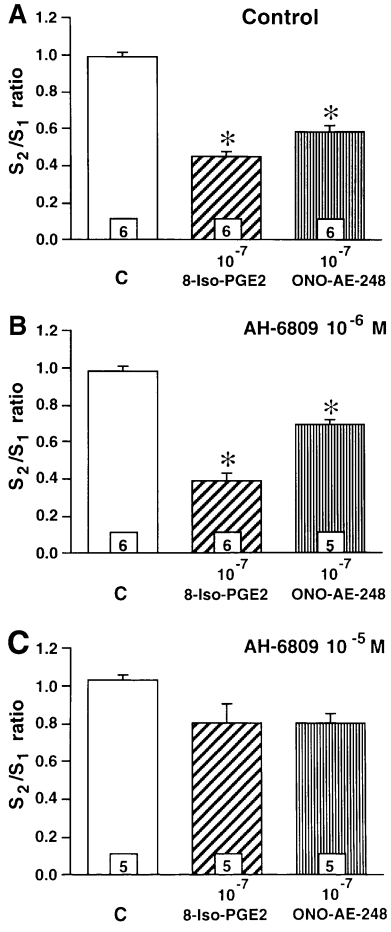

Fig. 4. Interaction of 8-iso-prostaglandin E_2 and ONO-AE-248 with AH-6809. AH-6809 (10^{-6} and 10^{-5} M) (an antagonist of EP_1/EP_2 receptors), 8-iso-prostaglandin E_2 (10^{-7} M), and ONO-AE-248 (a selective EP_3 receptor agonist) (10^{-7} M) were added during the second electrical stimulation. (A) Control experiments [data for control (C) and 8-iso-prostaglandin E_2 were cited from Fig. 3A]; (B) AH-6809 (10^{-6} M)-treated experiments; (C) AH-6809 (10^{-5} M)-treated experiments. *Significant difference ($P<0.05$) from vehicle-treated control (C). Other conditions were the same as those in Figs. 1–3.

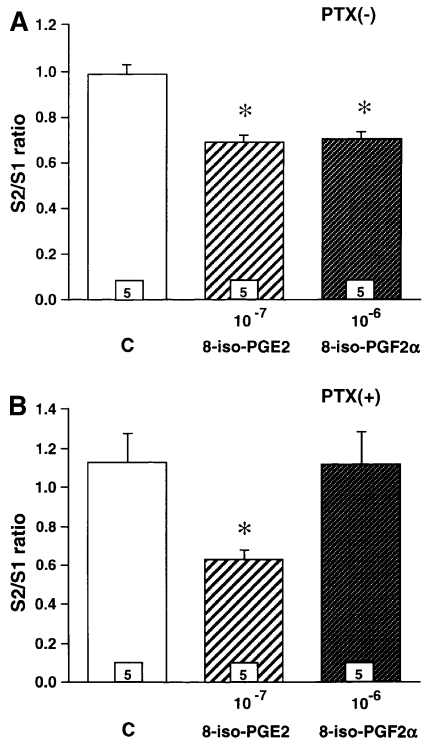

Fig. 5. Interaction of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ with pertussis toxin. Pertussis toxin (10 μg/animal, i.v.) or vehicle was administered 4 days before experiments. The periarterial nerves around the left gastric artery were electrically stimulated twice at 1 Hz for 1 min. 8-Iso-prostaglandin E_2 (8-iso-PGE_2) (10^{-7} M) and 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) (10^{-6} M) were administered during the second electrical stimulation. *Significant difference ($P<0.05$) from the respective control (C). Other conditions were the same as those in Figs. 1–4.

release of noradrenaline from the stomach was not influenced, however, the electrically evoked release of noradrenaline was slightly reduced by pretreatment with this toxin (Fig. 5). The inhibitory effects of 8-*iso*-prostaglandin $F_{2\alpha}$ (10^{-6} M) on the evoked release of noradrenaline were abolished by pertussis toxin, while those of 8-*iso*-prostaglandin E_2 (10^{-7} M) were not influenced by this toxin (Fig. 5A and B).

4. Discussion

Prostanoid TP-receptor antagonists, SQ-29548 or GR 32191, have been shown to reduce the 8-*iso*-prostaglandin $F_{2\alpha}$-induced evoked release of noradrenaline from the human iris-ciliary body (Awe et al., 2000). On the other hand, ICI 192605, a prostanoid TP receptor antagonist, had no effect on the 8-*iso*-prostaglandin $F_{2\alpha}$-induced inhibition of acetylcholine release from guinea pig trachea (Spicuzza et al., 2001), and there were no binding sites for 8-*iso*-prostaglandin $F_{2\alpha}$ on the rat mesangial cells displaying thromboxane A_2 binding sites (Fukunaga et al., 1997), suggesting the presence of a "unique" isoprostane receptor. In the present experiment, SQ-29548 abolished the 8-*iso*-prostaglandin $F_{2\alpha}$-induced inhibition of noradrenaline release from the rat stomach. The result suggests that 8-*iso*-prostaglandin $F_{2\alpha}$ exerts the inhibitory effect by interaction with prostanoid TP receptors located on the sympathetic nerve terminals in the rat stomach.

SQ-29548, a prostanoid TP receptor antagonist, has also been shown to abolish the 8-*iso*-prostaglandin E_2- and 8-*iso*-prostaglandin $F_{2\alpha}$-induced enhancement of noradrenaline release from bovine iris (Opere et al., 2001). The discrepancy of prostanoid TP receptor-mediated responses seems to be due to the differences in the tissue and species used in each experiment [augmentation of noradrenaline release from the human vas deferens and rabbit mesenteric artery (Trachte and Stein, 1989; Holmquist et al., 1991); inhibition of noradrenaline release from the rat hippocampus and stomach (Nishihara et al., 2000; Yokotani et al., 2003)].

Recently, we reported that ONO-AE-248, a selective prostanoid EP_3 receptor agonist (Suzawa et al., 2000), selectively inhibited noradrenaline release from the rat stomach (Yokotani et al., 2003), as shown in the cardiac adrenergic terminals (Mantelli et al., 1991), the rat and guinea pig saphenous veins (Molderings et al., 1992), the human pulmonary artery and vena cava (Molderings et al., 1994), and the rabbit aorta (Jensen and Nedergaard, 1997). In the present experiment, 8-*iso*-prostaglandin E_2 and ONO-AE-248 inhibited noradrenaline release from the rat stomach, and these inhibitions were attenuated by AH-6809, a prostanoid EP_1/EP_2 receptor antagonist (Funk et al., 1993; Woodward et al., 1995). AH-6809 has also been shown to reduce the 8-*iso*-prostaglandin E_2-induced contraction of human and canine pulmonary vessels (Janssen et al., 2001) and porcine pulmonary vein expressed excitatory EP_3 receptors (Janssen and Tazzeo, 2002). The evidence and our present results suggest that 8-*iso*-prostaglandin E_2 exerts the inhibitory effect by interaction with prostanoid EP_3 receptors located on the sympathetic nerve terminals in the rat stomach.

Previously, we reported that pertussis toxin abolished the U-46619 (a TP receptor agonist)-induced inhibition of noradrenaline release from the rat stomach, while ONO-AE-248-induced inhibition of noradrenaline release was not influenced by this toxin (Yokotani et al., 2003). In the present experiment, the 8-*iso*-prostaglandin $F_{2\alpha}$-, but not 8-*iso*-prostaglandin E_2-, induced response was abolished by pertussis toxin. These results further support the possibility that 8-*iso*-prostaglandin $F_{2\alpha}$ and 8-*iso*-prostaglandin E_2 exert their inhibitory effects by interaction with prostanoid TP and EP_3 receptors, respectively.

In conclusion, the present findings suggest that 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ inhibit noradrenaline release from the stomach by a respective activation of the prostanoid EP_3 and TP receptors located on the gastric sympathetic nerve terminals in rats.

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