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Prostanoid EP_3 and TP receptors-mediated inhibition of noradrenaline release from the isolated rat stomach

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Abstract

The postganglionic sympathetic nerves of the isolated rat stomach were electrically stimulated twice at 1 Hz for 1 min. Prostaglandin E_2 and ONO-AE-248 (16S-9-deoxy-9β-chloro-15-deoxy-16-hyfroxy-17,17-trimethylene-19,20-didehydro prostaglandin F_2) (an EP_3 receptor agonist) reduced the evoked noradrenaline release, while ONO-DI-004 (17S-2,5-ethano-6-oxo-17,20-dimethyl prostaglandin E_1) (an EP_1 receptor agonist), ONO-AE1-259-01 (11,15-*O*-dimethyl prostaglandin E_2) (an EP_2 receptor agonist) and ONO-AE1-329 [16-(3-methoxymethyl)phenyl-ω-tetranor-3,7-dithia prostaglandin E_1] (an EP_4 receptor agonist) had no effect. U-46619 (9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$) and I-BOP (7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2,2,1] hept-2-yl]-, [1S[1α,2α(Z),3β(1E,3S)4α]]-5-heptenoic acid) (TP receptor agonists) also reduced the noradrenaline release and these inhibitory effects were abolished by SQ-29548 (7-[3-[[2-[(phenylamino) carbonyl] hydrazino]methyl]-7-oxabicyclo[2,2,1]hept-2-yl][1S(1α,2α(Z), 3α,4α]-5-heptenoic acid) (a TP receptor antagonist). The inhibitory effect of U-46619, but not ONO-AE-248, was abolished by pertussis toxin. These results suggest that the prostanoid EP_3 and TP receptors mediate the inhibition of gastric noradrenaline release; TP, but not EP_3, receptor-mediated inhibition is mediated by a pertussis toxin-sensitive mechanism in rats.

Keywords: EP_3 receptor; TP receptor; Noradrenaline release; Stomach, rat; Pertussis toxin

1. Introduction

Prostanoids have been shown to modulate the depolarization-evoked release of noradrenaline from central noradrenergic nerve fibres and peripheral sympathetic neurons (Hedqvist, 1977; Güllner, 1983; Starke et al., 1989; Malik and Sehic, 1990; Exner and Schlicker, 1995). In peripheral nervous system, prostaglandin E_2 has been shown to reduce the stimulation-evoked noradrenaline release from blood vessels in rat, rabbit and human (Molderings et al., 1992, 1994; Jensen and Nedergaard, 1997), while prostaglandin D_2 enhances the stimulation-evoked noradrenaline release in blood vessels from dog (Nakajima and Toda, 1984) and humans (Molderings et al., 1994).

The action of prostanoids are mediated via specific receptors that have been classified on the basis of their sensitivity to the naturally occurring eicosanoids (prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2 and thromboxane A_2) and named DP, EP, FP, IP and TP, respectively (Kennedy et al., 1982). A lack of a variety of subtype-selective antagonists and insufficient agonist specificity for the subtypes have so far hampered an unequivocal receptor classification (Fuder and Muscholl, 1995). With the use of synthetic prostaglandin analogs, four subtypes of EP receptors have been characterized and named as EP_1, EP_2, EP_3 and EP_4 (Coleman et al., 1994). The aims of this study were to determine which types of prejunctional prostanoid receptors are involved in regulation of the release of noradrenaline from the gastric sympathetic nerve terminals 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

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stomach preparations were made as described previously (Yokotani et al., 1992). 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 maintained at pH 7.4 was perfused with a constant flow rate of 2.5 ml per 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, 3 μM indomethacin and 1 μM phentolamine. The tube was inserted into the lumen of the stomach via a 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 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 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 adrenaline and 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 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, 2, 3A and 4. Student's *t*-test was used for evaluating the significant difference between two values in Fig. 3B. *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, indomethacin, pargyline hydrochloride, pertussis toxin, phentolamine hydrochloride, prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2 (Sigma, St. Louis, MO, USA); 7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2,2,1] hept-2-yl]-,[1S[1α,2α(Z),3β(1E,3S)4α]]-5-heptenoic acid (I-BOP),

9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U-46619) and 7-[3-[[2-[(phenylamino) carbonyl] hydrazino]-methyl]-7-oxabicyclo[2,2,1]hept-2-yl][1S(1α,2α(Z), 3α,4α]-5-heptenoic acid (SQ-29548) (Cayman Chemical, Ann Arbor, MI, USA); 17S-2,5-ethano-6-oxo-17,20-dimethyl prostaglandin E_1 (ONO-DI-004) (an EP_1 receptor agonist), 11,15-*O*-dimethyl prostaglandin E_2 sodium salt (ONO-AE1-259-01) (an EP_2 receptor agonist), 16S-9-deoxy-9β-chloro-15-deoxy-16-hyfroxy-17,17-trimethylene-19,20-didehydro prostaglandin F_2 (ONO-AE-248) (an EP_3 receptor agonist) and 16-(3-methoxymethyl)phenyl-ω-tetra-nor-3,7-dithia prostaglandin E_1 (ONO-AE1-329) (an EP_4 receptor agonist) were kind gifts from Ono Pharmaceuticals (Osaka, Japan); alumina activated (Wako, Osaka, Japan). All other reagents were of the highest grade available (Nacalai Tesque).

Prostaglandins and related compounds were dissolved in ethanol and stored at −20 °C. More dilute aqueous solution were made daily and the final concentration of ethanol was 0.1%. ONO-AE1-259-01 and I-BOP were dissolved in water.

3. Results

3.1. Effects of prostaglandin D_2, E_2, $F_{2\alpha}$ and I_2 on the electrically evoked release of noradrenaline from the isolated rat stomach

The amount of noradrenaline remaining in the stomach was 732 ± 14 ng (n = 185). 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. 1A). 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 prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2 at a concentration of 10^{-7} M (Fig. 1A). Prostaglandin E_2 and prostaglandin $F_{2\alpha}$ inhibited the evoked release of noradrenaline, but prostaglandin D_2 and prostaglandin I_2 had no effect. Furthermore, prostaglandin E_2 inhibited the evoked release of noradrenaline in a concentration dependent manner (10^{-8}–10^{-6} M) (Fig. 1B). The maximal inhibition was obtained at 10^{-6} M.

3.2. Effects of ONO-DI-004, ONO-AE1-259-01, ONO-AE-248 and ONO-AE1-329 on the electrically evoked release of noradrenaline from the isolated rat stomach

We examined the effects of selective agonists of each prostanoid EP receptor subtypes (EP_1, EP_2, EP_3 and EP_4) on the electrically evoked release of noradrenaline from the stomach. ONO-AE-248 (an EP_3 receptor agonist) inhibited

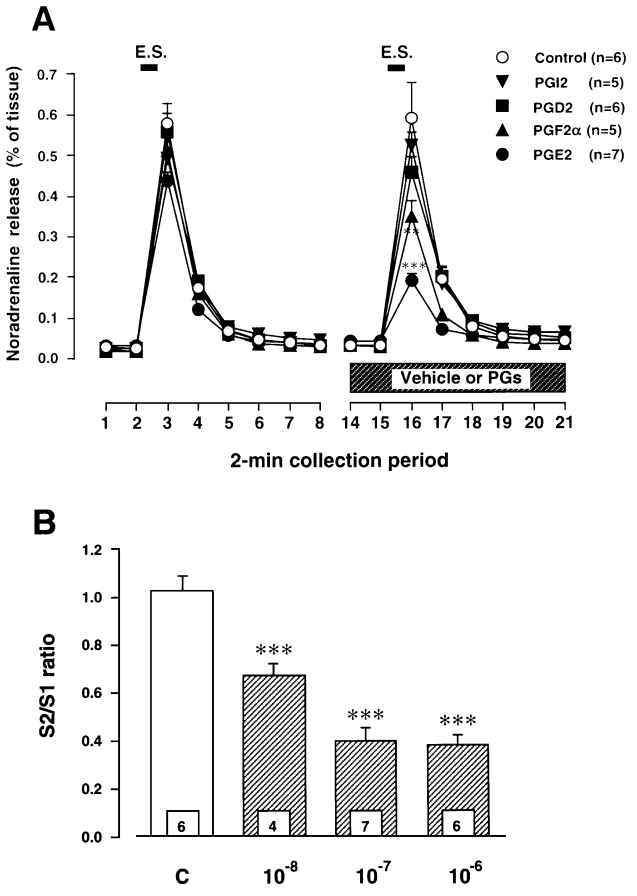

Fig. 1. Effects of prostaglandin D_2, E_2, $F_{2\alpha}$ and I_2 on the electrically evoked release of noradrenaline from the rat stomach. The gastric sympathetic nerves were electrically stimulated twice at 1 Hz for 1 min. The first stimulation was carried out in the normal medium and the second stimulation was carried out in the medium containing prostaglandins (D_2, E_2, $F_{2\alpha}$ and I_2) or vehicle. E.S., electrical stimulation of the gastric sympathetic nerves. The release of noradrenaline is expressed as percentage of its tissue content per 2 min in (A). The effect of prostaglandin E_2 on the evoked release of noradrenaline was expressed as S_2/S_1 ratio in (B). Values are the means ± S.E.M. Asterisks indicate significant difference (*P < 0.05, **P < 0.01, ***P < 0.001) from values of the vehicle-treated control.

the evoked release of noradrenaline in a concentration-dependent manner (10^{-8}–3×10^{-7} M) (Fig. 2C).

On the other hand, ONO-DI-004 (an EP_1 receptor agonist), ONO-AE1-259-01 (an EP_2 receptor agonist) and ONO-AE1-329 (an EP_4 receptor agonist) (10^{-8}–3×10^{-7} M) had no effect on the evoked release of noradrenaline from the stomach (Fig. 2A, B and D).

3.3. Effects of I-BOP and U-46619 on the electrically evoked release of noradrenaline from the isolated rat stomach

We examined the effects of I-BOP and U-46619, thromboxane A_2 mimetics, on the electrically evoked release of noradrenaline from the stomach. I-BOP inhibited the evoked release of noradrenaline from the stomach in a concentration dependent manner (10^{-8}–3×10^{-7} M) (Fig. 3A, upper

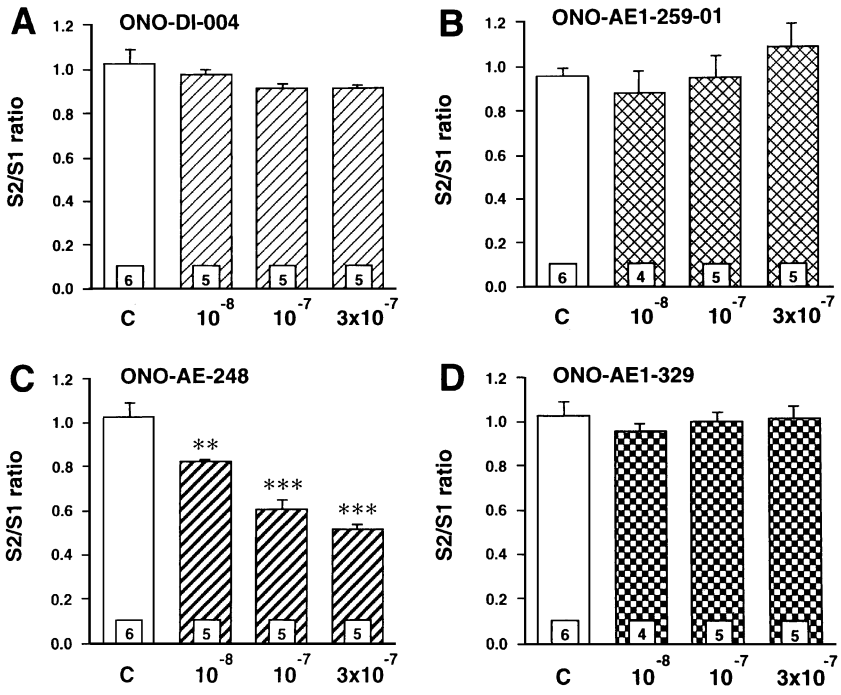

Fig. 2. Effects of selective agonists of prostanoid EP_{1-4} receptors on the electrically evoked release of noradrenaline from the rat stomach. ONO-DI-004 (an EP_1 receptor agonist), ONO-AE1-259-01 (an EP_2 receptor agonist), ONO-AE-248 (an EP_3 receptor agonist), ONO-AE1-329 (an EP_4 receptor agonist) or vehicle was added during the second electrical stimulation. Controls in (A), (C) and (D) were cited from Fig. 1A. Asterisks indicate significant difference (*$P<0.05$, **$P<0.01$, ***$P<0.001$) from values of the vehicle-treated control. Other conditions were the same as those in Fig. 1.

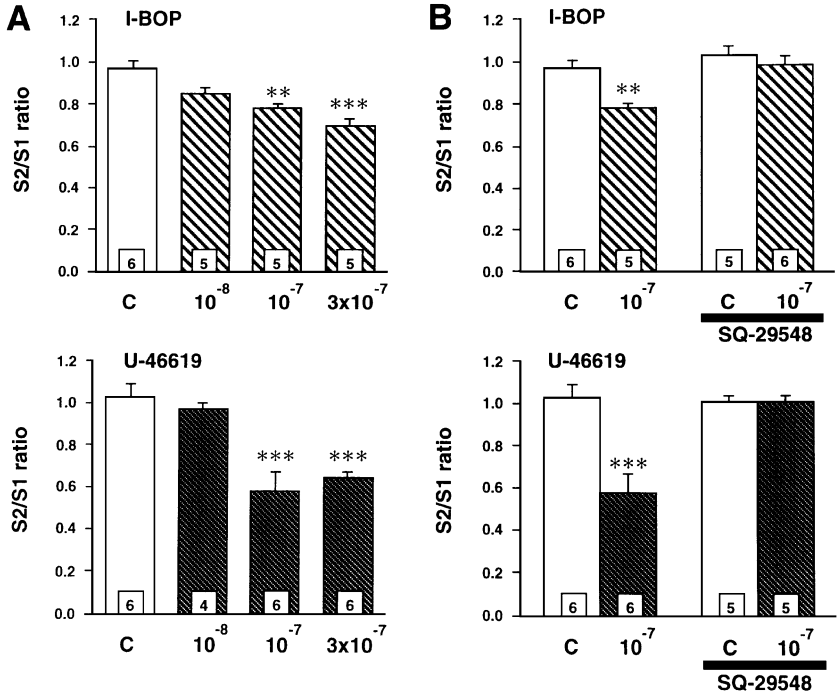

Fig. 3. Effects of I-BOP and U-46619, selective prostanoid TP receptor agonists, on the electrically evoked release of noradrenaline from the rat stomach. (A) I-BOP and U-46619 were added during the second electrical stimulation. Controls in upper and lower panels were cited from Figs. 2B and 1B, respectively. (B) Effects of SQ-29548 (10^{-6} M), a prostanoid TP receptor antagonist, on the I-BOP (10^{-7} M)- and U-46618 (10^{-7} M)-induced inhibition of noradrenaline release. SQ-29548 was added during the second electrical stimulation. Data in the absence of SQ-29548 were cited from (A). Asterisks indicate significant difference (*$P<0.05$, **$P<0.01$, ***$P<0.001$) from values of the vehicle-treated control. Other conditions were the same as those in Figs. 1 and 2.

panel). U-46619 also inhibited the evoked release of noradrenaline from the stomach; the maximal inhibitory response was obtained at 10^{-7} M (Fig. 3A, lower panel).

SQ-29548, an antagonist of prostanoid TP receptors, at 10^{-6} M had no effect on the basal and evoked release of noradrenaline from the stomach (Fig. 3B). I-BOP (10^{-7} M)- and U-46619 (10^{-7} M)-induced inhibition of the evoked release of noradrenaline from the stomach were abolished by 10^{-6} M SQ-29548 (Fig. 3B).

3.4. Effect of pertussis toxin on the ONO-AE-248- and U-46619-induced inhibition of noradrenaline release from the isolated rat stomach

Rats were pretreated with pertussis toxin (10 μg per rat, i.v., 4 days before experiments) or vehicle. The basal release of noradrenaline was not influenced, however, the electrically evoked release of noradrenaline was slightly, but not significantly, reduced by pretreatment with the toxin.

U-46619-induced inhibition of the evoked release of noradrenaline was abolished by the pretreatment with pertussis toxin, but ONO-AE-248-induced inhibition was not influenced by the toxin (Fig. 4).

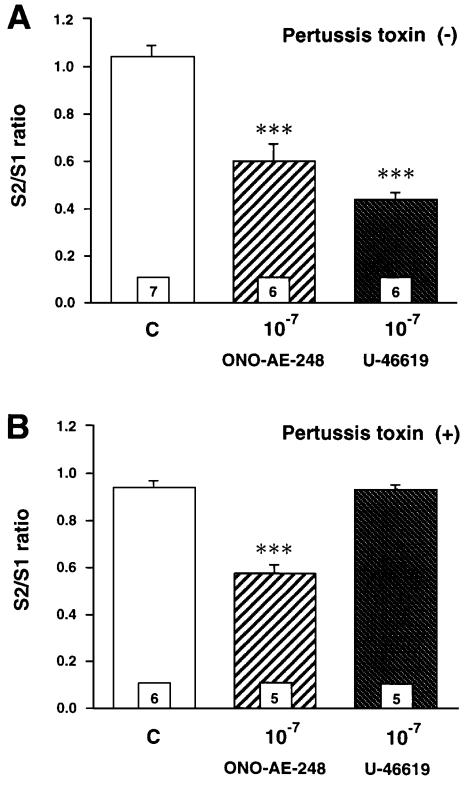

Fig. 4. Effect of pertussis toxin on the ONO-AE-248- and U-46618-induced inhibition of noradrenaline release from the rat stomach. Pertussis toxin (10 μg/animal, i.v.) or vehicle was administered 4 days before experiments. ONO-AE-248 (10^{-7} M) and U-46618 (10^{-7} M) was administered during the second electrical stimulation. (A) The vehicle-treated group; (B) the pertussis toxin-treated group. Asterisks indicate significant difference ($*P<0.05$, $**P<0.01$, $***P<0.001$) from the values of the vehicle-treated control. Other conditions were the same as those in Figs. 1–3.

4. Discussion

Transmitter release from the noradrenergic neurons is modulated via presynaptic receptors for noradrenaline itself (α_2-autoreceptors), in addition to presynaptic receptors for other neurotransmitters (heteroreceptors) (Starke et al., 1989; Fuder and Muscholl, 1995; Schlicker and Marr, 1997). We have already reported that presynaptic inhibitory α_2 adrenoceptors are involved in noradrenaline release from the rat stomach (Yokotani et al., 1992). Therefore, phentolamine, a non-selective α-adrenoceptor antagonist, was administered in the perfusion medium to block prejunctional α_2-autoreceptors in the present experiments.

Two prostanoids (prostaglandin E_2>prostaglandin $F_{2\alpha}$), but not prostaglandin D_2 and prostaglandin I_2, reduced the electrically evoked release of noradrenaline. These results are consistent with the previous reports in which prostaglandin E_2 reduces the stimulation-evoked noradrenaline release from blood vessels in rat, rabbit and human (Molderings et al., 1992, 1994; Jensen and Nedergaard, 1997). On the other hand, prostaglandin D_2 has been reported to enhance the evoked release of noradrenaline from the dog mesenteric arteries (Nakajima and Toda, 1984), human saphenous vein (Molderings et al., 1994), and human right atria (Molderings et al., 1998). However, the prostanoid had no effect in the present experiment.

With the use of synthetic prostaglandin analogs, four subtypes of prostanoid EP receptors have been characterized and named as EP_1, EP_2, EP_3 and EP_4 (Coleman et al., 1994). In previous papers, the EP_1-/EP_3-receptor agonist sulprostone and the EP_2-/EP_3-receptor agonist misoprostol have been shown to mimic the inhibitory effect of prostaglandin E_2 on the release of noradrenaline from the guinea-pig atria (Mantelli et al., 1991), rat vena cava (Molderings et al., 1992), human saphenous vein and atria (Molderings et al., 1994, 1998), rabbit aorta (Jensen and Nedergaard, 1999) and rat stomach (Racké et al., 1995). In addition, the EP_1 receptor antagonist AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) had no effect on the prostaglandin E_2- and sulprostone-induced inhibition of noradrenaline release (Molderings et al., 1994; Racké et al., 1995; Jensen and Nedergaard, 1999). From these results, it is suggested that the postganglionic sympathetic neurons are endowed with prejunctional inhibitory EP_3 receptors.

In the present experiment, we used selective agonists to each EP receptors (EP_{1-4}) to clarify the EP receptor subtype endowed with the gastric sympathetic nerve terminals in rats (Suzawa et al., 2000; Minami et al., 2001; Borgland et al., 2002; Cao et al., 2002). The K_i values of ONO-DI-004 (an EP_1 receptor agonist), ONO-AE1-259 (an EP_2 receptor agonist), ONO-AE-248 (an EP_3 receptor agonist) and ONO-AE1-329 (an EP_4 receptor agonist) to the respective EP receptors expressed in Chinese hamster ovary cells are 150, 3, 7.5 and 9.7 nM, respectively (Suzawa et al., 2000). Furthermore, the K_i values of ONO-AE-248 (an EP_3 receptor agonist) to EP receptors are as follows: >10,000 nM for

EP_1, 3,700 nM for EP_2, 7.5 nM for EP_3 and 4,200 nM for EP_4 (Suzawa et al., 2000). In the present experiments, ONO-AE-248 (an EP_3 receptor agonist) reduced the evoked release of noradrenaline, while ONO-DI-004 (an EP_1 receptor agonist), ONO-AE1-259-01 (an EP_2 receptor agonist) and ONO-AE1-329 (an EP_4 receptor agonist) had no effect. These results clearly indicate the presence of prejunctional inhibitory EP_3 receptors in the rat stomach.

Effects of thromboxane A_2 on the release of noradrenaline have been examined, however the results are controversial. U-46619, a stable analogue of thromboxane A_2 mimetic (Coleman et al., 1981), has been shown to augment the electrically evoked release of noradrenaline from the human vas deferens and rabbit mesenteric artery (Trachte and Stein, 1989; Holmquist et al., 1991), while this reagent had no effect on the evoked release of noradrenaline from the human atria (Molderings et al., 1998). Recently we reported that U-46619 reduced the high K^+-evoked release of noradrenaline from the rat hippocampus (Nishihara et al., 2000). In the present experiment, we examined the effect of two thromboxane A_2 mimetics, I-BOP (Morinelli et al., 1990) and U-46619, on the electrically evoked release of noradrenaline from the rat stomach. Both TP receptor agonists reduced the evoked release of noradrenaline from the stomach, and these inhibitory effects were abolished by SQ-29548, a TP receptor antagonist (Naka et al., 1992). These results suggest that the sympathetic neurons are endowed with prejunctional inhibitory TP receptors in the rat stomach. The differences on the effects of TP agonists on the release of noradrenaline are probably due to the difference of organs used in each experiment.

The transduction of extracellular signals to specific intracellular events often involves the participation of regulatory GTP-binding proteins. Pertussis toxin has been shown in ADP-ribosylates, a subunit of some GTP-binding proteins ($G_{i/o}$), to cause their inactivation (Katada and Ui, 1981; Bokoch et al., 1983). Pertussis toxin inhibited the sulprostone-induced activation of Gi proteins in membranes from the human erythroleukaemia cell line, HEL (Schwaner et al., 1995), while prostaglandin E_2-induced inhibition of cyclic AMP content in the rat renal tubule was not influenced by the toxin (Aarab et al., 1999). On the other hand, the toxin did not significantly affect the suppression of the Ca^{2+} currents induced by U-46619 and I-BOP in the rat hippocampal CA1 neurons (Hsu et al., 1996).

Previously we reported that the pretreatment with pertussis toxin (10 μg/rat, for 4 days) abolished the negative chronotropic and inotropic effects of oxotremorine (a muscarinic receptor agonist) on the isolated, spontaneously beating rat atria (Yokotani and Osumi, 1993). Therefore, pretreatment with the same dose of the toxin seems to be enough to block pertussis toxin-sensitive GTP-binding proteins in the rat stomach. In the present experiment, U-46619 (an TP receptor agonist)-induced inhibition of the evoked release of noradrenaline was abolished by the toxin, while ONO-AE-248 (an EP_3 receptor agonist)-induced inhibition was not influenced. These results suggest the difference of inhibitory mechanisms of U-46619 and ONO-AE-248 on the release of noradrenaline from the rat stomach. We have already reported the presence of N-type voltage-activated Ca^{2+} channels involved in the release of noradrenaline from the gastric sympathetic nerves in rats (Yokotani et al., 1998). From these evidences, it is suggested that U-46619 and ONO-AE-248 attenuate the opening of the Ca^{2+} channels with a pertussis toxin-sensitive or pertussis toxin-insensitive manner, thereby inhibiting the gastric noradrenaline release. The inhibitory mechanisms of U-46619 and ONO-AE-248 on the release of noradrenaline from the stomach remains to be elucidated.

In conclusion, the present findings indicate that the activation of prostanoid EP_3 and TP receptors located on the gastric sympathetic nerve terminals inhibits the release of noradrenaline in rats; EP_3 receptor-mediated inhibition is mediated by a pertussis toxin-insensitive mechanism and TP receptor-mediated inhibition is mediated by a pertussis toxin-sensitive mechanism in rats.

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