British Journal of Pharmacology (1996) 117, 653-656

Inhibition of vagally mediated gastric acid secretion by activation of central prostanoid EP_3 receptors in urethane-anaesthetized rats

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1 We studied the effects of intracerebroventricular (i.c.v.) administration of prostanoid EP receptor ligands on vagally stimulated gastric acid secretion in rats anaesthetized with urethane.

2 Administration of misoprostol (EP_3/EP_2 receptor agonist) and sulprostone (EP_3/EP_1 receptor agonist) reduced vagally mediated gastric acid secretion in a dose-dependent manner (0.1, 0.3 and 1.0 nmol per animal). Butaprost (EP_2 receptor agonist) (0.3 and 3.0 nmol per animal) was without effect. 17-Phenyl-ω-trinor PGE_2 (EP_1/EP_3 receptor agonist) attenuated vagally mediated gastric acid secretion only at its highest dose (1.0 nmol per animal); this antisecretory effect was not prevented by pretreatment with SC-19220 (selective EP_1 receptor antagonist) (20 nmol per animal, i.c.v.).

3 The potency of these test agents in attenuation of vagally mediated gastric acid secretion was as follows: misoprostol $\geqslant$ sulprostone $>>$ 17-phenyl-ω-trinor $PGE_2 >>>$ butaprost. These results suggest that activation of central prostanoid EP_3 receptors induces inhibition of vagally mediated gastric acid secretion in rats.

Keywords: Central nervous system; EP_3 receptor; gastric acid secretion

Introduction

Prostaglandins are produced in the central nervous system of mammals (Wolfe, 1982) and are implicated in central regulation of a variety of functions, including body temperature (Coceani *et al.*, 1988), hypothalamic and pituitary hormone release (Behrman, 1979), some behavioural activities (Johnson *et al.*, 1993) and the control of gastric functions (Saperas *et al.*, 1991; Sautereau *et al.*, 1991). Receptors activated by prostaglandin E_2 (PGE_2) are pharmacologically divided into at least three subtypes (EP_1, EP_2 and EP_3). Many prostanoid ligands with affinity for the EP receptors have been produced, but none shows absolute selectivity for each of the EP receptor subtypes (Coleman *et al.*, 1994). Recently, we reported that i.c.v. administered sulprostone (EP_3/EP_1 receptor agonist) and misoprostol (EP_3/EP_2 receptor agonist) elevated plasma noradrenaline levels in urethane-anaesthetized rats, but butaprost (EP_2 receptor agonist) had no effect (Yokotani *et al.*, 1995). From these results, we concluded that central prostanoid EP_3 receptors affect the central sympathetic outflow in rats.

With regard to gastric functions, we previously reported that i.c.v. administered PGE_2 inhibited the vagally stimulated gastric acid secretion and this antisecretory effect was abolished both by bilateral splanchnectomy and by pretreatment with phentolamine (Yokotani *et al.*, 1988). This evidence suggests that activation of central prostanoid EP receptors induces a sympathetic outflow, thereby inhibiting the vagally mediated gastric acid secretion by activation of α-adrenoceptors in the stomach. However, the EP receptor subtypes mediating this effect have not been characterized. In the present study, therefore, we made an attempt to clarify central EP receptor subtypes mediating the inhibition of vagally mediated gastric acid secretion using several kinds of EP receptor ligands in urethane-anaesthetized rats.

Methods

Procedures

Male Wistar rats weighing 350 to 400 g were deprived of food for 16 h but were allowed free access to tap water. Details of the experimental procedures were as described in our previous papers (Yokotani *et al.*, 1988). Briefly, under urethane anaesthesia (1.2g kg^{-1}, i.p.), the oesophagus was ligated and the trachea was cannulated through a cervical incision. Bilateral vagus nerves were carefully separated from the carotid arteries and cut at the cervical portion. The peripheral end of the left side vagus nerve was placed on platinum ring electrodes and covered with cotton wool soaked in paraffin oil. The cervical incision was then sutured. The femoral vein was cannulated for infusion of saline (1.5 ml h^{-1}). The abdomen was opened by a midline incision and a round-tip polyethylene cannula (3.5 cm in length and 0.4 cm in diameter) inserted into the stomach via an incision in the duodenum. The cannula was held in place by two ligatures around the duodenum, one at the rostral site and the other at the caudal site of the duodenal incision, and the abdominal incision was sutured. After these procedures, the animal was placed in a stereotaxic apparatus.

Measurement of gastic acid secretion

One hour was allowed to elapse before the start of each experiment, for stabilization of the basal acid secretion. Two ml of gastric solution prewarmed to 38°C was instilled and replaced at intervals of 15 min. The solution consisted of a 1/5 (v/v) mixture of glycine and mannitol adjusted to 300 mOsmol and pH 3.5 by the addition of 0.1 N HCl, according to Blair *et al.* (1975). After two 15 min collections at the basal state, gastric acid secretion was elicited by continuous electrical stimulation of the left vagus nerve using the electronic stimulator (Nihon-Kohden SEN-7103, Tokyo, Japan) and isolator (Nihon-Kohden SS-102J). Stimulation was with square-wave pulses of 0.5 ms duration, at 3 Hz and supramaximal intensity (1 mA). Gastric acid secretion was determined by titration with 0.01 N NaOH to pH 7.0 and expressed as μEq 15 min^{-1}

I.c.v. administration of prostanoid EP receptor agonists and antagonists

For i.c.v. administration of prostanoid EP receptor agonists (sulprostone, misoprostol, butaprost and 17-phenyl-ω-trinor PGE_2) or their vehicles in all experiments, a stainless steel cannula (0.35 mm outer diameter) was inserted into the lateral cerebral ventricle at co-ordinates AP -0.8 mm from the

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bregma, L 1.5 mm from the midline, H 4.0 mm below the surface of the brain according to the rat brain atlas of Paxinos & Watson (1986). Vehicle or prostanoid EP receptor agonist dissolved in saline was slowly injected into the right lateral ventricle in a volume of 10 μl with a 50 μl Hamilton syringe 60 min after the start of the vagal stimulation. In some experiments, SC-19220 was administered i.c.v. in a volume of 10 μl 30 min before the beginning of vagal stimulation.

Compounds

The following drugs were used: 17-phenyl-ω-trinor PGE_2 was purchased from Cayman Chemicals, U.S.A. The following compounds were gifts which we gratefully acknowledge: butaprost from Bayer, U.K.; sulprostone from Schering AG, Germany; misoprostol and SC-19220 (1-acetyl-2-(8-chloro-10, 11-dihydrodibenz (b.f) (1,4) oxazepine-10-carbonyl)-hydrazine) from G.D. Searle, U.S.A. Prostanoid EP receptor agonists were stored at −20°C in 99% ethanol and diluted with saline to 0.5% ethanol concentration whenever they were used (saline containing 0.5% of ethanol was used as vehicle for control experiments). SC-19220 was dissolved in saline containing 5% dimethylsulphoxide.

Treatment of data and statistics

Because the absolute values of acid secretion varied with individual animals, the effects of prostanoid EP receptor agonists on gastric acid secretion were expressed as percentages of the value obtained immediately before i.c.v. administration of these ligands. Results were expressed as the mean ± s.e.mean. Statistical analysis was carried out using the Bonferroni method for comparing a control to all other means after one-way analysis of variance (ANOVA) in Figures 1 and 2 and Student's unpaired t test in Figure 3. P values of less than 0.05 were taken to indicate significant differences.

Results

Effects of misoprostol (EP_3/EP_2 receptor agonist) and sulprostone (EP_3/EP_1 receptor agonist) on the gastric acid secretion induced by vagus nerve stimulation

Continuous electrical stimulation of the vagus nerve at 3 Hz evoked an increase in gastric acid secretion which lasted more than 60 min (Figure 1a). Acid secretion at 60 min after the intracerebroventricular (i.c.v.) administration of vehicle was 102.4 ± 6.5% of the preadministered values at 0 min ($n=4$). The actual values of the evoked gastric acid secretion at 0 min were 92.6 ± 4.9 μEq 15 min^{-1} ($n=44$).

Misoprostol (0.1, 0.3 and 1.0 nmol per animal, i.c.v.) dose-dependently reduced the vagally stimulated gastric acid secretion and these inhibitory effects lasted for more than 60 min after its administration (Figure 1a). Acid secretion at 45 min after administration of misoprostol (0.1, 0.3 and 1.0 nmol per animal, i.c.v.) was 57.8 ± 5.7 ($n=4$), 43.7 ± 5.4% ($n=4$), and 37.3 ± 3.9% ($n=4$) of the preadministered values, respectively. On the other hand, intravenous administration of misoprostol (1.0 nmol per animal) was without effect on the vagally stimulated gastric acid output, indicating that the sites of action of the i.c.v. administered misoprostol are probably within the brain but not in peripheral organs after its leakage into systemic circulation (data not shown).

Sulprostone (0.1, 0.3 and 1.0 nmol per animal, i.c.v.) also dose-dependently reduced the vagally stimulated gastric acid secretion and these inhibitory effects lasted over 60 min after its administration (Figure 1b). Acid secretion at 45 min after administration of sulprostone (0.1, 0.3 and 1.0 nmol per animal, i.c.v.) was 61.7 ± 6.3% ($n=4$), 41.1 ± 9.2% ($n=4$), and 41.8 ± 5.1% ($n=4$) of the preadministered values, respectively.

Effects of butaprost (EP_2 receptor agonist) and 17-phenyl-ω-trinor-PGE_2 (EP_1/EP_3 receptor agonist) on gastric acid secretion induced by vagus nerve stimulation

Butaprost (0.3 and 3.0 nmol per animal, i.c.v.) did not significantly affect the vagally stimulated gastric acid secretion (Figure 2a).

17-Phenyl-ω-trinor-PGE_2 (1.0 nmol per animal, i.c.v.) significantly ($P<0.05$) reduced the vagally stimulated gastric acid secretion and this inhibitory effect lasted for 45 min after its administration (Figure 2b). A lower dose of this agent (0.3 nmol per animal, i.c.v.) was without effect.

Effects of SC-19220 (selective EP_1 receptor antagonist) on 17-phenyl-ω-trinor-PGE_2-induced inhibition of gastric acid secretion

After animals had been treated with SC-19220 (20 nmol per animal, i.c.v.) at −90 min, vagal stimulation was initiated at −60 min. I.c.v. administration of SC-19220 had no effect on either the basal or vagally-induced gastric acid secretion (Figure 3). The actual values of the evoked gastric acid secretion at 0 min in SC-19220-treated animals were 84.1 ± 6.2 μEq 15 min^{-1} ($n=8$). 17-Phenyl-ω-trinor-PGE_2 (1.0 nmol per animal) or vehicle were subsequently adminis-

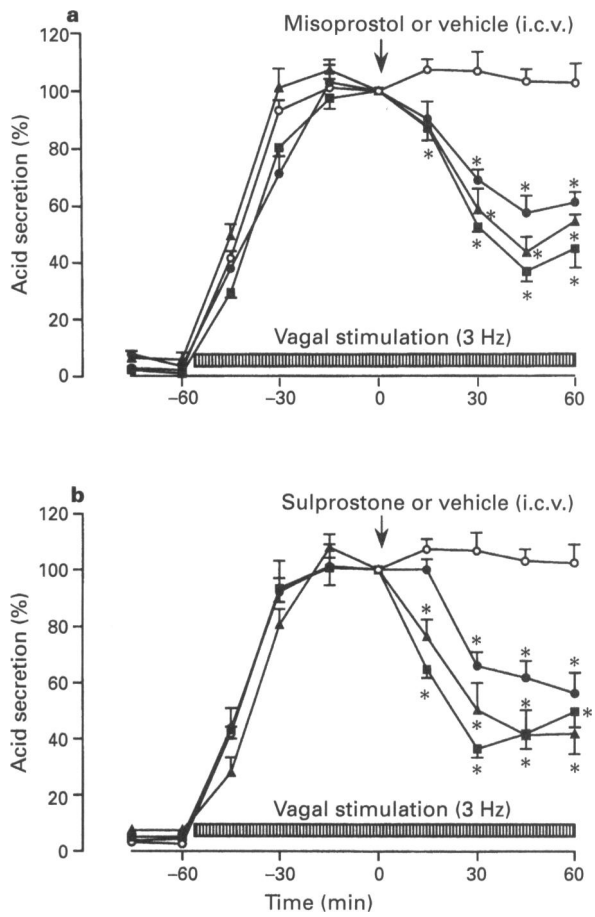

Figure 1 Effects of intracerebroventricularly (i.c.v.) administered misoprostol and sulprostone on vagally stimulated gastric acid secretion. Arrow indicates i.c.v. administration of test substances. In (a), vehicle (○), $n=4$; misoprostol 0.1 nmol per animal (●), $n=4$; misoprostol 0.3 nmol per animal (▲), $n=4$; misoprostol 1.0 nmol per animal (■), $n=4$. In (b), vehicle (○) (as in a), $n=4$; sulprostone 0.1 nmol per animal (●), $n=4$; sulprostone 0.3 nmol per animal (▲), $n=4$; sulprostone 1.0 nmol per animal (■), $n=4$. Each point represents the mean ± s.e.mean. *Significantly different ($P<0.05$) from vehicle-treated control.

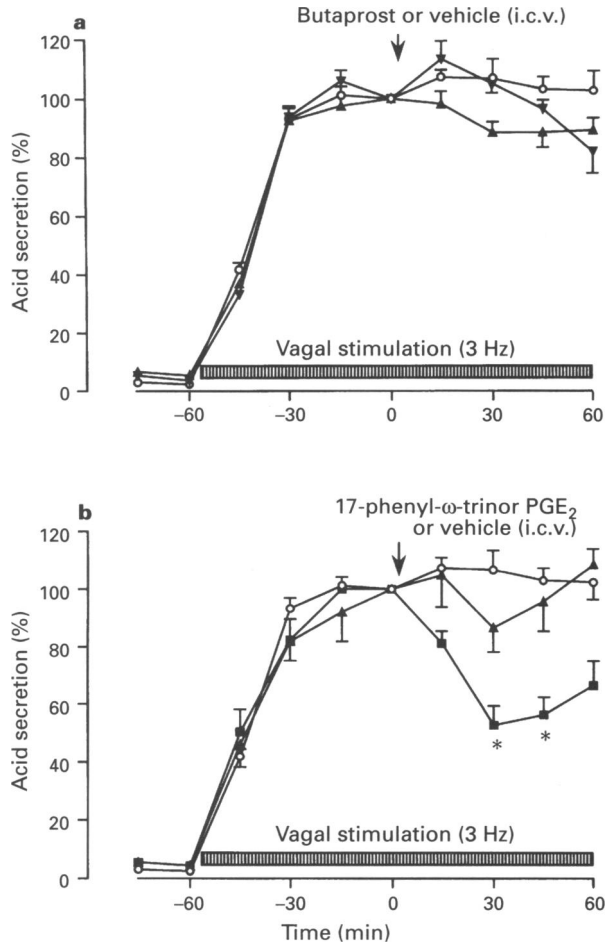

Figure 2 Effects of i.c.v. administered butaprost and 17-phenyl-ω-trinor-PGE_2 on vagally stimulated gastric acid secretion. Arrow indicates i.c.v. administration of test substances. In (a), vehicle (○), $n=4$ (as in Figure 1); butaprost 0.3 nmol per animal (▲), $n=4$; butaprost 3.0 nmol per animal (▼), $n=4$. In (b), vehicle (○) (as in Figure 1); 17-phenyl-ω-trinor-PGE_2 0.3 nmol per animal (▲), $n=4$; 17-phenyl-ω-trinor-PGE_2 1.0 nmol per animal (■), $n=4$. *Significantly different ($P<0.05$) from vehicle-treated control. Other conditions were the same as those in Figure 1.

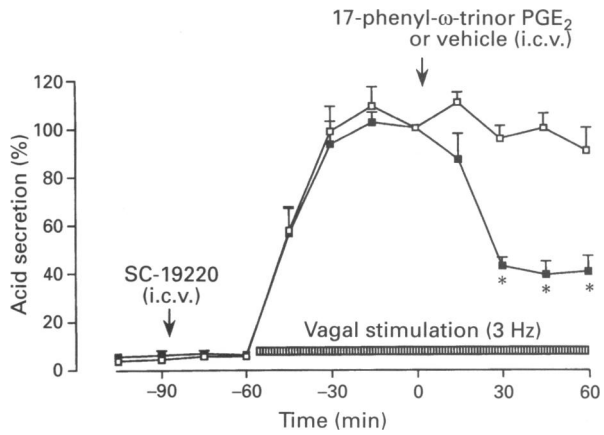

Figure 3 Effects of SC-19220 on the 17-phenyl-ω-trinor-PGE_2-induced inhibition of gastric acid secretion. The first arrow (at −90 min) indicates the administration of SC-19220 (20 nmol per animal, i.c.v.). The second arrow (at 0 min) indicates the administration of 17-phenyl-ω-trinor-PGE_2 (1.0 nmol per animal, i.c.v.) or vehicle alone (i.c.v.). Vehicle (□), $n=4$; 17-phenyl-ω-trinor-PGE_2 1.0 nmol per animal (■), $n=4$. *Significantly different ($P<0.05$) from SC-19220 plus vehicle-treated control. Other conditions were the same as in Figures 1 and 2.

tered i.c.v. (at 0 min); 17-phenyl-ω-trinor-PGE_2 reduced the vagally stimulated gastric acid secretion. Acid secretion at 45 min after i.c.v. administration of 17-phenyl-ω-trinor-PGE_2 (1.0 nmol per animal) was 39.0 ± 5.6% of the preadministered values.

Discussion

Puurunen (1983; 1984) demonstrated that i.c.v. administered PGE_2 (3–10 μg per animal) decreased gastric acid secretion in conscious, pylorus-ligated rats. Since this inhibition was prevented by hypophysectomy or intravenous administration of a vasopressin antagonist, he concluded that the central antisecretory action of PGE_2 was due to the release of vasopressin from the pituitary gland, In our previous report, however, i.c.v. but not i.v. administered small doses of PGE_2 (0.05–0.5 μg per animal) also effectively inhibited the vagally stimulated gastric acid secretion in urethane-anaesthetized rats (Yokotani *et al.*, 1988). This PGE_2-induced antisecretory effect was abolished both by bilateral splanchenectomy and by pretreatment with phentolamine, an α-adrenoceptor blocking agent. Activation of α-adrenoceptors in the stomach inhibits the vagally stimulated gastric acid secretion by reducing acetylcholine release from parasympathetic nerve terminals in the stomach (Yokotani *et al.*, 1984; 1993). This evidence suggests that centrally administered PGE_2 inhibits gastric acid secretion by activation of central sympathetic outflow. Furthermore, vasopressin released from the pituitary gland with a relatively large dose of PGE_2 also inhibits gastric acid secretion as suggested by Puurunen (1983, 1984).

In the present experiments, we used various agonists selective for EP receptor subtypes to characterize the central EP receptor which mediates inhibition of gastric acid secretion. Misoprostol is a potent EP_3 receptor agonist and also has moderate EP_2 and weak EP_1 receptor agonist activities (agonist potencies: $EP_3 > EP_2 > > > EP_1$) (Lawrence *et al.*, 1992; Mantelli *et al.*, 1991). Sulprostone was first identified as a potent EP_1 receptor agonist, but it is more potent at EP_3 receptors (agonist potencies: $EP_3 > EP_1 > > EP_2$) (Coleman *et al.*, 1987a,b; Bunce *et al.*, 1990). Butaprost behaves as a selective EP_2 receptor agonist with relatively low potency (agonist potencies: $EP_2 > > > EP_1$) (Gardiner, 1986). In the present experiments, misoprostol and sulprostone (0.1, 0.3 and 1.0 nmol per animal, i.c.v.) both effectively reduced vagally mediated gastric acid secretion in a dose-dependent manner. However, butaprost was ineffective on the vagally mediated gastric acid secretion. These results suggest that EP_3 receptors in the brain are probably involved in the PGE_2-induced antisecretory effects. However, there was a possibility that central EP_1 receptors are also involved in these antisecretory effects.

We finally examined the role of EP_1 receptors in the PGE_2-induced antisecretory effect. 17-Phenyl-ω-trinor PGE_2 is an EP_1 receptor agonist, with some weaker EP_3 agonist activity (agonist potencies: $EP_1 > EP_3 > > EP_2$) (Dong *et al.*, 1986; Lawrence *et al.*, 1989; 1992). In the present study, the dose of 17-phenyl-ω-trinor PGE_2 (1.0 nmol per animal, i.c.v.), sufficient to reduce the vagally mediated gastric acid secretion, was relatively large. Furthermore, SC-19220, a highly selective and competitive albeit rather weak EP_1 receptor antagonist (Sanner, 1969; Kennedy *et al.*, 1982; Coleman *et al.*, 1985), failed to attenuate 17-phenyl-ω-trinor PGE_2-induced inhibition of gastric acid secretion at a dose of 20 nmol per animal (i.c.v.). This dose of SC-19220 is probably sufficient to block central EP_1 receptors (Ferreira *et al.*, 1978; Kandasamy & Williams, 1982). The present results therefore suggest that an antisecretory effect of a large dose of 17-phenyl-ω-trinor PGE_2 (1.0 nmol per animal, i.c.v.) is produced by activation of central EP_3-receptors.

In the central nervous suystem, EP_3 receptor mRNA is highly expressed in brain regions such as the preoptic area, hypothalamus, locus coeruleus and raphe nuclei (Sugimoto *et al.*, 1994). Administration of PGE_2 into specific brain areas of the hypothalamus such as ventromedial hypothalamus and paraventricular nucleus of hypothalamus has been shown to inhibit stimulated gastric acid secretion (Barocelli *et al.*, 1991).The paraventricular nucleus of the hypothalamus is a major site sending signals to the spinal sympathetic preganglionic neurones (Swanson & Sawchenko, 1983). Recently we reported that activation of central EP_3 receptors elevates plasma noradrenaline levels (Yokotani *et al.*, 1995). It is interesting to note that the potencies of misoprostol, sulprostone and 17-phenyl-ω-trinor PGE_2 in elevating plasma noradrenaline levels were almost the same as those for inhibition of gastric acid secretion. Furthermore, misoprostol- and sulprostone-induced antisecretory effects were abolished by pretreatment with phentolamine (5 mg kg^{-1}, i.m.) (data not shown). It is therefore likely that the PGE_2-induced antisecretory effect is mediated by central prostanoid EP_3 receptors through activation of central sympathetic outflow in urethane-anaesthetized rats.

In summary, we demonstrate here that EP_3-receptors in the rat brain are involved in the PGE_2-induced antisecretory effects on vagally mediated gastric acid secretion.

This study was supported in part by a grant from The Smoking Research Foundation, Japan.

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(Received July 27, 1995
Revised October 13, 1995
Accepted October 24, 1995)