Dual action of prostaglandin E₂ on gastric acid secretion through different EP-receptor subtypes in the rat

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Kato, Shinichi, Eitaro Aihara, Katsuhide Yoshii, and Koji Takeuchi. Dual action of prostaglandin E₂ on gastric acid secretion through different EP-receptor subtypes in the rat. Am J Physiol Gastrointest Liver Physiol 289: G64–G69, 2005; doi:10.1152/ajpgi.00397.2004.—We examined the role of prostaglandin E (EP) receptor subtypes in the regulation of gastric acid secretion in the rat. Under urethane anesthesia, the stomach was superfused with saline, and the acid secretion was determined at pH 7.0 by adding 50 mM NaOH. The acid secretion was stimulated by intravenous infusion of histamine or pentagastrin. Various EP agonists were administered intravenously, whereas EP antagonists were given subcutaneously 30 min or intravenously 10 min before EP agonists. PGE₂ suppressed the acid secretion stimulated by either histamine or pentagastrin in a dose-dependent manner. The acid inhibitory effect of PGE₂ was mimicked by sulprostone (EP₁/EP₃ agonist) but not butaprost (EP₂ agonist) or AE1–329 (EP₄ agonist). The inhibitory effect of sulprostone, which was not affected by ONO-8711 (EP₁ antagonist), was more potent against pentagastrin (50% inhibition dose: 3.6 μg/kg) than histamine-stimulated acid secretion (50% inhibition dose: 18.0 μg/kg). Pentagastrin increased the luminal release of histamine, and this response was also inhibited by sulprostone. On the other hand, AE1–329 (EP₄ agonist) stimulated the acid secretion in vagotomized animals with a significant increase in luminal histamine. This effect of AE1–329 was totally abolished by cimetidine as well as AE3–208 (EP₃ antagonist). These results suggest that PGE₂ has a dual effect on acid secretion: inhibition mediated by EP₁ and EP₃ receptors and stimulation through EP₄ receptors. The former effect may be brought about by suppression at both parietal and enterochromaffin-like cells, whereas the latter effect may be mediated by histamine released from enterochromaffin-like cells.

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Allowing a 30- to 40-min stabilization of basal secretion, gastric acid secretion was stimulated by intravenous (IV) infusion of pentagastrin (100 µg·kg\(^{-1}·h\(^{-1}\)) or histamine (4 mg·kg\(^{-1}·h\(^{-1}\)) at the submaximal dose via a tail vein. After the acid secretion stimulated by pentagastrin or histamine had reached a plateau, various EP agonists, PGE\(_2\) (EP\(_1,\) EP\(_2,\) EP\(_3\) agonist: 0.1–1 mg/kg), sulprostone (EP\(_1,\) EP\(_3\) agonist: 1–30 µg/kg), butaprost (EP\(_2\) agonist: 3 mg/kg), and ONO-AE1–329 (EP\(_1\) agonist: 10 µg/kg), were administered IV via a tail vein as a single injection. EP antagonists ONO-8711 (EP\(_1\) antagonist: 30 mg/kg) and ONO-AE3–208 (EP\(_4\) antagonist: 1 mg/kg) were administered subcutaneously 30 min or IV 10 min before the administration of sulprostone (10 µg/kg), respectively. The 50% inhibition dose (ID\(_{50}\)) of PGE\(_2\) and sulprostone against the acid secretion stimulated by pentagastrin or histamine was calculated from values of the maximal inhibition observed after the injection of these agents.

In a separate experiment, we also examined the effect of various EP agonists on basal acid secretion in normal and vagotomized rats. Vagotomy was performed bilaterally at the cervical portion 1 h before the onset of the experiment. At least 1 h after basal acid secretion had stabilized, various EP agonists, such as PGE\(_2\) (1 mg/kg), sulprostone (30 µg/kg), butaprost (3 mg/kg), and ONO-AE1–329 (1–10 µg/kg), were administered IV via a tail vein as a single injection.

Measurement of histamine release into the gastric lumen. A polyethylene tube was inserted into the stomach through the pylorus, and 2 ml of saline were instilled in the stomach. Thirty minutes later, the gastric contents were recovered from the stomach through the tube. This procedure was repeated every 30 min. The amount of histamine in the gastric content was determined by enzyme-immunoassay (Histamine EIA kit, Oxford Biomedical Research, Oxford, MI). One hour after the onset of IV infusion of pentagastrin (100 µg·kg\(^{-1}·h\(^{-1}\)), PGE\(_2\) (1 mg/kg) and sulprostone (10 µg/kg) were administered IV as a single injection.

Preparation of drugs. Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), pentagastrin (Sigma Chemicals, St. Louis, MO), histamine dihydrochloride (Nacali Tesque, Kyoto, Japan), PGE\(_2\), sulprostone, butaprost (Cayman Chemical, Ann Arbor, MI), ONO-AE1–329, ONO-8711, and ONO-AE3–208 (kindly supplied by Ono Pharmaceutical, Osaka, Japan). Histamine was dissolved in saline. Pentagastrin was first dissolved in 0.05% ammonia solution and then diluted with saline to the desired concentration. ONO-AE3–208 was first dissolved in 1 M NaOH and then diluted with saline to the desired concentration, and ONO-8711 was suspended in a 0.5% carboxymethylcellulose (Nacali Tesque) solution. Each drug was prepared immediately before use and administered IV in a volume of 1 ml/kg, subcutaneously in a volume of 5 ml/kg, and IV infusion in a volume of 5 ml·kg\(^{-1}·h\(^{-1}\).

Statistical analysis. Data are presented as the means ± SE from five to six rats per group. Statistical analyses were performed using a two-tailed Student’s t-test and Dunnnett’s multiple-comparison test, and values of P < 0.05 were regarded as significant.

RESULTS

Effects of various EP agonists on pentagastrin-stimulated acid secretion. IV infusion of pentagastrin (100 µg·kg\(^{-1}·h\(^{-1}\)) produced a progressive increase in acid secretion, with a plateau value reached within 1 h after the onset of the infusion, and the acid secretion was 19.0 ± 1.5 µeq/10 min. The acid secretory response induced by pentagastrin was significantly attenuated by a single IV injection of PGE\(_2\) (1 mg/kg) and sulprostone (10 µg/kg) but not butaprost (3 mg/kg) or AE1–329 (10 µg/kg) (Fig. 1A). PGE\(_2\) (0.1, 0.3, and 3 mg/kg) dose-dependently suppressed the acid secretion, and a significant effect was obtained even at 0.1 µg/kg (Fig. 1B). A dose-dependent suppression of the acid output was also observed by sulprostone (1, 3, and 10 µg/kg), and the effect was significant at all doses. The ID\(_{50}\) values (95% confidence interval) of PGE\(_2\) and sulprostone determined from the maximal inhibition were 0.6 (0.1–1.4) mg/kg and 3.6 (1.5–9.1) µg/kg, respectively. On the other hand, the inhibitory effect of sulprostone (10 µg/kg) on pentagastrin-stimulated acid secretion was not affected by prior subcutaneous administration of ONO-8711 (EP\(_1\) antagonist; 30 mg/kg), and the net acid output was −72.1 ± 1.8 µeq/h, the value being almost equivalent to that of control animals (−81.7 ± 4.7 µeq/h) (Fig. 1B).

Effects of various EP agonists on histamine-stimulated acid secretion. Gastric acid secretion was also stimulated by IV infusion of histamine (4 mg·kg\(^{-1}·h\(^{-1}\)) at the submaximal dose, reaching a plateau value 1 h later, and the total acid out was 27.0 ± 2.2 µeq/10 min. Both PGE\(_2\) (1 mg/kg) and sulprostone (10 µg/kg) significantly attenuated the acid secretory response induced by histamine, whereas neither butaprost (3 mg/kg) nor AE1–329 (10 µg/kg) had any effect (Fig. 2A). As shown in Fig. 2B, PGE\(_2\) (0.1, 0.3, and 1 mg/kg) decreased the net acid output in a dose-dependent manner, and a significant effect was obtained at all doses. Sulprostone (1, 3, and 10 µg/kg) also produced a dose-dependent suppression of the histamine-induced acid secretion, and the effect was significant at all doses used. The
Because basal acid secretion depends largely on the vagus nerves, it is possible that any stimulatory effect of the EP₄ agonist is underestimated. Then we further examined the effects of various EP agonists on acid secretion in vagotomized stomachs, where the basal level of acid secretion was low (2.8 ± 0.4 µeq/10 min). IV administration of both PGE₂ (1 mg/kg) and sulprostone (10 µg/kg) caused a slight but significant decrease in the acid secretion in vagotomized rats, whereas AE₁–329, the EP₄ agonist (10 µg/kg), apparently increased the acid secretion (Fig. 5A). The net acid output for 1 h after the administration of AE₁–329 (1, 3, and 10 µg/kg) was 4.0 ± 1.4, 8.4 ± 2.0, and 10.2 ± 2.3 µeq/1 h, respectively, and a significant increase was observed even at 1 µg/kg (Fig. 5B). The acid stimulatory effect of AE₁–329 (10 µg/kg) was totally abolished by prior administration of AE₃–208 (EP₃ antagonist; 10 µg/kg) or cimetidine (200 mg/kg), the inhibition being 117.7 and 97.6%, respectively.

Effect of AE₁–329 on pentagastrin-induced histamine release in vagotomized stomachs. The amount of luminal histamine release in the vagotomized stomach was 3.2 ± 0.2 pg/30 min (Fig. 6). IV administration of PGE₂ (1 mg/kg) slightly suppressed the release of histamine to 2.7 ± 0.1 pg at 30- to 60-min intervals after the injection, whereas AE₁–329 (10 µg/kg), given IV, significantly enhanced the luminal release of histamine to 5.8 ± 0.5 pg for the first 30 min.

**DISCUSSION**

Exogenously administered PGE and its analogs have been reported to suppress gastric acid secretion in rats (12, 13) and dogs (38) as well as humans (2). Way and Durbin (37) showed that PGE₁ inhibited acid secretion stimulated by histamine but not by cAMP in the isolated amphibian fundic mucosa. Soll (27) demonstrated, using isolated canine parietal cells, that PGE₂ directly suppressed the parietal cell activity stimulated by histamine but not by carbachol or gastrin. These studies in vitro suggest that PGE directly suppresses the activity of
parietal cells, especially that stimulated by histamine. In the present study, we observed that PGE2 given IV dose dependently suppressed acid secretion stimulated by not only histamine but also pentagastrin in anesthetized rats. Because gastrin stimulates acid secretion mediated by endogenous histamine released from ECL cells (1, 7, 8, 20, 23, 36), it is assumed that the inhibitory effect of PGE2 on acid secretion is specific for the histamine-induced response of the parietal cell.

We further observed that the inhibitory effect of PGE2 on acid secretion was mimicked by the EP1/EP3 agonist sulprostone but not the EP2 agonist butaprost or the EP4 agonist AE1–329. In addition, ONO-8711, a specific EP1 antagonist, did not affect the inhibitory effect of sulprostone on pentagastrin-stimulated acid secretion. Our laboratory previously showed that ONO-8711 at the dose used in the present study totally abolished adaptive gastroprotection induced by mild irritants, the phenomenon mediated by EP1 receptors (34). These findings suggest that the inhibitory effect of PGE2 on acid secretion is mainly mediated by EP3 receptors. Yokotani et al. (39) reported that the activation of central EP3 receptors inhibited vagally mediated gastric acid secretion through the increase of central sympathetic outflow in rats. Sympathetic neurons functionally antagonize the actions mediated by the vagal-cholinergic system, by inhibiting the release of acetylcholine. We observed, however, in the present study, that IV injection of PGE2 and sulprostone significantly suppressed acid secretion, even in vagotomized rats, suggesting the inhibitory effect through EP3 receptors on acid secretion, independent of vagal innervation. Because we did not examine the effect of sympathectomy on the acid secretory changes caused by PGE2 in the present study, a possibility cannot be totally excluded that the inhibitory effect of PGE2 originates centrally, in addition to that which occurred peripherally. We also found that the inhibitory effects of PGE2 and sulprostone were much more potent when acid secretion was stimulated by pentagastrin rather than histamine. These results may be related to the different mechanisms in the process of acid secretion induced by these secretagogues. Several studies showed that PGs inhibited histamine release from isolated perfused stomach (24), isolated canine oxyntic mucosal cells (27), and isolated rat ECL cells (19). In the present study, sulprostone significantly

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**Fig. 4.** Effects of various EP agonists on basal gastric acid secretion in rats. PGE2 (1 mg/kg), butaprost (3 mg/kg), and AE1–329 (10 μg/kg) were administered intravenously as a single injection. A: values are presented as the means ± SE of values determined every 10 min from 5–6 rats. B: values represent the net gastric acid output obtained for 1 h after the injection of EP agonist and are presented as the means ± SE from 5–6 rats. *Statistically significant difference from control (vehicle alone), at P < 0.05.

**Fig. 5.** Effects of various EP agonists on gastric acid secretion in vagotomized rats. Vagotomy was performed acutely at the cervical portion 1 h before the injection of EP agonists. PGE2 (1 mg/kg), sulprostone (10 μg/kg), butaprost (3 mg/kg), and AE1–329 (1–10 μg/kg) were administered intravenously as a single injection. AE1–208 (EP 4 antagonist: 10 μg/kg) was administered intravenously 10 min before, whereas cimetidine (200 mg/kg) was administered subcutaneously 30 min before the injection of AE1–329. A: values are presented as the means ± SE of values determined every 10 min from 5–6 rats. B: values represent the net gastric acid output obtained for 1 h after the injection of EP agonist and are presented as the means ± SE from 5–6 rats. *Statistically significant difference at P < 0.05; #from control (vehicle alone); †from values treated with AE1-329 (10 μg/kg).
suppressed the luminal histamine release induced by pentagastrin, suggesting that this inhibitory effect may be brought about by suppression of histamine release from ECL cells, in addition to inhibition of the parietal cell activity, both mediated by EP3 receptors.

It has been reported that PGE-induced inhibition of acid secretion is mediated by somatostatin released from D cells in the stomach (21, 29). Somatostatin is known to be an endogenous inhibitor of acid secretion, the effect being caused by both suppression of the parietal cell activity (3) and inhibition of the histamine release from ECL cells (10, 19, 24). Lindstrom and Hakanson (11), however, reported that the amount of somatostatin was very small in gastric mucosa and that the immunonneutralization of somatostatin failed to prevent the inhibitory action of a PGE analog on ECL cells. Thus it is unlikely that the inhibitory effect of PGE2 is mediated by endogenous somatostatin, although this possibility cannot be completely excluded at present.

One of the present authors previously reported that 16,16-dimethyl-PGE2 stimulated acid secretion at high concentrations, despite inhibiting acid secretion at low concentrations in an isolated preparation of amphibian fundic mucosa, and the stimulatory response was totally abolished by either a histamine H2 receptor antagonist or compound 48/80, a histamine-depleting agent (31). These results indicate that PGE2 at high doses stimulates acid secretion through the release of endogenous histamine. Similarly, Sernka and Caplan (25) showed, in the isolated rat gastric mucosa, that PGE2 stimulated oxygen consumption and H+ transport, indicating an enhancement of parietal cell activity by this agent. In contrast, Nylander et al. (17) reported that PGE has two opposing effects: liberation of endogenous histamine and inhibition of the histamine action on the parietal cell. In the present study, we observed that basal acid secretion was significantly suppressed by PGE2 but slightly augmented by AE1–329, the EP4 agonist. Because basal acid secretion depends largely on the vagus nerves, it is possible that the acid stimulatory effect of the EP4 agonist is underestimated. As expected, we found that the acid secretion was dose dependently enhanced by AE1–329 in the vagotomized stomach, where the basal level of secretion was very low, and this effect was totally abolished by cimetidine as well as AE2–208, the specific EP3 antagonist. Furthermore, AE1–329 significantly increased the release of luminal histamine in the vagotomized stomach. These results strongly suggest that the stimulatory effect of PGE2 on acid secretion is brought about by the release of endogenous histamine from ECL cells, the process being mediated by EP4 receptors. However, the stimulatory effect of PGE2 through EP4 receptors seems not to be so potent, as the EP4 agonist did not significantly increase basal acid secretion in normal rats and as PGE2 itself inhibited basal acid secretion mediated by the activation of EP3 receptors. It is assumed that the stimulatory effect of PGE2 through EP4 receptors is overcome by the inhibitory effect mediated by EP3 receptors and that the former effect is predominant. Anyhow, the present results confirmed the stimulatory effect of PGE2 observed in previous studies in vitro, where basal secretion was made scanty by excluding extrinsic nerves, as in vagotomized stomachs used in the present study.

Ding et al. (5) showed, using Northern blot analysis, that both the EP3- and EP4-receptor genes were mainly expressed in the parietal cell. The EP3 receptor has at least four splicing variants coupled with different signaling pathways (14). The EP3A receptor is linked to the activation of Gs protein, resulting in a suppression of intracellular cAMP levels, whereas EP3B and EP3C are coupled with the activation of Gi protein, resulting in an increase of intracellular cAMP. In addition, activation of the EP3D receptor causes an elevation of intracellular Ca2+ by stimulating Gq protein. It has also been reported that PGE2 and its analog inhibited acid secretion as well as cAMP production stimulated by histamine in parietal cells (27, 35). It is likely that the inhibitory effect of PGE2 on acid secretion is mediated by activation of EP3, especially EP3A, receptors through inhibition of intracellular cAMP in the parietal cell. On the other hand, Naribayashi-Inoue et al. (15) reported that EP2 and EP3 receptors were found on ECL carcinoid tumor cells of Mastomys, African rodents. They also showed that PGE2 enhanced the generation of cAMP through EP2 receptors while inhibiting the cAMP production induced by forskolin through EP3 receptors. Thus the EP3A receptor may also be involved in the inhibitory effect of PGE2 on histamine release from ECL cells. In the present study, however, the EP2 agonist butaprost had no influence on acid secretion, and the EP4 agonist stimulated acid secretion through the release of histamine from ECL cells in vagotomized rats. The reason for these different results remains unclear, yet the difference may be due to different experimental conditions, such as animal species.

The physiological implication of the present findings in the regulation of acid secretion remains unknown. Our laboratory previously reported that gastric acid secretion was decreased in the damaged stomach following the barrier disruption (16, 30). This response is mediated by enhanced release of PGE2 and plays a role in maintaining the microclimate for restitution of the injured tissue. We also reported that damage in the stomach enhances the acid stimulatory pathway, in addition to the PG-dependent inhibitory pathway, although the latter effect normally overcomes the former, resulting in a decrease of acid secretion (32). This stimulatory acid response is mediated by release of endogenous histamine, the process being also modified by a PG-dependent mechanism (33). It is assumed that
endogenous PGs may have a dual role in the regulation of acid secretion in the damaged stomach. The stimulatory effect mediated by EP4 receptors, although not so potent, may contribute to prevent the excessive inhibition of acid secretion caused by activation of EP3 receptors. Certainly, there is a possibility that the histamine release from ECL cells mediated by EP4 receptors may serve another function than stimulation of acid secretion.

Taken together, it is concluded that PGE2 has a dual effect on the regulation of acid secretion: inhibition mediated by EP3 receptors and stimulation through EP4 receptors. The former effect may be brought about by suppression at both parietal and ECL cells, whereas the latter effect may be mediated by histamine release from ECL cells.

REFERENCES


