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Effects of Endothelin-1 on Duodenal Bicarbonate Secretion and Mucosal Integrity in Rats

Koji Takeuchi, Shinichi Sugamoto, Keizo Suzuki, Shoji Kawauchi and Osamu Furukawa

Department of pharmacology and Experimental Therapeutics
Kyoto Pharmaceutical University
Misasagi, Yamashina
Kyoto 607-8414, Japan

Abstract

Effects of endothelin-1 on gastric acid secretion, duodenal HCO_3^- secretion, and duodenal mucosal integrity were investigated in anesthetized rats, in comparison with those of TY-10957, a stable analogue of prostacyclin. A rat stomach mounted on an *ex-vivo* chamber or a proximal duodenal loop was perfused with saline, and gastric acid or duodenal HCO_3^- secretion was measured using a pH-stat method and by adding 100 mM NaOH or 10 mM HCI, respectively. Duodenal lesions were induced by mepirizole (200 mg/kg) given subcutaneously. Intravenous administration of endothelin-1 (0.6 and 1 nmol/kg) caused an increase of duodenal HCO_3^- secretion with concomitant elevation of blood pressure; this effect was antagonized by co-administrahon of BQ-123 (ET_A antagonist; 3 mg/kg, i.v.) and significantly mitigated by vagotomy. Likewise, endothelin-1 caused a significant decrease in histamine-stimulated acid secretion, and this effect was also significantly antagonized by BQ-123. Although TY-10957 (10 and 30 mg/kg, i.v.) produced a temporal decrease of blood pressure, this agent caused not only an increase of duodenal HCO_3^- secretion, independent of vagal nerves, but also a decrease of acid secretion as well. In addition, both endothelin-l and TY-10957 significantly prevented mepirizole-induced duodenal lesions at the doses that caused an increase of duodenal HCO_3^- secretion and a decrease of gastric acid secretion. These results suggest that endothelin-1 affects the duodenal mucosal integrity by modifying both gastric acid and duodenal HCO_3^- secretions, the effects being mediated by ETA receptors.

Key Word: endothelin-1, prostacyclin, acid secretion, HCO_3^- secretion, duodenal lesion

Introduction

Endothelium-derived substances such as nitric oxide (NO), endothelins and prostaglandins (PGs), espeeially prostacyclin (PGI_2), are involved in regulation of the vascular smooth muscle tone and the arterial blood pressure (9, 13, 14). Both PGs and NO are important in maintaining the gastroduodenal mucosal integrity against noxious stimuli by modulating various functions such as mucosal blood flow and mucus secretion (1, 10, 11, 13, 14, 31). These substances also play roles in regulation of acid secretion in the damaged stomach and contribute to maintaining the microclimate for repair of the injury (26). However, only few study has been reported dealing with the relation of endothelins to gastric lesions induced by ethanol (15) and ischemia-reperfusion (12), or its interactive action with NO and PGs in the stomach (32).

We recently reported that the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) markedly stimulates gastroduodenal HCO_3^- secretion, similar to PGs, and protects the duodenal mucosa against acid injury (23, 25). The HCO_3^- stimulatory effect of L-NAME occurs with a concomitant increase of arterial blood pressure and is significantly attenuated by vagotomy, suggesting the importance of the vagal neuronal activation due to the presser response in this phenomenon (23, 24, 27). Because endothelin-1 is known to elevate the blood

Corresponding author: Dr. K. Takeuchi, Tel: 81-595-4699, Fax: 81-595-4774, E-mail: takeuchi@mb.kyoto-phu.ac.jp
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pressure, it is possible that this substance may also affect the mucosal integrity of the duodenum by altering the HCO_3^- secretion.

In the present study, we thus investigated the effects of endothelin-1 on duodenal HCO_3^- secretion and ulcerogenic response as well as gastric acid secretion, in comparison with TY- 10957, a stable analogue of PGI_2.

Materials and Methods

Male Sprague-Dawley GS rats (250~300 g, Nippon Charles River, Shizuoka, Japan) were kept in individual cages with raised mesh bottoms to prevent coprophagia and deprived of food but allowed free access to tap water for 18 hr before the experiments. All studies were carried out using 5~6 rats per group under urethane anesthetized conditions (1.25 g/kg, i.p.). All experimental procedures described were approved by the Experimental Animal Research Committee of the Kyoto Pharmaceutical University.

Determination of Duodenal HCO_3^- Secretion

Duodenal HCO_3^- secretion was measured in a proximal loop according to the previously published method (22, 23). Briefly, the abdomen was incised, and both the stomach and duodenum were exposed. The duodenal loop was made between the pyloric ring and the area proximal to the outlet of the common bile duct and perfused at a flow rate 0.8 ml/min with saline that was gassed with 100% O_2, heated at 37°C and kept in a reservoir. The HCO_3^- secretion was measured at pH7.0 using a pH-stat method (Hiranuma Comtite-7, Mito, Japan) and by adding 10 mM HCI to the reservoir. In some animals, the loop was perfused with slightly acidified saline (pH4.5), and the pH of the perfusate was continuously monitored using a flow-type pH glass electrode (6901-25T; Horiba, Kyoto, Japan) (22). The femoral artery was cannulated and the arterial blood pressure was monitored by a pressure transducer and polygraph device (Sanei CASE-7903, Tokyo, Japan). After basal HCO_3^- secretion had well stabilized, endothelin-1 (0.6 and 1 nmol/kg) and TY-10957 (10 and 30 μg/kg) were administered i.v. as a single injection. Vagotomy was performed bilaterally at cervical portion 1 hr before administration of endothelin-1 or PGI_2. In some animals, BQ-123 (1 and 3 mg/kg), an ETA antagonist (5), was given s.c. 30 min before i.v. administrahon of endothelin-1.

Determination of Gastric Acid Secretion

Gastric acid secretion was measured in a chambered stomach, according to the previously published method (26). In brief, the abdomen was incised, the stomach exposed and mounted in an *ex-vivo* chamber (an exposed area: 3.14 cm^2). The gastric mucosa was perfused at a flow rate of 0.8 ml/min with saline that was gassed with 100% O_2, heated at 37°C and kept in a reservoir. The acid secretion was measured at pH 7.0 using a pH-stat method (Hiranuma Comtite-8, Tokyo, Japan) by adding 100 mM NaOH to the reservoir. After basal acid secretion had well stabilized, histamine (4 mg/kg/hr) was continuously infused i.v. from a tail vein. Endothelin-1 (1 nmol/kg) and TY-10957 (10 μg/kg) were administered i.v. as a single injection after the acid secretion had reached a plateau level. In some animals, BQ-123 (1 mg/kg) was given s.c. 30 min before i.v. administration of endothelin-1.

Induction of Duodenal Lesions

Animals were given mepirizole s.c. in a dose of 200 mg/kg, without any surgical manipulation, and they were killed 6 hr later (23). Then, both the stomachs and duodenums were removed, inflated by injecting 8 ml of 2% formalin, immersed in 2% formalin for 10 min to fix both the inner and outer layers of the tissues, and opened along the greater curvature in the stomach or along the antimesenteric attachment in the duodenum. The area (mm^2) of each lesion developed in the duodenal mucosa was measured under a dissecting microscope with a square grid (×10), summed per each tissue, and used as lesion score. Endothelin-1 (1 nmol/kg) and TY-10957 (10 and 30 μg/kg) were administered i.v. twice, 10 min before and 3 hr after administration of mepirizole. In some animals, BQ-123 (1 mg/kg) was given s.c. twice 30 min before each administrahon of endothelin- 1.

Preparation of Drugs

Drugs used were urethane (Tokyo Kasei, Kyoto, Japan), endothelin-1, BQ-123 (Banyu Pharm. Co., Tokyo, Japan), TY-10957 (Toha-Eiyo, Aichi, Japan) and histamine 2HCI (Nakarai Tesque, Kyoto, Japan). BQ-123 was suspended in saline with a drop of Tween 80 (Wako, Osaka, Japan), while other agents were dissolved in saline. Each agent was prepared immediately before use and was administered i.p. or s.c. in a volume of 0.5 ml per 100 g body wt., or i.v. in a volume of 0.1 ml/100 g body wt. Control animals received saline as the vehicle.

Statistics

Data are presented as the means±SE from 4~6

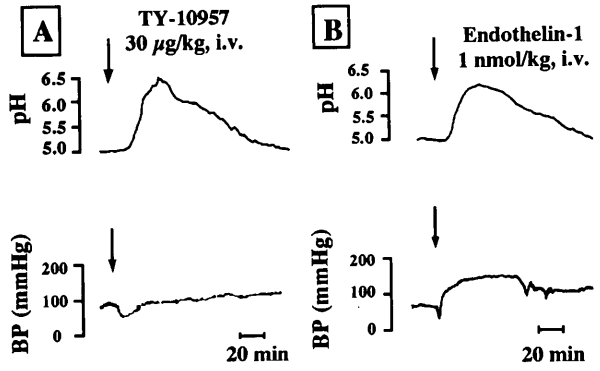

Fig. 1. Representative recordings showing changes in duodenal luminal pH and blood pressure in anesthetized rats after administration of endothelin-1 (1 nmol/kg) or TY- 10957 (30 μg/kg). These agents were given i .v. as a single bolus injection. Note that both of these agents increased the pH of the luminal perfusate due to HCO_3^- secretion, while the arterial blood pressure was increased by endothelin-1 and decreased by TY- 10957.

rats from each group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of $P<0.05$ were considered as significant.

Results

Effects of Endothelin-1 and TY-10957 on Luminal pH in The Duodenum

In the anesthetized rat duodenum perfused with slightly acidified saline (pH 4.5), the pH of luminal perfusate remained in the range of 5.0~5.3, with the arterial blood pressure of about 80~90 mmHg. Intravenous administration of TY-10957 (30 μg/kg) caused a marked increase of the pH of luminal perfusate, with slight decrease of arterial blood pressure; the pH reached a maximal value within 20 min and remained elevated for about 1 hr (Fig. 1A). Similarly, endothelin-1 (1 nmol/kg) given i.v. as a single injection produced an apparent increase of the pH, reaching a maximal value of 6.1, and gradually returned to a baseline level within 1 hr (Fig. 1B). Endothelin-1 also caused a marked and persistent elevation of the arterial blood pressure, and this change preceded the increase of the luminal pH.

Effects of Endothelin-1 and TY-10957 on Duodenal HCO_3^- Secretion

The proximal duodenum spontaneously secreted HCO_3^- at a steady rate of 0.2~0.4 μEq/5 min during a test period. Intravenous administration of TY-10957 (10 and 30 μg/kg) caused a dose-dependent increase of duodenal HCO_3^- secretion, and at 30 μg/kg a maximal response was obtained within 30 min, reaching 1.0±0.1 μEq/5 min, the total net HCO_3^- output being 7.2±1.5 μEq/hr (Fig. 2). Likewise, the duodenal HCO_3^- secretion was also dose-dependently increased in response to endothelin-1 (0.6 and 1 nmol/kg, i.v.), reaching a maximal value of 1.1±0.3 μEq/5 min within 20 min at 1 nmol/kg; the total net HCO_3^- output was 2.8±0.7 μEq/hr and 7.9± 1.3 μEq/hr, respectively, at 0.6 nmol/kg and 1 nmol/kg (Fig. 3). The HCO_3^- stimulatory action of endothelin-1 was dose-dependently attenuated by prior s.c. administration of BQ-123, an ET-1 receptor antagonist, the inhibition being almost complete at 3 mg/kg.

Effects of Vagotomy on Duodenal HCO_3^- Secretory Response to Endothelin-1 and TY-10957

Bilateral vagotomy did not affect basal HCO_3^- secretion in the rat duodenum, the total net HCO_3^- output being -0.9±0.1 μEq/hr, which is not significantly different from that (-0.8±0.1 μEq/hr) in the animals with intact vagus nerves (Fig. 4). However, in the animals subjecting to vagotomy, endothelin-1 (

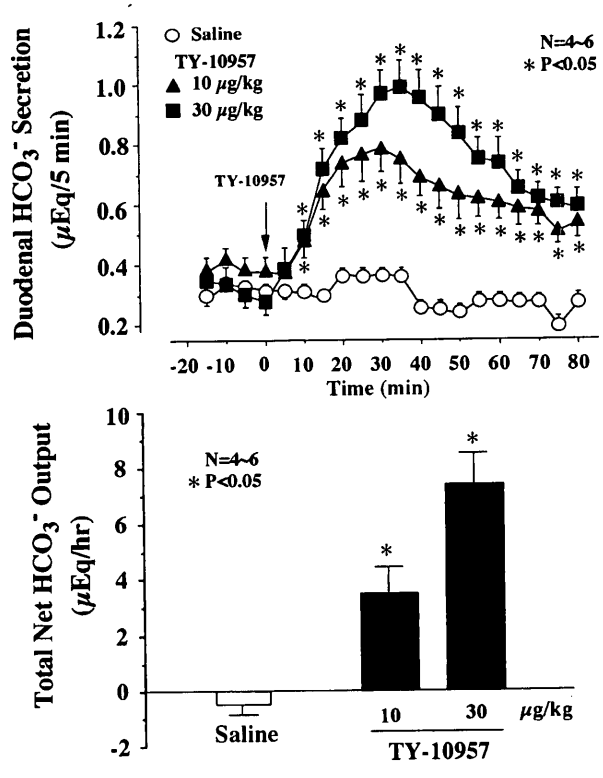

Fig. 2. Effect of TY-10957 on duodenal HCO_3^- secretion in anesthetized rats. TY-10957 (10 and 30 μg/kg) was given i.v. as a single bolus injection. Lower panel shows the total net HCO_3^- output for 1 hr after administration of TY- 10957. Data are presented as the means±SE from 4~6 rats per group. *Significantly different from control at $P<0.05$.

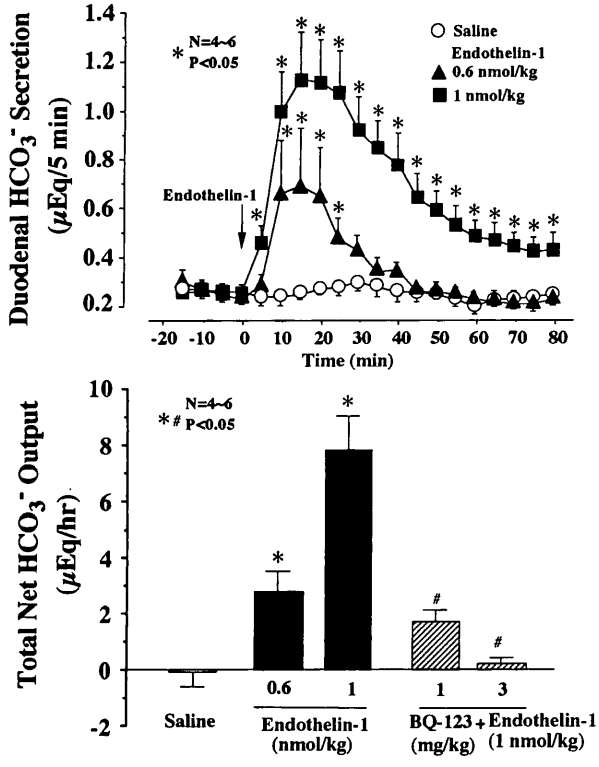

Fig. 3. Effect of endothelin- 1 on duodenal HCO_3^- secretion in anesthetized rats, and the reveral of this effect by BQ-123, an ET_A antagonist. Endothelin-1 (0.6 and 1 nmol/kg) was given i.v. as a single bolus injection. BQ-123 (1 and 3 mg/kg) was administered s.c. 30 min before administration of endothelin-1 (1 nmol/kg). Lower panel shows the total net HCO_3^- output for 1 hr after administrahon of endothelin-1. Data are presented as the means±SE from 4~6 rats per group. *Significantly different from control at P<0.05.

1 nmol/kg, i.v.) totally failed to stimulate duodenal HCO_3^- secretion. The total net HCO_3^- output induced by endothelin-1 in the animals with or without vagotomy was 9.1±1.8 μEq/hr and 1.0±0.8 μEq/hr, respectively. By contrast, a PGI_2 derivative TY-10957 (30 μg/kg, i.v.) caused a marked increase of duodenal HCO_3^- secretion, irrespective of whether or not the animals were subjected to vagotomy, and the total net HCO_3^- output was 11.0±1.28 μEq/hr and 10.5±1.9 μEq/hr, respectively.

Effects of Endothelin-1 and TY-10957 on Gastric Acid Secretion

Under urethane anesthetized conditions, the stomach spontaneously secreted acid at rates of 3~4 μEq/5 min as basal secretion. The acid secretion increased in response to i.v. infusion of histamine (4 mg/kg/hr), reached a plateau level of approximately 8~10 μEq/5 min, and remained elevated during a 2 hr-test period; total acid output after reaching a plateau

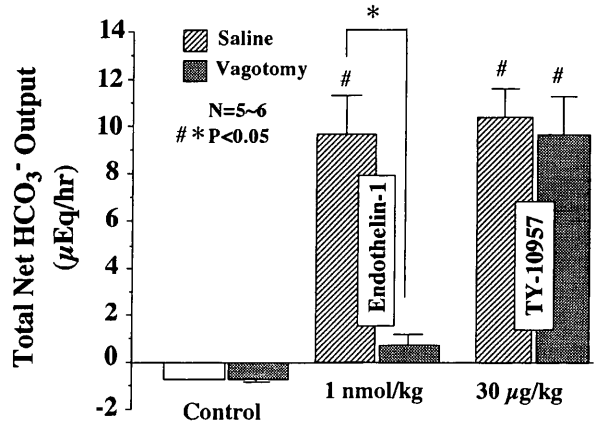

Fig. 4. Influence of vagotomy on the duodenal HCO_3^- stimulatory action of endothelin-1 and TY-10957 in anesthetized rats. Endothelin-1 (1 nmol/kg) and TY-10957 (30 lug/kg) were given i.v. as a single bolus injection. Bilateral vagotomy was performed bilaterally at the cervical portion 1 hr before administration of these agents. Data show the total net HCO_3^- output for 1 hr after administration of these agents and represent the means±SE from 5~6 rats. *Statistically significant difference from saline, at P<0.05.

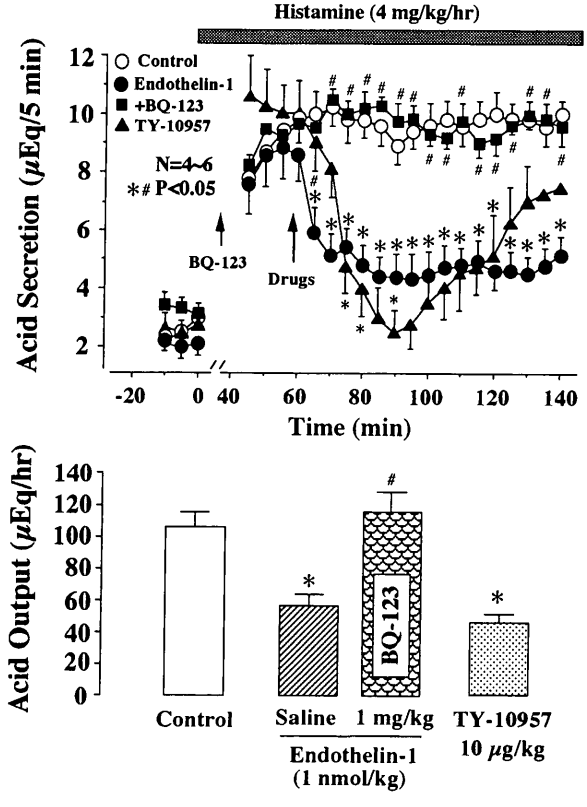

Fig. 5 . Effects of endothelin-1 and TY-10957 on histamine-stimulated gastric acid secretion in anesthetized rats. Acid secretion was stimulated by a continuous, i.v. infusion of histamine (4 mg/kg/hr). Endothelin-1 (1 nmol/kg) or TY-10957 (10 μg/kg) was given i.v. as a single bolus injection. BQ-123 (1 mg/kg) was given S.C. 30 min before administration of endothelin-1. Values in Fig. B show the total acid output for 1 hr after administration of these agents. Data are presented as the means±SE from 4~6 rats per group. *Statistically significant difference from saline, at P<0.05.

was 109±13 μEq/hr (Fig. 5). Endothelin-1 (1 nmol/kg, i.v.) caused a significant reduction in the histamine-stimulated acid secretion from 8.3±0.8 μEq/5 min to the lowest values of 4.1±0.7 μEq/5 min within 15 min, the total acid output being 58.1±7.2 μEq/hr. TY-10957 also decreased the acid secretory response to histamine; the total acid output was 51.7±6.1 μEq/hr, the reduction being 68.2%. On the other hand, BQ-123 (1 mg/kg, s.c.) given prior to endothelin-1, completely prevented the reduction of histamine-stimulated acid secretion following administration of endothelin-1. In these animals, the total acid output was 116±18 μEq/hr, which is not significantly different from that in control rats.

Effect of Endothelin-1 and TY-10957 on Duodenal Ulcerogenic Response

The animals given mepirizole (200 mg/kg, s.c.) developed hemorrhagic lesions in the proximal duodenum with minimal damage in the stomach, when examined macroscopically 6 hr after administration. The duodenal damage consisted of one or two lesions, and the lesion score was 16.2±3.6 mm^2. Prior administration of 1 nmol/kg of endothelin-1 significantly reduced the seventy of duodenal damage, the lesion score being 5.0±1.0 mm^2 (Fig. 6). However, pretreatment of BQ-123 almost completely reversed the prophylactic effect of endothelin-1 on the duodenal damage. Likewise, TY-10957 (10 and 30 μg/kg) also prevented the development of duodenal lesion in a dose-related manner. At 30 μg/kg of this agent, the lesion score was 5.0±1.2 mm^2, which was significantly lower than control values.

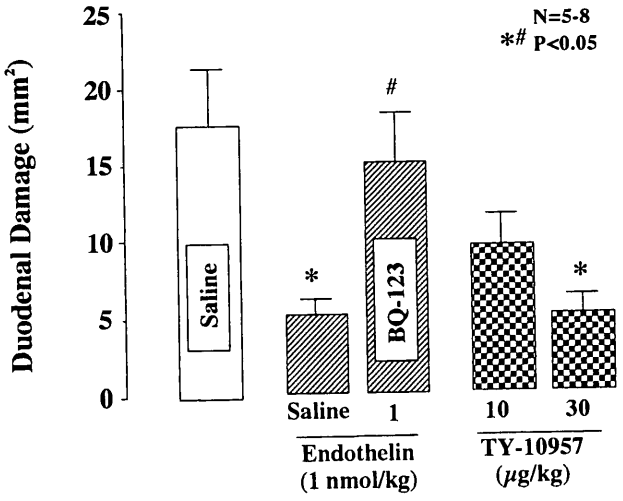

Fig. 6. Effects of endothelin-1 and TY-10957 on mepirizole-induced duodenal lesions in anesthetized rats. The animals were administered mepirizole s.c. in a dose of 200 mg/kg, and they were killed 8 hr later. Endothelin-1 (1 nmol/kg) or TY-10957 (30 μg/kg) was given i.v. twice, 10 min before and 3 hr after mepirizole treatment. BQ- 123 (1 mg/kg) was given s.c. 30 min before each administration of endothelin-1. Data are presented as the means±SE from 5~8 rats. Statistically significant difference at $P<0.05$; *from saline; #from endothelin-1 alone.

Discussion

Endothelium-derived substances such as NO and PGs are involved in regulation of gastroduodenal function and in modulating the mucosal integrity of these tissues (1, 9, 10, 11, 14, 31). However, only few study has been reported dealing with the influence of endothelin, the endogenous substance also produced by endothelium (12, 15, 32). In the present study, we demonstrated that endothelin-1 caused apparent effects on the duodenal mucosal integrity by modifying acid and HCO_3^- secretions, similar to PGI_2.

TY-10957, a stable analogue of PGI_2, has been previously shown to have various actions in the stomach and duodenum, such as inhibition of gastric acid secretion, stimulation of gastroduodenal HCO_3^- secretions, and gastroprotection against ethanol (18). The present study confirmed that TY-10957 inhibited acid secretion and stimulated duodenal HCO_3^- secretion, and further showed a protective effect of this PGI_2 analogue on the duodenum against mepirizole-induced ulceration. Firstly, many studies showed that duodenal HCO_3^- secretion is stimulated by endogenous and exogenous PGs of various types, including PGI_2 (4, 8). We have recently reported that PGE_2 stimulates HCO_3^- secretion in the duodenum through EP_3 receptor subtype, mediated with adenylate cyclase/cyclic adenosine monophosphate (cAMP) (28). Since the IP receptor is linked with Gs protein or Gq protein, increasing cAMP or Ca^{2+}, respectively (2, 16), it may be assumed that TY-10957 stimulates duodenal HCO_3^- secretion, mediated by cAMP, similar to PGE_2 (28). Secondly, the inhibition of acid secretion by TY-10957 is in agreement with previous findings by Soll et al. (21), who reported that PGI_2 inhibited histamine-stimulated acid secretion in isolated parietal cells as determined by aminopyrine uptake. It is unlikely that this action is mediated by IP receptors, because they are not coupled with Gi protein, resulting in a decrease in cAMP in the parietal cell. PGE_2 is, however, coupled with Gi protein through EP_3 receptors in the parietal cell and inhibits histamine-induced acid secretion (16, 33). Thus, it may be assumed that TY-10957, an PGI_2 analogue, has also affinity to EP_3 receptors, leading to suppression of gastric acid secretion.

Of the most interest is the finding that endothelin-1 also caused an increase of duodenal HCO_3^- secretion in addition to inhibition of acid secretion, resulting in protection of the duodenal mucosa against mepirizole-induced ulceration. Because these actions were all

significantly antagonized by BQ-123, an ET_A antagonist (5), it is likely that endothelin-1 elicits both inhibition of gastric acid secretion and stimulation of duodenal HCO_3^- secretion mediated by ET_A receptors. Koduru et al. (7) recently reported that endothelin-1 has dual effects on gastric acid secretion, the inhibitory action mediated by ET_B receptors and the stimulatory action by ET_A receptors:, though the former overcomes the latter, leading to acid inhibition. Although the ET receptors responsible for the antisecretory action of endothelin-1 are not without controversy between their study and ours, overall results suggest that endothelin-1 causes a dose-dependent inhibition of acid secretion. Endothelin-1 inhibited histamine-stimulated acid secretion, excluding the possibility that the antisecretory effect is due to inhibition of histamine release from the enterochromaffin-like cells. In addition, because endothelin-1 may reduce mucosal blood flow in the stomach due to contraction of the vascular smooth muscle, it is possible that the acid inhibition is secondary to a decrease of mucosal blood flow. Furthermore, Shimomura et al (19) reported that the endothelin effect on stomach smooth muscles is inhibited by indomethacin, suggesting involvement of PGs in its action. De Nucci et al. (3) also reported that the pressor effect of circulating endothelin is limited by the release of PGI_2 and NO. Thus, it is possible that the present results observed by endothelin- 1 may also be mediated partly by endogenous NO as well as PGs, probably PGI_2.

On the other hand, the stimulation by endothelin-1 of duodenal HCO_3^- secretion was attenuated by not only BQ-123 but also by vagotomy, suggesting an involvement of vagus nerves in the stimulatory pathway. We previously reported that the inhibition of NO production by L-NAME stimulates duodenal HCO_3^- secretion in anesthetized rats, and this effect was accompanied by increase of arterial blood pressure and significantly mitigated by vagotomy (23, 24, 27). We also reported that the HCO_3^- stimulatory action of L-NAME was also inhibited by prior administration of α-blockers such as yohimbine and prazosin (24). These α-blockers alone lowered blood pressure and reduced the magnitude of the blood pressure response to L-NAME. These all data suggest that the mechanism by which L-NAME stimulates the HCO_3^- secretion is mediated by a neural reflex through the vagal efferent nerve, resulting from the pressor response to L-NAME. As shown in this study, endothelin-1 caused a persistent elevation of arterial blood pressure, and this change preceded the increase of duodenal HCO_3^- secretion, similarly to the phenomenon observed by L-NAME. Thus, it is possible that endothelin-1 shares the same mechanism with L-NAME for stimulating duodenal HCO_3^- secretion, ie., the agent first elevates arterial blood pressure, causing the neuronal reflex, and stimulates the HCO_3^- secretion through vagal efferent nerves. It should be noted that duodenal HCO_3^- response to TY-10957 was not affected by vagotomy, suggesting a direct action of PGI_2 on the epithelial cells to stimulate HCO_3^- secretion.

It is not surprising that TY-10957 as well as endothelin-1 inhibited duodenal ulcerogenic response to mepirizole, since these substances produced an increase of duodenal HCO_3^- secretion and a decrease of acid secretion, both factors being involved in the pathogenesis of duodenal ulceration (17, 29). Indeed, many studies demonstrated that mepirizole-induced duodenal lesions are prevented by various analogues of PGs by increasing the HCO_3^- secretion (17, 30). We also reported that L- NAME increased duodenal HCO_3^- secretion and prevented the mucosal ulcerogenic response to mepirizole, without inhibiting acid secretion (25). In the present study, we did not examine the effects of TY-10957 and endothelin-1 on gastric acid secretory response to mepirizole. However, since these two agents inhibited the acid secretion induced by histamine, it is possible to speculate that they are effective in reducing mepirizole-induced acid secretion. Thus, the mechanism underlying duodenal protection by TY-10957 may be accounted for at least partly by stimulation of HCO_3^- secretion in the duodenum. The protective effect of endothelin- 1 in the duodenum is in contrast to the deleterious effect of this substance on various lesions in the stomach (12,15). However, we have obtained similar results by the NO synthase inhibitor L-NAME, the proulcerogenic effect in the stomach (6) and the protective effect in the duodenum (25).

In conclusion, the present study showed that endothelin-1, similar to an PGI_2 analogue TY-10957, caused an increase of duodenal HCO_3^- secretion in addition to a decrease of histamine-induced gastric acid secretion, and protected the duodenal mucosa against ulcerogenic stimulation by mepirizole. These data suggest that endothelium-derived substances including endothelium and NO as well as PGI_2 may play roles in modulation of the mucosal integrity of the duodenum, similarly to the stomach.

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