

Stimulation of Gastroduodenal HCO₃⁻ Secretion by Lubiprostone in Rats Mediated by Different EP Receptor Subtypes

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Abstract

We examined the stimulatory effects of lubiprostone, a bicyclic fatty acid derived from prostaglandin E₁ and a Chloride Channel type-2 opener (ClC-2), on HCO₃⁻ secretion in the rat stomach and duodenum, with a focus on the EP receptor subtypes involved in this action. Under urethane anesthesia, an ex-vivo chambered stomach or a duodenal loop was perfused with saline, and HCO₃⁻ secretion was measured at pH 7.0 using a pH stat-method. Lubiprostone (0.1-30 μM) was perfused in the chamber or loop for 10 min. Indomethacin, ONO-8711 (an EP1 antagonist), or AE5-599 (an EP3 antagonist) was given s.c. 1 h before the lubiprostone treatment, while AE3-208 (an EP4 antagonist) or CFTR_{inh}-172 (a CFTR inhibitor) was given i.p. 30 min before. Lubiprostone dose-dependently and significantly increased HCO₃⁻ secretion in both the stomach (≥10 μM) and duodenum (≥1 μM). The stimulatory effect in the stomach was significantly abrogated by a pretreatment with the EP1 antagonist, but not the EP3/EP4 antagonists or CFTR inhibitor, while that in the duodenum was significantly attenuated by the EP3/EP4 antagonists as well as the CFTR inhibitor. Indomethacin had no effect on the response of either tissue to lubiprostone. These results suggest that lubiprostone stimulated HCO₃⁻ secretion in the stomach

and duodenum in a manner that was mediated by different EP receptor subtypes; the former was mediated by EP1 receptors while the latter was mediated by both EP3 and EP4 receptors. CFTR/ClC-2 may be involved in the response observed in the duodenum, but not in the stomach.

Keywords: Lubiprostone; HCO₃⁻ secretion; Prostaglandin EP receptor subtypes; Stomach; Duodenum; Rat

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Introduction

Lubiprostone, a bicyclic fatty acid derived from ProstaGlandin (PG) E₁, has been used to treat chronic constipation and irritable bowel syndrome with constipation [1]. Its mechanism of action has been attributed to the stimulation of intestinal fluid secretion via the activation of ClC-2 chloride channels, which are located in the apical membranes of epithelial cells as a Cystic Fibrosis Transmembrane Regulator

(CFTR) bypass channel in Cystic Fibrosis [2, 3]. Previous studies demonstrated that lubiprostone activated PGE receptors [4-6]. These receptors have been pharmacologically subdivided into four subtypes, EP1-EP4 [7]. Among them, the EP4 receptor appears to be the main target for lubiprostone. Cuthbert [6] demonstrated that EP4 receptors in sheep were the major target for lubiprostone to stimulate the secretion of anions in ovine airways. In addition, several studies showed that lubiprostone activated ClC-2/CFTR chloride channels via EP4 receptors [3, 4, 8]. We previously reported that PGE_2 ameliorated indomethacin-induced small intestinal damage via the activation of EP4 receptors [9-11]. Consistent with these findings we recently confirmed that lubiprostone also prevented indomethacin-induced enteropathy via an EP4 receptor-dependent mechanism [12].

The secretion of HCO_3^- from surface epithelial cells is one of the main processes involved in mucosal defense and plays an important role in protecting the gastro duodenal mucosa against acid [13-15]. The physiological regulation of HCO_3^- secretion involves several factors such as PGs, nitric oxide, and neuronal factors [13, 16-20]. Mizumori et al. [5] reported that lubiprostone stimulated CFTR-dependent duodenal HCO_3^- secretion in the rat, and this action was mediated by the activation of EP4 receptors. PGE_2 has been shown to stimulate HCO_3^- secretion in the stomach and duodenum in a manner that is mediated by different EP receptors; EP1 receptors in the stomach and EP3/EP4 receptors in the duodenum [20-24]. However, it currently remains unknown whether lubiprostone stimulates HCO_3^- secretion in the stomach similar to PGE_2 and which EP receptors are responsible for its effects in the stomach.

In the present study, we examined the stimulatory effects of lubiprostone on HCO_3^- secretion in the rat stomach and duodenum with a focus on the EP receptor subtypes involved in these effects. Since lubiprostone is a ClC-2 chloride channel opener [2, 3], we also determined the contribution of ClC-2/CFTR channels to its HCO_3^- stimulatory effects in these tissues.

Materials and Methods

Animals

Male Sprague-Dawley rats (200-260 g; Nippon Charles River, Shizuoka, Japan) were acclimated to standard laboratory conditions (12:12 h light-dark cycle, temperature of $22\pm 1^\circ\text{C}$). Animals were kept in individual cages with raised mesh bottoms and deprived of food, but allowed free access to tap water for 18 h before the experiments. Experiments were carried out using four to six rats per group under urethane anesthesia (1.25 g/kg, i.p.). Body temperature was monitored intermittently using a rectal thermometer (Natsume, Tokyo, Japan) and maintained at $\sim 35^\circ\text{C}$ by placing the animals on a heat pad and exposing them to an external heat lamp (40 W) [15, 20]. All experimental procedures described were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Determination of HCO_3^- Secretion

The secretion of HCO_3^- was measured in a chambered stomach or duodenal loop as described previously [20, 25]. The abdomen was incised, and the stomach was exposed and mounted on a chamber (exposed area: 3.1 cm^2), while a duodenal loop (17 mm) was made between the pyloric ring and area just above the outlet of the common bile duct to exclude the influences of bile and pancreatic juice (Figure 1). The ex-vivo chambered stomach or duodenal loop was perfused at a rate of 0.2 ml/min with saline, which was gassed with 100% O_2 and kept in a reservoir. The secretion of HCO_3^- was measured at pH 7.0 using a pH-stat method (Hiranuma Comtite-8, Mito, Japan) and by the addition of 2 mM HCl to the reservoir. To unmask HCO_3^- in the stomach, the secretion of acid was completely inhibited by omeprazole, which was administered i.p. at a dose of 60 mg/kg [26]. Omeprazole at this dose has been shown to have no influence on gastric HCO_3^- secretion in rats [26]. After the basal secretion of HCO_3^- had been stabilized, the chamber or loop was perfused at a rate of 0.2 ml/min for 10 min with lubiprostone (0.1~30 μM) made isotonic with NaCl. In some cases, HCO_3^- secretion was

stimulated in both the stomach and duodenum by PGE_2 (1 mg/kg) given intravenously (i.v.). Indomethacin (a cyclooxygenase inhibitor: 5 mg/kg), ONO-8711 (an EP1 antagonist: 10 mg/kg), or AE5-599 (an EP3 antagonist: 10 mg/kg) was given subcutaneously (s.c.) 1 h before the application of lubiprostone or administration of PGE_2 , while

AE3-208 (an EP4 antagonist: 3 mg/kg) or CFTR_{inh}-172 (an inhibitor of CFTR: 1 mg/kg) was given intraperitoneally (i.p.) 30 min before. The doses of these EP and CFTR antagonists were selected in order to induce the respective pharmacological actions according to the findings of previous studies [12, 24, 27, 28].

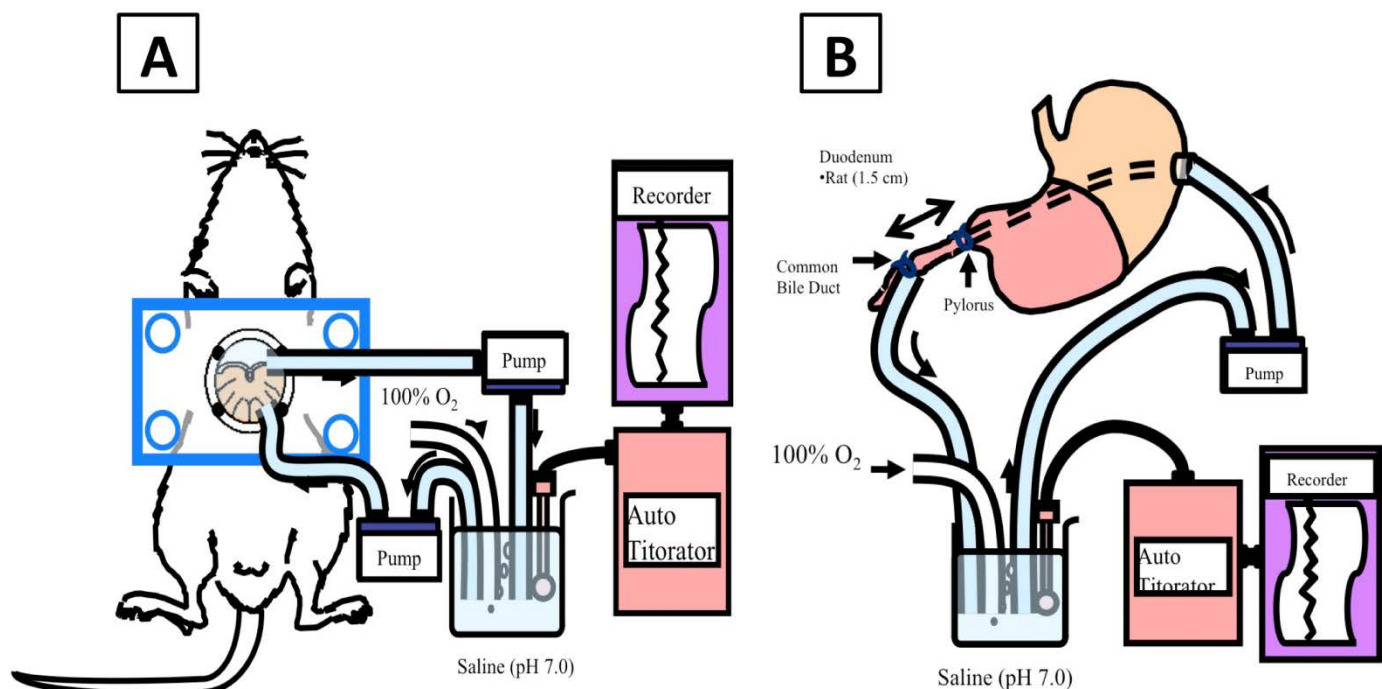


Figure 1: Schematic illustration of the perfusion system and order of connection of the loop to determine HCO_3^- secretion in the whole stomach (A) or proximal duodenum (B) of an anesthetized rat. The tissue was continuously perfused at a rate of 0.2 ml/min with saline, which was gassed with 100% O_2 , heated at 37°C , and kept in a reservoir. HCO_3^- secretion was measured at pH 7.0 using a pH-stat method.

Determination of Gene Expression of the CLC-2 Chloride Channel and EP1-4 Receptors

The gene expression of the CLC-2 chloride channel and EP1-4 receptors was measured in the gastric and duodenal mucosa by a Reverse Transcriptional Polymerase Chain

Reaction (RT-PCR). The stomach or duodenum was removed under deep ether anesthesia, and stored at -80°C prior to use. Total RNA was extracted from tissue samples using Sepasol RNA I (Nacalai Tesque, Kyoto, Japan). Total RNA was reverse-transcribed with a first strand cDNA synthesis kit (ReverTra Ace alpha, TOYOBIO, Osaka, Japan). The sequences of the sense and antisense primers for the rat CLC-2 chloride channel and EP1-4 receptors, and each product size, are shown in Table 1. An aliquot of the RT reaction product served as a template in 35 cycles of PCR with 0.5 min of denaturation at 95°C and 1 min of extension at 68°C using the Advantage 2 polymerase mixture (CLONTECH, Mountain View, CA) in a thermal cycler (PC-806, ASTEC, Fukuoka, Japan). A portion of the PCR mixture was electrophoresed in 1.5% agarose gel in Tris-acetic

acid-EDTA buffer (40 mM Tris, 20 mM acetic acid, and 2 mM EDTA; pH 8.1), and the gel was stained with ethidium bromide

and photographed (Bio Doc-It Imaging System; UVP, Upland, CA, USA). Images were analyzed with Image J (version 1.39).

Table 1: Sequences of Sense and Antisense Primers for ClC-2 and EP1-EP4 Receptors

Gene	Primer Sequence 5'-3'	PCR Product
ClC-2		499 bp
Forward	CAAGTTCCTCTCCCTCTTTG	
Reverse	GAAGTGTCCAAAGCCAGGG	
EP1		778 bp
Forward	CCCAGGGTCCCCAATACATCT	
Reverse	GGCAGCTGTGGTTGAAG	
EP2		1178 bp
Forward	CGCCCTCCACCATGGACAAT	
Reverse	AAGCAGCGCATGCTCACAAC	
EP3		666 bp
Forward	TGGCTGGCGCTCACCGACTTG	
Reverse	GCATTGCTCTACTGACATCTG	
EP4		488 bp
Forward	CCCTGCAGCGCCTCAGTGACTTT	
Reverse	CTTGCCCTCCGAGGCTGCTTTCAGT	
GAPDH		331 bp
Forward	GAACGGGAAGCTCACTGGCATGGC	
Reverse	TGAGGTCCACCACCCTGTTGCTG	

Preparation of Drugs

The drugs used were prostaglandin E₂, indomethacin (Sigma Chemicals, St. Louis, MO), lubiprostone (Abbott Japan Co., Ltd. Tokyo, Japan), ONO-8711 (an EP1 antagonist), AE5-599 (an EP3 antagonist), AE3-208 (an EP4 antagonist) (Ono Pharmaceutical Co., Ltd., Osaka, Japan), CFTR(inh)-172 (a CFTR inhibitor; Wako Pure Chemicals, Osaka, Japan), and urethane (Tokyo Kasei, Tokyo, Japan). Prostanoids, including lubiprostone, were dissolved in absolute ethanol and diluted with saline to the desired concentrations. Indomethacin was suspended in a 0.5% hydroxy propyl cellulose solution (Wako Pure Chemicals). Other drugs were dissolved in saline. All

drugs were prepared immediately before use, perfused intraluminally at a rate of 0.2 ml/min, and administered subcutaneously (s.c.) or intraperitoneally (i.p.) in a volume of 0.5 ml/100 g body weight or intravenously (i.v.) in a volume of 0.1 ml/100 g body weight. Control animals received the vehicle alone.

Statistical Analyses

Data are presented as means ± SE for four to eight rats per group. Statistical analyses were performed using a two-tailed unpaired t-test and Dunnett's multiple comparison test, and values of P<0.05 were considered significant.

Results

Effects of Lubiprostone on Gastric HCO_3^- Secretion

Under urethane anesthesia, the rat chambered stomach spontaneously secreted HCO_3^- at a steady rate of $0.1\sim 0.2 \mu\text{Eq}/10 \text{ min}$, and secretion remained unaltered after the perfusion of saline for 10 min at a rate of $0.2 \text{ ml}/\text{min}$. However, the perfusion of the chambered stomach with lubiprostone ($1\text{--}30 \mu\text{M}$) for 10 min increased the secretion of HCO_3^- in a

concentration-dependent manner, and the secretion of HCO_3^- was significantly greater at concentrations of $10 \mu\text{M}$ or higher than that in the saline-perfused stomach; ΔHCO_3^- outputs at 1 , 10 , and $30 \mu\text{M}$ were 1.11 ± 0.42 , 2.15 ± 0.36 , and $3.53\pm 0.37 \mu\text{Eq}/\text{h}$, respectively (Figure 2A and Figure 2B). The HCO_3^- response to lubiprostone in the stomach persisted for approximately 2 h. Based on these results, lubiprostone was perfused in the chambered stomach at $30 \mu\text{M}$ in subsequent experiments.

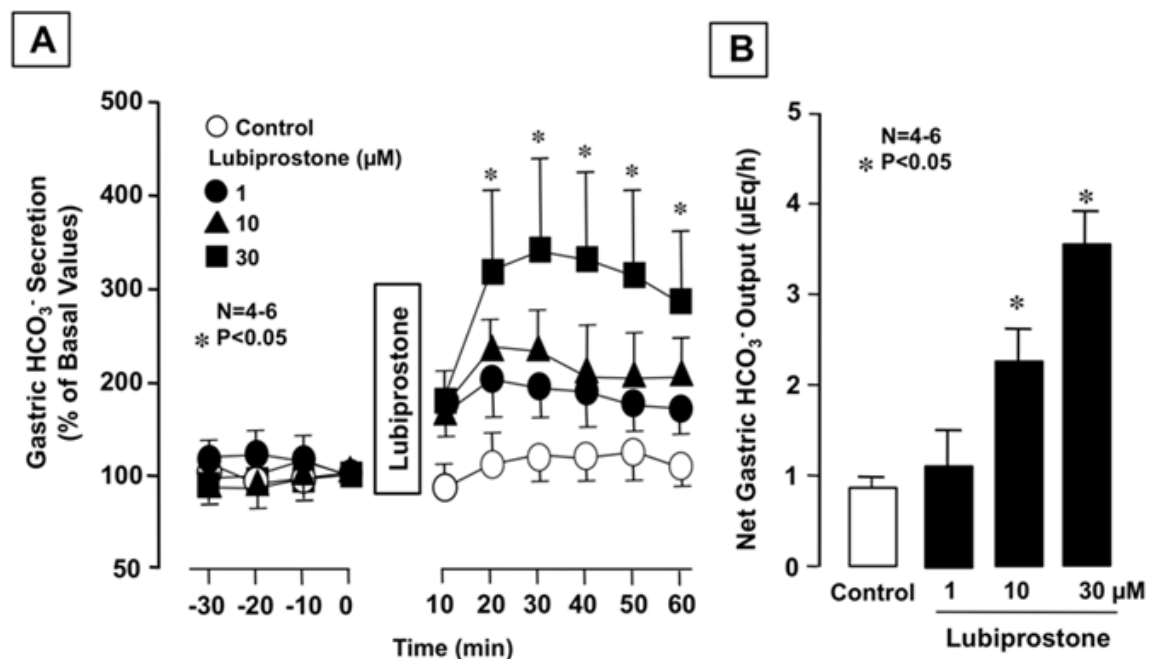


Figure 2: Effects of lubiprostone on gastric HCO_3^- secretion in anesthetized rats. The chambered stomach was perfused at a rate of $0.2 \text{ ml}/\text{min}$ for 10 min with lubiprostone ($1\text{--}30 \mu\text{M}$), and HCO_3^- secretion was measured before and after the perfusion of lubiprostone. Data are presented as the mean $\pm$ SE of the values determined every 10 min from 4-6 rats. * Significantly different from the control, at $P<0.05$. Figure B shows the net HCO_3^- output for 1 h after the perfusion of lubiprostone, and the data represent the mean $\pm$ SE from 4-6 rats. * Significantly different from the control, at $P<0.05$.

Effects of Lubiprostone on Duodenal HCO_3^- Secretion

The rat duodenum spontaneously secreted HCO_3^- at a steady rate of $0.3\sim 0.5 \mu\text{Eq}/10 \text{ min}$ under urethane anesthesia, and its secretion remained unaltered after the perfusion of saline for 10 min at a rate of $0.2 \text{ ml}/\text{min}$. However, lubiprostone ($0.1\text{--}10 \mu\text{M}$) perfused luminally in the duodenal loop for 10 min increased the secretion of HCO_3^- in a concentration-dependent manner, and its secretion was significantly greater at concentrations of $1 \mu\text{M}$ or higher than that in the saline-perfused duodenum; ΔHCO_3^- outputs at 0.1 , 1 , and $10 \mu\text{M}$ were

1.62 ± 0.42 , 5.35 ± 0.31 , and 4.78 ± 0.32 $\mu\text{Eq/h}$, respectively (Figure 3A and Figure 3B). Based on these results, lubiprostone

was perfused in the duodenal loop at 1 μM in subsequent experiments.

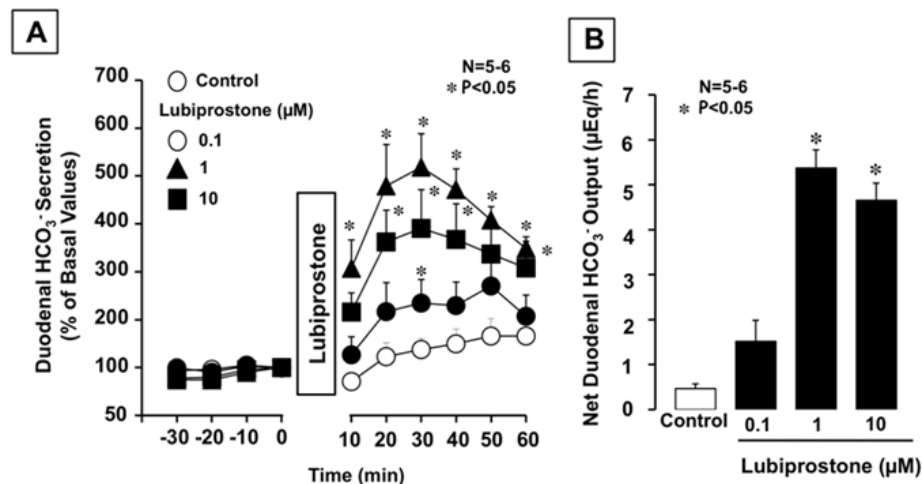


Figure 3: Effects of lubiprostone on duodenal HCO_3^- secretion in anesthetized rats. The duodenal loop was perfused at a rate of 0.2 ml/min for 10 min with lubiprostone (0.1-10 μM), and HCO_3^- secretion was measured before and after the perfusion of lubiprostone. Data are presented as the mean $\pm$ SE of the values determined every 10 min from 5-6 rats. * Significantly different from the control, at $P < 0.05$. Figure B shows the net HCO_3^- output for 1 h after the perfusion of lubiprostone, and the data represent the mean $\pm$ SE from 4-6 rats. * Significantly different from the control, at $P < 0.05$.

Effects of EP1, EP3, and EP4 Antagonists on Lubiprostone-Stimulated Gastric HCO_3^- Secretion

The luminal perfusion of lubiprostone (30 μM) in the chambered stomach for 10 min potently increased HCO_3^- secretion, with ΔHCO_3^- output being 3.73 ± 0.41 $\mu\text{Eq/h}$, which was significantly higher than that (0.96 ± 0.08 $\mu\text{Eq/h}$) in the control. The stimulatory effects of lubiprostone were significantly attenuated by the pretreatment of animals with ONO-8711 (10 mg/kg, s.c.), the EP1 antagonist, but not by either AE5-599 (10 mg/kg), the EP3 antagonist, or AE3-208 (3 mg/kg), the EP4 antagonist, with the degrees of inhibition being 70.3%, 16.2%, and -16.1%, respectively (Figure 4).

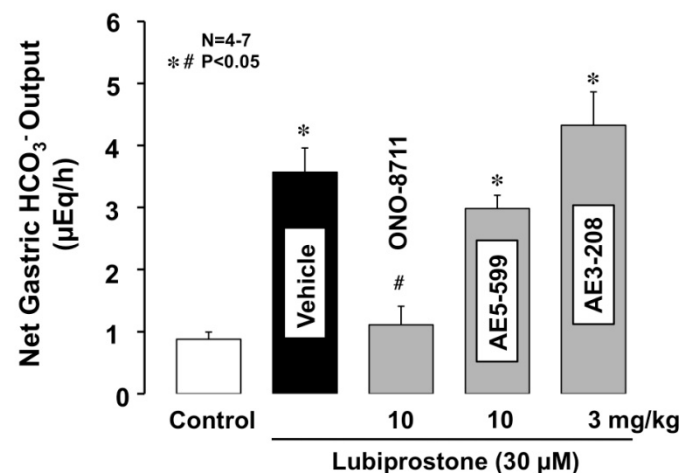


Figure 4: Effects of various subtype-selective EP antagonists on lubiprostone-stimulated gastric HCO_3^- secretion in anesthetized rats. Lubiprostone (30 μM) was perfused in the chambered stomach at a rate of 0.2 ml/min for 10 min. ONO-8711 (an EP1 antagonist: 10 mg/kg) or AE5-599 (an EP3 antagonist: 10 mg/kg) was given s.c. 1 h before the perfusion of lubiprostone, while AE3-208 (an EP4 antagonist: 3 mg/kg) was given i.p. 30 min before. Data are presented as the mean $\pm$ SE for 4-7 rats. Significant difference at $P < 0.05$; * from the control; # from the vehicle.

Effects of EP1, EP3, and EP4 Antagonists on Lubiprostone-Stimulated Duodenal HCO_3^- Secretion

The luminal perfusion of lubiprostone (1 μM) in the duodenal loop for 10 min significantly elevated HCO_3^- secretion over that in the control group treated with saline; ΔHCO_3^- output was 5.38 ± 0.41 $\mu\text{Eq/h}$. The HCO_3^- stimulatory effect of lubiprostone was significantly attenuated by the pretreatment of animals with AE5-599 (10 mg/kg, s.c.) and AE3-208 (3 mg/kg, i.p.), but not with ONO-8711 (10 mg/kg, s.c.), and the degrees of inhibition were 48.2%, 75.9% and 5.5%, respectively (Figure 5).

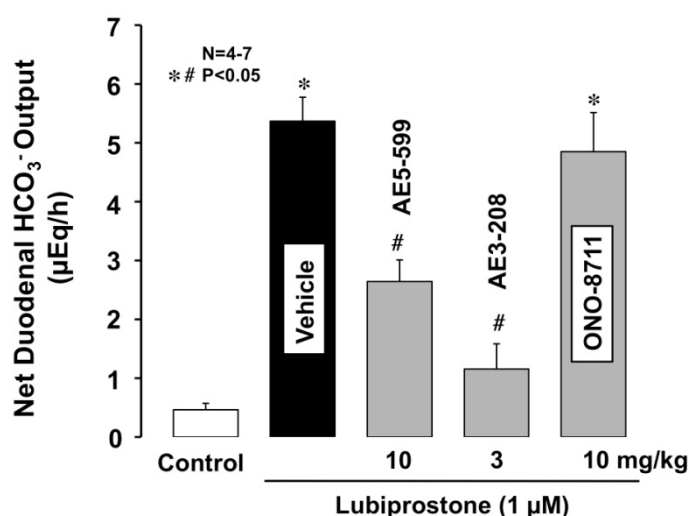


Figure 5: Effects of various subtype-selective EP antagonists on lubiprostone-stimulated duodenal HCO_3^- secretion in anesthetized rats. Lubiprostone (1 μM) was perfused in the duodenal loop at a rate of 0.2 ml/min for 10 min. ONO-8711 (an EP1 antagonist: 10 mg/kg) or AE5-599 (an EP3 antagonist: 10 mg/kg) was given s.c. 1 h before the perfusion of lubiprostone, while AE3-208 (an EP4 antagonist: 3 mg/kg) was given i.p. 30 min before. Data are presented as the mean $\pm$ SE for 4-7 rats. Significant difference at $P < 0.05$; * from the control; # from the vehicle.

Effects of Indomethacin and CFTR (inh)-172 on HCO_3^- Responses Induced by Lubiprostone in the Stomach and Duodenum

Since the HCO_3^- response to lubiprostone was significantly attenuated by EP antagonists, this effect may be mediated by endogenous PGs. Lubiprostone has also been shown to activate ClC-2 chloride channels [29, 30]. In order to

investigate the possible involvement of endogenous PGs and ClC-2 chloride channels in the HCO_3^- stimulatory action of lubiprostone, we examined the effects of indomethacin and CFTR (inh)-172 on HCO_3^- responses to lubiprostone in the stomach and duodenum.

When lubiprostone was perfused for 10 min into the chambered stomach or duodenal loop at 30 μM or 1 μM , respectively, the secretion of HCO_3^- was significantly increased; ΔHCO_3^- output was 3.68 ± 0.46 $\mu\text{Eq/h}$ in the stomach or 5.50 ± 0.32 $\mu\text{Eq/h}$ in the duodenum, respectively. As shown in Figure 6A, HCO_3^- responses in the stomach and duodenum were not significantly affected by the pretreatment of animals with indomethacin (5 mg/kg, s.c.); the responses observed were similar to those in control tissues. On the other hand, CFT(inh)-172 (1 mg/kg, i.p.), the inhibitor of CFTR, significantly attenuated the HCO_3^- response to lubiprostone (1 μM) in the duodenum, but not in the stomach, with inhibition being 41.6% in the former and 23.6% in the latter (Figure 6B).

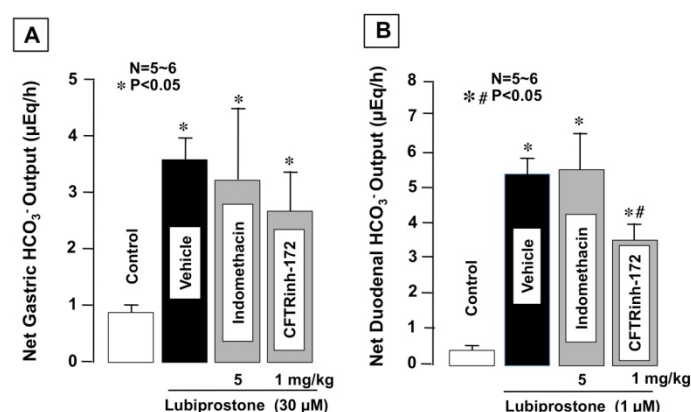


Figure 6: Effects of indomethacin and CFTRinh-172 on lubiprostone-stimulated HCO_3^- secretion in the stomach (A) and duodenum (B) of anesthetized rats. The chambered stomach or duodenal loop was perfused at a rate of 0.2 ml/min for 10 min with lubiprostone at 30 μM or 1 μM , respectively. Indomethacin (5 mg/kg) was given s.c. 1 h before the perfusion of lubiprostone while CFTRinh-172 (1 mg/kg), the CFTR inhibitor, was given i.p. 30 min before. Data are presented as the mean $\pm$ SE for 4-7 rats. Data are presented as the mean $\pm$ SE for 5-6 rats. Significant difference at $P < 0.05$; * from the control; # from the vehicle.

Effects of EP1, EP3, and EP4 Antagonists on PGE_2 -Induced Gastric and Duodenal HCO_3^- Secretion

To confirm the involvement of specific EP receptor subtypes in the HCO_3^- response to PGE_2 in the stomach and duodenum, we examined the effects of various EP antagonists on PGE_2 -induced HCO_3^- secretion in these tissues. The intravenous administration of PGE_2 (1 mg/kg) significantly increased the

secretion of HCO_3^- in the stomach and duodenum; ΔHCO_3^- output was $3.48 \pm 0.96 \mu\text{Eq/h}$ and $5.43 \pm 0.62 \mu\text{Eq/h}$, respectively (Figure 7). The response in the stomach was significantly inhibited by ONO-8711 (10 mg/kg, s.c.), but not by AE5-599 (10 mg/kg, s.c.) or AE3-208 (5 mg/kg, i.p.), while the response in the duodenum was significantly attenuated by both AE5-599 and AE3-208, but not by ONO-8711.

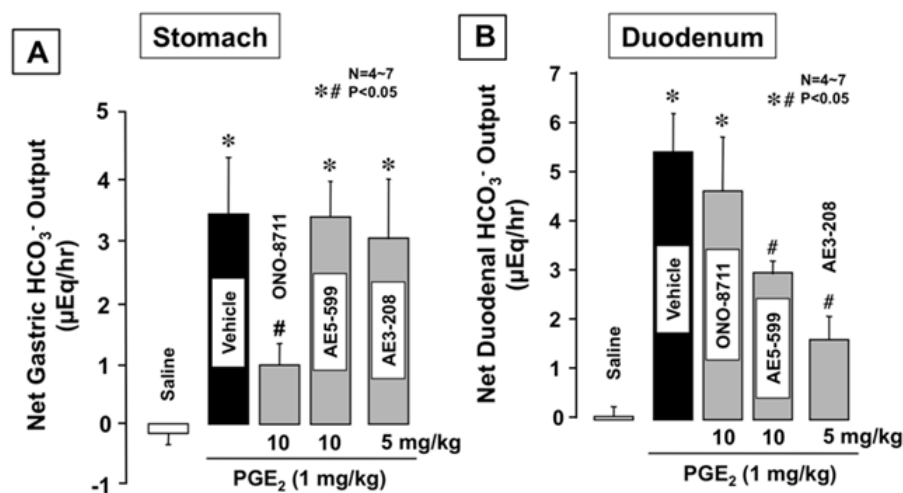


Figure 7: Effects of various subtype-selective EP antagonists on PGE_2 -stimulated HCO_3^- secretion in the stomach (A) and duodenum (B) of anesthetized rats. PGE_2 was administered i.v. at 1 mg/kg. ONO-8711 (an EP1 antagonist: 10 mg/kg) or AE5-599 (an EP3 antagonist: 10 mg/kg) was given s.c. 1 h before the administration of PGE_2 , while AE3-208 (an EP4 antagonist: 3 mg/kg) was given i.p. 30 min before. Data are presented as the mean $\pm$ SE for 4-7 rats. Significant difference at $P < 0.05$; * from the control; # from the vehicle.

Gene Expression of the ClC-2 Chloride Channels and EP1-EP4 Receptors in Rat Gastric and Duodenal Mucosa

Since EP1 and EP3/EP4 receptors were found to be involved in the HCO_3^- stimulatory action of lubiprostone in the stomach and duodenum, respectively, we examined the gene expression of various EP receptor subtypes (EP1-4) in addition to the ClC-2 chloride channel. As shown in Figure 8, EP1-4 receptors were expressed in both the gastric and duodenal mucosa, although

differences were observed in the intensities of their expression. The gene expression of the ClC-2 chloride channel was also clearly detected in both tissues.

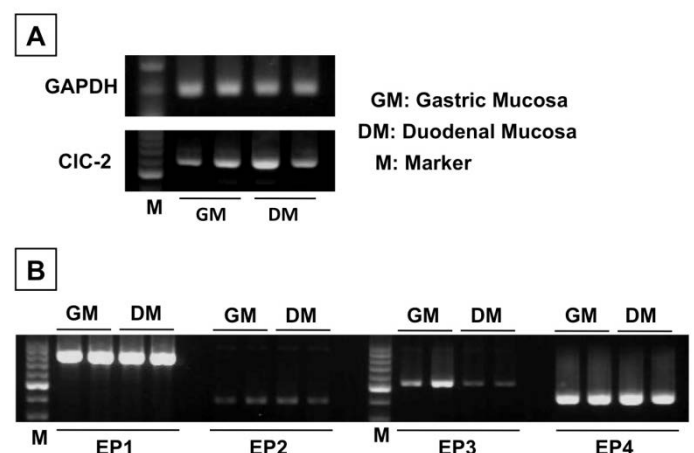


Figure 8: Gene expression of ClC-2 and EP receptor subtypes (EP1-EP4) in the rat stomach and duodenum.

Discussion

Lubiprostone has been used to treat chronic constipation [1], and its mechanism of action has been attributed to the stimulation of intestinal fluid secretion via the activation of ClC-2 chloride channels [2, 3]. This drug is a bicyclic fatty acid derived from PGE_1 and has been shown to activate PGE receptors [4-6]. We recently reported that lubiprostone prevented indomethacin-induced small intestinal damage via the activation of EP4 receptors, similar to PGE_2 [12, 13], suggesting the prophylactic use of this drug against NSAID-induced enteropathy. In the present study, we demonstrated for the first time that lubiprostone stimulated HCO_3^- secretion in both the stomach and duodenum via the different EP receptor subtypes; its effect in the stomach was mediated by EP1 receptors, while that in the duodenum was mediated by both EP3 and EP4 receptors.

The secretion of HCO_3^- from the surface epithelium is one of the mucosal defensive mechanisms and plays an important role in protecting the gastro duodenal mucosa. Various analogues of PGs or agents that enhance the biosynthesis of endogenous PGs stimulate HCO_3^- secretion, while nonsteroidal anti-inflammatory agents decrease the secretion of HCO_3^- by inhibiting PG generation [13-15, 24]. We previously reported that PGE_2 affected HCO_3^- secretion via distinctive mechanisms in the stomach and duodenum concerning the EP receptor subtypes involved in this process; its effect in the stomach was mediated by EP1 receptors coupled with elevations in intracellular Ca^{2+} , while that in the duodenum was associated with the intracellular accumulation of both Ca^{2+} and 3', 5'-cyclic Adenosine Mono Phosphate (cAMP) caused by the activation of EP3/EP4 receptors [20-24].

Since lubiprostone is derived from PGE_1 and induces its pharmacological effects through EP receptors [2, 3], this drug may increase the secretion of HCO_3^- in the stomach and duodenum via EP receptors. Mizumori et al. [5] was the first to report that lubiprostone stimulated duodenal HCO_3^- secretion via the activation of EP4 receptors in rats, and

suggested the possibility of its protection of the duodenum from acid-induced injury. We confirmed the HCO_3^- stimulatory effect of lubiprostone in the duodenum via EP4 receptors and further showed that this effect was mediated by the activation of not only EP4 receptors, but also EP3 receptors. As expected, we also found that this drug increased the secretion of HCO_3^- in the stomach, and this effect was significantly attenuated by the pretreatment with ONO-8711, the EP1 antagonist, but not by the EP3 or EP4 antagonist, suggesting that its action in the stomach was mediated by the activation of EP1 receptors. Lubiprostone is unlikely to have stimulated HCO_3^- secretion by increasing endogenous PG levels because this effect was observed even under PG-deficient conditions caused by indomethacin. This was also supported by previous findings in which lubiprostone prevented the intestinal ulcerogenic response caused by indomethacin via EP4 receptors [11, 12]. In a preliminary study, we also observed the effects of lubiprostone in an isolated mouse stomach in vitro, which suggested its direct action on epithelial cells without involving intrinsic and extrinsic nerves [31].

Several studies demonstrated that lubiprostone activated ClC-2/CFTR chloride channels via EP4 receptors [4-6]. Lubiprostone has been shown to stimulate CFTR-dependent duodenal HCO_3^- secretion without changing net Cl^- secretion, which suggested that lubiprostone acts as a dual activator of CFTR-independent Cl^- secretion and as a PG receptor agonist [5]. In the present study, we confirmed the gene expression of ClC-2 chloride channels as well as EP1-EP4 receptors in both the rat stomach and duodenum, with some differences in the intensities of their expression. Although the cell types that express each EP receptor subtype and ClC-2 chloride channels have not yet been identified, we assumed that ClC-2/CFTR channels are expressed in epithelial cells, even in the stomach. However, we noted that the prior administration of CFTR (inh)-172, an inhibitor of CFTR, significantly attenuated the HCO_3^- stimulatory effect of lubiprostone in the duodenum, but not in the stomach. Since the activation of EP4 receptors increases intracellular cAMP [32], and elevations in cAMP, in turn,

activate CFTR [33], CFTR-dependent HCO_3^- secretion by lubiprostone appeared to be consistent with the activation of EP4 receptors by lubiprostone. Norimatsu et al. [28] also confirmed that CFTR was activated by lubiprostone via the EP4 receptor in oocytes, even though the drug had no direct effect on either ClC-2 or CFTR channels expressed in oocytes. It has not yet been determined why the effect of lubiprostone in the stomach was unaffected by the CFTR inhibitor; however, these results suggest that the direct activation of CFTR/ClC-2 chloride channels does not contribute to the HCO_3^- stimulatory action of lubiprostone in the stomach.

Another interesting result in this study was that the effective dose of lubiprostone markedly differed between the stomach and duodenum; the stimulation of HCO_3^- secretion was observed at $\geq 10 \mu\text{M}$ in the stomach and at $\geq 1 \mu\text{M}$ in the duodenum. Consistent with our previous findings [20-24], PGE_2 stimulated HCO_3^- secretion in both the stomach and duodenum at the same dose level (1 mg/kg, i.v.); however, these effects were mediated via different EP receptors in these tissues, similar to those of lubiprostone. Although this difference remains unexplained, it may have been due to different affinities to the EP receptor subtypes and/or ClC-2/CFTR- dependency; higher affinity to both EP3/EP4 receptors than EP1 receptors and ClC-2/CFTR- dependency in the duodenum, but not in the stomach.

The present results suggest that lubiprostone, a bicyclic fatty acid derived from PGE_1 , stimulated HCO_3^- secretion in the stomach and duodenum, similar to PGE_2 , and these effects were mediated by different EP receptor subtypes in these tissues; the effect observed in the stomach was mediated by EP1 receptors while that in the duodenum was mediated by both EP3 and EP4 receptors. In addition, CFTR was involved in modulating HCO_3^- secretion in the duodenum, but not in the stomach. Considering the findings in the present study, it is assumed that beyond treatment of constipation, irritable bowel syndrome and enteropathy, lubiprostone may have potential to be used more for protection against gastritis and peptic ulcer diseases, since it does stimulate the secretion of HCO_3^- in both the stomach and

duodenum. Furthermore, because duodenal HCO_3^- secretion was shown to be impaired in patients with *Helicobacter pylori* [34, 35], it is also possible that lubiprostone may be useful for treatment of *Helicobacter pylori*-related diseases.

Conclusion

Lubiprostone stimulated gastro duodenal HCO_3^- secretion and these stimulatory effects differed in the two tissues examined; the effect observed in the stomach was mediated by EP1 receptors and independent of CFTR channels while that in the duodenum was mediated by both EP3 and EP4 receptors and dependent on CFTR channels. Lubiprostone appeared to protect the stomach and duodenum against acid injury by stimulating the secretion of HCO_3^- .

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