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Stimulation of gastroduodenal HCO_3- secretion by lubiprostone mediated by different prostaglandin EP receptor subtypes

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Abstract. We examined the stimulatory effects of lubiprostone, a bicyclic fatty acid derived from prostaglandin E_1 and a chloride channel type-2 opener (ClC-2), on HCO_3- 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 HCO_3- 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 EP_1 antagonist), or AE5-599 (an EP_3 antagonist) was given s.c. 1 h before the lubiprostone treatment, while AE3-208 (an EP_4 antagonist) or $CFTR_{inh}$-172 (a cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor) was given i.p. 30 min before. Lubiprostone dose-dependently and significantly increased HCO_3- 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 EP_1 antagonist, but not the EP_3/EP_4 antagonists or CFTR inhibitor, while that in the duodenum was significantly attenuated by the EP_3/EP_4 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 HCO_3- secretion in the stomach and duodenum in a manner that was mediated by different EP receptor subtypes; the former was mediated by EP_1 receptors, while the latter was mediated by both EP_3 and EP_4 receptors. CFTR/ClC-2 may be involved in the response observed in the duodenum, but not in the stomach.

Keywords: lubiprostone, HCO_3- secretion, prostaglandin EP receptor subtypes, stomach, duodenum, rat.

Introduction

Lubiprostone, a bicyclic fatty acid derived from prostaglandin (PG) E_1, has been used to treat chronic constipation and irritable bowel syndrome with constipation (Schey, Rao 2011). 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 (Schwiebert et al. 1998; Cuppoletti et al. 2004). Previous studies demonstrated that lubiprostone activated PGE receptors (Bassil et al. 2008; Mizumori et al. 2009; Cuthbert 2011). These receptors have been pharmacologically subdivided into four subtypes, EP_1-EP_4 (Woodward et al. 2011). Among them, the EP_4 receptor appears to be the main target for lubiprostone. Cuthbert demonstrated that EP_4 receptors in sheep were the major target for lubiprostone to stimulate the secretion of anions in ovine airways (Cuthbert 2011). In addition, several studies showed that lubiprostone activated ClC-2/CFTR chloride channels via EP_4 receptors (Cuppoletti et al. 2004; Bassil et al. 2008; Bao et al. 2008). We previously reported that PGE_2 ameliorated indomethacin-induced small intestinal damage via the activation of EP_4 receptors (Kunikata et al. 2002; Hatazawa et al. 2006; Takeuchi 2014). In consistence with these findings, we recently

confirmed that lubiprostone also prevented indomethacin-induced enteropathy via an EP_4 receptor-dependent mechanism (Hayashi et al. 2014).

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 gastroduodenal mucosa against acid (Flemström, Garner 1982; Flemström, Turnberg 1984; Takeuchi et al. 1986). The physiological regulation of HCO_3^- secretion involves several factors such as PGs, nitric oxide, and neuronal factors (Flemström, Garner 1982; Heylings et al. 1984; Takeuchi et al. 1991; Hogan et al. 1993; Sugamoto et al. 2001; Takeuchi et al. 1997). Mizumori et al. (2009) reported that lubiprostone stimulated CFTR-dependent duodenal HCO_3^- secretion in rats, and this action was mediated by the activation of EP_4 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; EP_1 receptors in the stomach and EP_3/EP_4 receptors in the duodenum (Takeuchi et al. 1997; Takeuchi et al. 1999; Aoi et al. 2004; Aihara et al. 2007). 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 (Schwiebert et al. 1998; Cuppoletti et al. 2004), we also determined the contribution of ClC-2/CFTR channels to its HCO_3^- stimulatory effects in these tissues.

Materials and methods

1. 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 ± 1° 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 ~35 °C by placing the animals on a heat pad and exposing them to an external heat lamp (40 W) (Takeuchi et al. 1986; Takeuchi et al. 1997). All experimental procedures described were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

2. Determination of HCO_3^- secretion

The secretion of HCO_3^- was measured in a chambered stomach or duodenal loop as described previously (Takeuchi et al. 1997; Takeuchi et al. 1990). 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 (fig. 1).

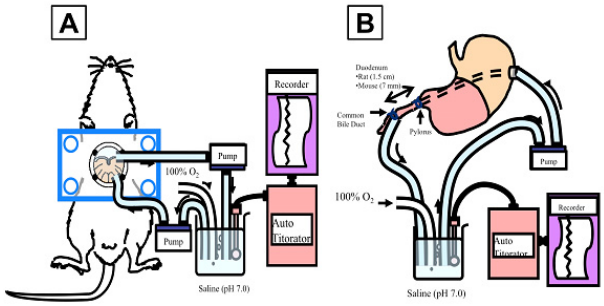

Fig. 1. Schematic illustration of the perfusion system and order of connection of the loop to determine HCO_3^- secretion in the whole stomach **(A)** or proximal duodenum **(B)** of an anesthetized rat. The tissue was continuously perfused at a rate of 0.2 ml/min with saline, which was gassed with 100 % 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

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. Omeprazole at this dose has been shown to have no influence on gastric HCO_3^- secretion in rats (Flemstrom, Mattsson 1986). 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 EP_1 antagonist: 10 mg/kg), or AE5-599 (an EP_3 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 EP_4 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 (Hayashi et al. 2014; Takeuchi et al. 2011; Takeuchi et al. 2002; Norimatsu et al. 2012).

3. Determination of gene expression of the ClC-2 chloride channel and EP_1-EP_4 receptors

The gene expression of the ClC-2 chloride channel and EP_1-EP_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, TOYOBO, Osaka, Japan). The sequences of the sense and antisense primers for the rat ClC-2 chloride channel and EP_1-EP_4 receptors, and each product size, are shown in Table 1. An aliquot

Table 1. Sequences of Sense and Antisense Primers for ClC-2 and EP_1-EP_4 Receptors

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

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).

4. Preparation of drugs

The drugs used were prostaglandin E_2, indomethacin (Sigma Chemicals, St. Louis, MO), lubiprostone (Abbott Japan Co., Ltd. Tokyo, Japan), ONO-8711 (an EP_1 antagonist), AE5-599 (an EP_3 antagonist), AE3-208 (an EP_4 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 % hydroxypropylcellulose 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.

5. 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

1. Effects of lubiprostone on gastric HCO_3^- secretion

Under urethane anesthesia, the rat chambered stomach spontaneously secreted HCO_3^- at a steady rate of 0.1~0.2 μEq/10 min, and secretion remained unaltered after the perfusion of saline for 10 min at a rate of 0.2 ml/min. The perfusion of the chambered stomach with lubiprostone (1–30 μM) for 10 min increased the secretion of HCO_3^- in a concentration-dependent manner. The rate of HCO_3^- secretion was significantly increased by lubiprostone perfusion at 30 μM compared to saline (fig. 2A), while the net HCO_3^- output was significantly greater at 10 μM and 30 μM than that in the saline-perfused stomach; ΔHCO_3^- outputs at 1, 10, and 30 μM were 1.11 ± 0.42, 2.15 ± 0.36, and 3.53 ± 0.37 μEq/h, respectively (fig. 2B). The HCO_3^- response to lubiprostone in the stomach persisted for ap-

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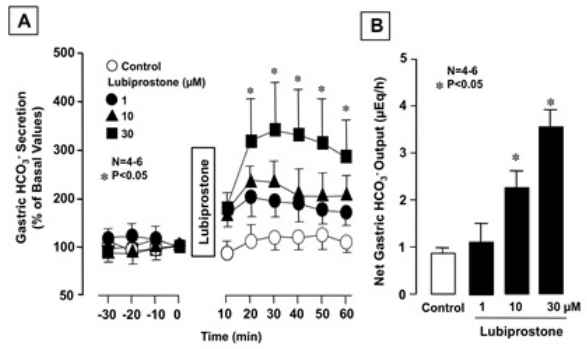

Fig. 2. Effects of lubiprostone on gastric HCO_3^- secretion in anesthetized rats. The chambered stomach was perfused at a rate of 0.2 ml/min for 10 min with lubiprostone (1–30 μM), and HCO_3^- secretion was measured before and after the perfusion of lubiprostone. Figure A: Data are presented as the mean ± 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 ± SE from 4–6 rats. *Significantly different from the control, at $P < 0.05$

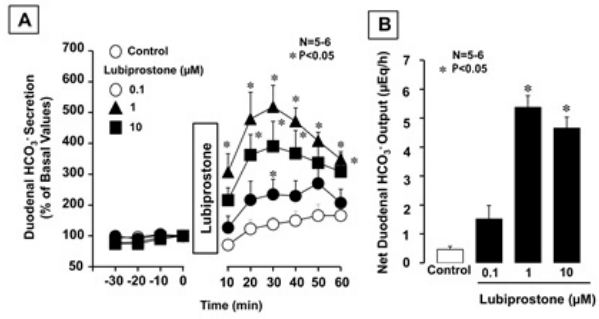

Fig. 3. Effects of lubiprostone on duodenal HCO_3^- secretion in anesthetized rats. The duodenal loop was perfused at a rate of 0.2 ml/min for 10 min with lubiprostone (0.1–10 μM), and HCO_3^- secretion was measured before and after the perfusion of lubiprostone. Figure A: Data are presented as the mean ± 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 ± SE from 4–6 rats. *Significantly different from the control, at $P < 0.05$

proximately 2 h. Based on these results, lubiprostone was perfused in the chambered stomach at 30 μM in subsequent experiments.

2. Effects of lubiprostone on duodenal HCO_3^- secretion

The rat duodenum spontaneously secreted HCO_3^- at a steady rate of 0.3~0.5 μEq/10 min under urethane anesthesia, and its secretion remained unaltered after the perfusion of saline for 10 min at a rate of 0.2 ml/min. However, lubiprostone (0.1–10 μM) 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 μM and 10 μM than that in the saline-perfused duodenum; ΔHCO_3^- outputs at 0.1, 1, and 10 μM were 1.62 ± 0.42, 5.35 ± 0.31, and 4.78 ± 0.32 μEq/h, respectively (figs. 3A and 3B). Based on these results, lubiprostone was perfused in the duodenal loop at 1 μM in subsequent experiments.

3. Effects of EP_1, EP_3, and EP_4 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 at 3.73 ± 0.41 μEq/h, which was significantly higher than that (0.96 ± 0.08 μEq/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 EP_1 antagonist, but not by either AE5-599 (10 mg/kg), the EP_3 antagonist, or AE3-208 (3 mg/kg), the EP_4 antagonist, with the degrees of inhibition being 70.3 %, 16.2 %, and -16.1 %, respectively (fig. 4).

4. Effects of EP_1, EP_3, and EP_4 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 μEq/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 (fig. 5).

5. 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 (De Lisle et al. 2010; Schiffhauer

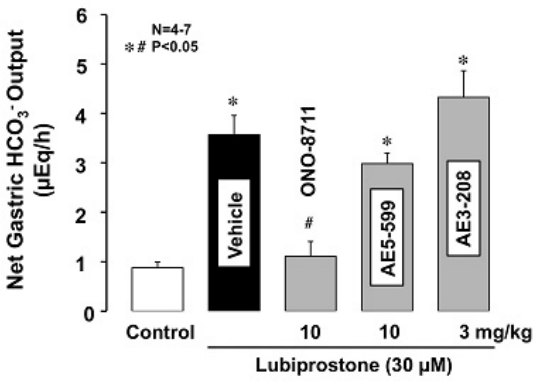

Fig. 4. Effects of various subtype-selective EP antagonists on lubiprostone-stimulated gastric HCO_3^- secretion in anesthetized rats. Lubiprostone (30 μM) was perfused in the chambered stomach at a rate of 0.2 ml/min for 10 min. ONO-8711 (an EP_1 antagonist: 10 mg/kg) or AE5-599 (an EP_3 antagonist: 10 mg/kg) was given s.c. 1 h before the perfusion of lubiprostone, while AE3-208 (an EP_4 antagonist: 3 mg/kg) was given i.p. 30 min before. Data are presented as the mean ± SE for 4–7 rats. Significant difference at P < 0.05; *from the control; # from the vehicle

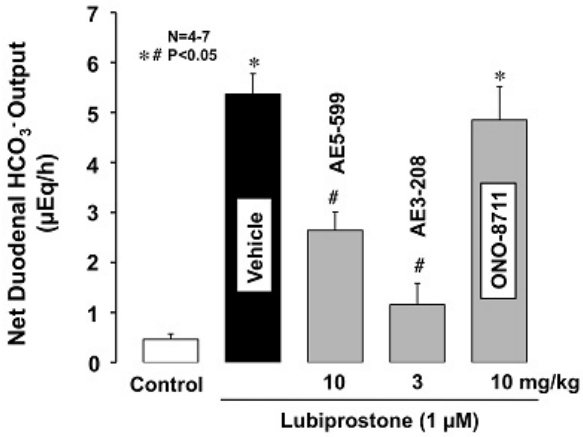

Fig. 5. Effects of various subtype-selective EP antagonists on lubiprostone-stimulated duodenal HCO_3^- secretion in anesthetized rats. Lubiprostone (1 μM) was perfused in the duodenal loop at a rate of 0.2 ml/min for 10 min. ONO-8711 (an EP_1 antagonist: 10 mg/kg) or AE5-599 (an EP_3 antagonist: 10 mg/kg) was given s.c. 1 h before the perfusion of lubiprostone, while AE3-208 (an EP_4 antagonist: 3 mg/kg) was given i.p. 30 min before. Data are presented as the mean ± SE for 4–7 rats. Significant difference at P < 0.05; *from the control; # from the vehicle

et al. 2013). 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 μEq/h in the stomach or 5.50 ± 0.32 μEq/h in the duodenum, respectively. As shown in Fig. 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 at 41.6 % in the former and 23.6 % in the latter (fig. 6B).

6. Effects of EP_1, EP_3, and EP_4 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 at 3.48 ± 0.96 μEq/h

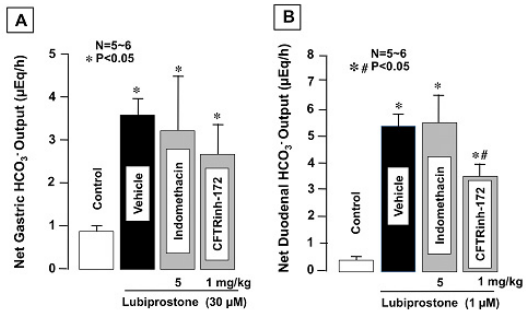

Fig. 6. Effects of indomethacin and $CFTR_{inh}$-172 on lubiprostone-stimulated HCO_3^- secretion in the stomach **(A)** and duodenum **(B)** of anesthetized rats. The chambered stomach or duodenal loop was perfused at a rate of 0.2 ml/min for 10 min with lubiprostone at 30 μm or 1 μM, respectively.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

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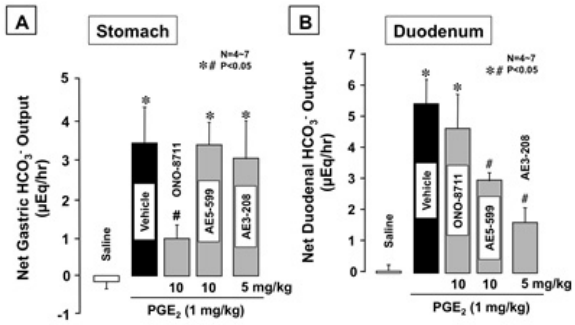

Fig. 7. Effects of various subtype-selective EP antagonists on PGE_2-stimulated HCO_3^- secretion in the stomach **(A)** and duodenum **(B)** of anesthetized rats. PGE_2 was administered i.v. at 1 mg/kg. ONO-8711 (an EP_1 antagonist: 10 mg/kg) or AE5-599 (an EP_3 antagonist: 10 mg/kg) was given s.c. 1 h before the administration of PGE_2, while AE3-208 (an EP_4 antagonist: 3 mg/kg) was given i.p. 30 min before. Data are presented as the mean ± SE for 4–7 rats. Significant difference at $P < 0.05$; *from the control; # from the vehicle

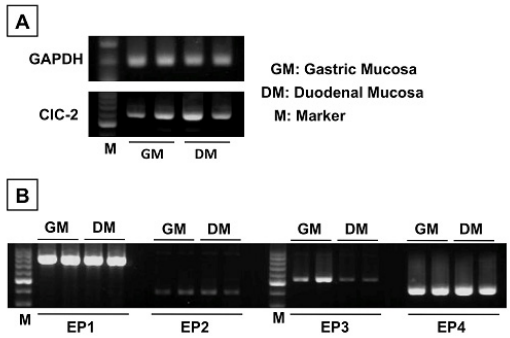

Fig. 8. Gene expression of EP receptor subtypes (EP_1-EP_4) **(A)** and ClC-2 **(B)** in the rat stomach and duodenum

and 5.43 ± 0.62 μEq/h, respectively (fig. 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.

7. Gene expression of the ClC-2 chloride channels and EP_1-EP_4 receptors in rat gastric and duodenal mucosa

Since EP_1 and EP_3/EP_4 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 (EP_1-EP_4) in addition to the ClC-2 chloride channel. As shown in fig. 8A, EP_1-EP_4 receptors were expressed in both the gastric and duodenal mucosa, although differences were observed in the intensity of their expression. The gene expression of the ClC-2 chloride channel was also clearly detected in both tissues (fig. 8B).

Discussion

Lubiprostone has been used to treat chronic constipation (Schey, Rao 2011), and its mechanism of action has been attributed to the stimulation of intestinal fluid secretion via the activation of ClC-2 chloride channels (Schwiebert et al. 1998; Cuppoletti et al. 2004). This drug is a bicyclic fatty acid derived from PGE_1 and has been shown to activate PGE receptors (Bassil et al. 2008; Mizumori et al. 2009; Cuthbert 2011). We recently reported that lubiprostone prevented indomethacin-induced small intestinal damage via the activation of EP_4 receptors, similar to PGE_2 (Hayashi et al. 2014; Flemström, Garner 1982), 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 different EP receptor subtypes; its effect in the stomach was mediated by EP_1 receptors, while that in the duodenum was mediated by both EP_3 and EP_4 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 gastroduodenal 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 (Flemström, Garner 1982; Flemström, Turnberg 1984; Takeuchi 1986; Takeuchi et al. 2011). 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 EP_1 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 monophosphate (cAMP) caused by the activation of EP_3/EP_4 receptors (Takeuchi et al. 1997; Takeuchi et al. 1999; Aoi et al. 2004; Aihara et al. 2007; Takeuchi et al. 2011).

Since lubiprostone is derived from PGE_1 and induces its pharmacological effects through EP receptors (Schwiebert et al. 1998; Cuppoletti et al. 2004),

this drug may increase the secretion of HCO_3^- in the stomach and duodenum via EP receptors. Mizumori et al. (2009) was the first to report that lubiprostone stimulated duodenal HCO_3^- secretion via the activation of EP_4 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 EP_4 receptors and further showed that this effect was mediated by the activation of not only EP_4 receptors, but also EP_3 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 EP_1 antagonist, but not by the EP_3 or EP_4 antagonist, suggesting that its action in the stomach was mediated by the activation of EP_1 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 EP_4 receptors (Takeuchi 2014; Hayashi et al. 2014). 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 (Takeuchi 2014).

Several studies demonstrated that lubiprostone activated ClC-2/CFTR chloride channels via EP_4 receptors (Bassil et al. 2008; Mizumori et al. 2009; Cuthbert 2011). 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 (Mizumori et al. 2009). In the present study, we confirmed the gene expression of ClC-2 chloride channels as well as EP_1-EP_4 receptors in both rat stomach and duodenum, with some differences in the intensity 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 EP_4 receptors increases intracellular camp (Regan 2003), and elevations in cAMP, in turn, activate CFTR (Li, Naren 2005), CFTR-dependent HCO_3^- secretion by lubiprostone appeared to be consistent with the activation of EP_4 receptors by lubiprostone. Norimatsu et al. (2012) also confirmed that CFTR was activated by lubiprostone via the EP_4 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 finding was that the effective dose of lubiprostone markedly differed between the stomach and duodenum; the stimulation of HCO_3^- secretion was observed at ≥ 10 μM in the stomach and at ≥ 1 μM in the duodenum. Consistent with our previous findings (Takeuchi et al. 1997; Takeuchi et al. 1999; Aoi et al. 2004; Aihara et al. 2007; Takeuchi et al. 2011), 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 EP_3/EP_4 receptors than EP_1 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 EP_1 receptors, while that in the duodenum was mediated by both EP_3 and EP_4 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* (Tuo et al. 2004; Tuo et al. 2009), it is also possible that lubiprostone may be useful for treatment of *Helicobater pylori*-related diseases.

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Conclusion

Lubiprostone stimulated gastroduodenal HCO_3^- secretion, and these stimulatory effects differed in the two tissues examined; the effect observed in the stomach was mediated by EP_1 receptors and independent of CFTR channels, while that in the duodenum was mediated by both EP_3 and EP_4 receptors and dependent on CFTR channels. Lubiprostone appeared to protect the stomach and duodenum against acid injury by stimulating the secretion of HCO_3^-.

Conflict of interest statement

No potential conflict of interest relevant to this article was reported.

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