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PROSTAGLANDIN E RECEPTOR SUBTYPES INVOLVED IN STIMULATION OF GASTRODUODENAL BICARBONATE SECRETION IN RATS AND MICE

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We investigated prostaglandin E (EP) receptor subtypes responsible for the HCO$_3^-$ stimulatory action of prostaglandin E$_2$ (PGE$_2$) in the gastroduodenal mucosa, by examining the effects of various prostanoids with subtype specific EP receptor agonists in rats and those of PGE$_2$ in knockout mice lacking EP$_1$ or EP$_3$ receptors. In rats, gastric HCO$_3^-$ secretion was stimulated by i.v. administration of PGE$_2$, 17-phenyl PGE$_2$ the selective EP$_1$ agonist as well as sulprostone the EP$_1$ and EP$_3$ agonist, but was not affected by other EP agonists such as butaprost the selective EP$_2$ agonist, ONO-NT-012 the selective EP$_3$ agonist or 11-deoxy PGE$_1$ the EP$_1$ and EP$_3$ agonist. In contrast, the HCO$_3^-$ secretion in rat duodenum was stimulated by PGE$_2$, sulprostone, ONO-NT-012 as well as 11-deoxy PGE$_1$ but not affected by either 17-phenyl PGE$_2$ or butaprost. The HCO$_3^-$ stimulatory effect of sulprostone in the stomach was significantly inhibited by ONO-AE-829, the selective EP$_1$ antagonist. On the other hand, PGE$_2$ applied topically to the mucosa for 10 min caused a dose-dependent increase of HCO$_3^-$ secretion in both the stomach and duodenum of wild-type mice. The HCO$_3^-$ stimulatory action of PGE$_2$ in the stomach was also observed dose-dependently in knockout mice lacking EP$_3$-receptors but was absent in EP$_1$-receptor knockout mice, while the stimulatory effect in the duodenum was observed in EP$_1$-receptor knockout mice, similar to wild-type animals, but not in knockout mice lacking EP$_3$-receptors. These results indicate that PGE$_2$ stimulates HCO$_3^-$ secretion via different EP receptor subtypes in the stomach and duodenum; the former is mediated by EP$_1$-receptors, while the latter mediated by EP$_3$-receptors.

Key words: EP receptor subtype, PGE$_2$, gastroduodenal HCO$_3^-$ secretion rat, Ep receptor knockout mice.
INTRODUCTION

The secretion of $\text{HCO}_3^-$ in the surface epithelial cell is one of the main defense mechanisms of the gastroduodenal mucosa against acid (1). Mucus adherent to the luminal surface of the mucosa provides a zone of low turbulence (unstirred layer), allowing the development of a gradient for $\text{HCO}_3^-$ from the luminal side (2). Small amounts of $\text{HCO}_3^-$ protect the mucosa against large amounts of acid by neutralizing $\text{H}^+$ ions that diffuse back into the mucus layer, and hence the $\text{HCO}_3^-$ secretion is thought to play an important role in maintaining the integrity of the gastroduodenal mucosa (3, 4). Although the physiological regulation of $\text{HCO}_3^-$ secretion involves prostaglandins (PGs) as well as neuro-humoral factors (3—7), it is thought that endogenous PGs are particularly important in the local control of this secretion. Indeed, $\text{PGE}_2$ and its analogues, whether applied luminally or vascularly, stimulate duodenal $\text{HCO}_3^-$ secretion in vivo and in vitro, in a variety of species including man and in this way may contribute to protection of the mucosa against acid-induced injury (3, 4). On the other hand, recent studies showed that the receptors activated by $\text{PGE}_2$ are pharmacologically subdivided in 4 subtypes, $\text{EP}_1$ - $\text{EP}_4$ (8—10), but those mediating the $\text{HCO}_3^-$ stimulatory action of $\text{PGE}_2$ remain to be much characterized.

The present study was designed to determine the EP receptor subtypes responsible for the $\text{HCO}_3^-$ stimulatory action of $\text{PGE}_2$ in the gastroduodenal mucosa, by examining the effects of various subtype specific EP agonists on the $\text{HCO}_3^-$ secretion in rats and by evaluating the $\text{HCO}_3^-$ response to $\text{PGE}_2$ in knockout mice lacking $\text{EP}_1$- and $\text{EP}_3$-receptors.

MATERIALS AND METHODS

Animals

Male SD rats (200—220 g) or C57BL/6 mice (25—30 g) were used. Mice lacking the EP or EP receptors were generated as described previously (11, 12). In brief, the mouse genes encoding the EP and EP receptors were individually disrupted, and chimaeric mice were generated. These animals were then back crossed with C57BL/6 mice, and the resulting heterozygous litter mates [EP$^+/-$ or EP$^+/-$] were bred to produce homozygous EP$^-/-$ or EP$^-/-$ mice. Distribution of the EP and EP receptor genes was verified by northern blot hybridization, which failed to detect messenger RNAs encoding the respective receptors in EP$^-/-$ and EP$^-/-$ mice. These knockout mice and rats were deprived of food but allowed free access to tap water for 18 hr before the experiments. All studies were performed under urethane anesthetized conditions (1.25 g/kg, IP).

Determination of $\text{HCO}_3^-$ secretion

The $\text{HCO}_3^-$ secretion was measured in both rats and mice under urethane anesthetized conditions, according to our previously published method (13). In brief, the stomach mounted on
ex-vivo chamber or the duodenal loop that was between the pylorus and the outlet of the common bile duct (1.5 cm in the rat and 0.8 cm in the mouse) was perfused at a flow rate of 1 ml/min in rats or 0.2 ml/min in mice, with saline that was gassed with 100% O₂, heated at 37°C and kept in a reservoir. The HCO₃⁻ secretion was titrated at pH 7.0 using a pH-stat method and by adding 5 mM HCL to the reservoir. In the stomach preparation, acid secretion had been inhibited by pretreatment of the animals with omeprazole given i.p. in a dose of 60 mg/kg. After basal HCO₃⁻ secretion had well stabilized, the following prostanoids were administered i.v. as a single bolus injection in rats, and the HCO₃⁻ secretion was measured for 1 hr thereafter; PGE₂ (EP₁/EP₂/EP₃/EP₄ agonist: 0.1 ~ 3 mg/), sulprostone (EP₁/EP₃ agonist: 0.3 ~ 3 mg/kg), enprostil (EP₁/EP₃ agonist: 0.001 ~ 0.03 mg/kg), (misoprostol (EP₂/EP₃ agonist: 0.01 ~ 1 mg/kg), butaprost (EP₂ agonist: 1 ~ 10 mg/kg), 11-deoxy PGE₁ (EP₃/EP₄ agonist: 0.01 ~ 1 mg/kg), 17-phenyl-trinor PGE₂ (17-phenyl PGE₂: EP₃ agonist: 0.3 ~ 3 mg/kg), and ONO-NT-012 (EP₃ agonist: 0.1 ~ 61 mg/kg). These prostanoids are considered to be EP receptor subtype specific agonists (13—15). In some animals, the effect of ONO-AE-829 (3 and 10 mg/kg), an EP₁ receptor antagonist, on gastroduodenal HCO₃⁻ secretion induced by PGE₂ was examined. ONO-AE-829 was administered s.c. 30 min before PGE₂. In mice, PGE₂ (0.01 ~ 0.3 mg/ml) was applied topically to the gastroduodenal mucosa for 10 min (16, 17).

Preparation of drugs

Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), prostaglandin E₂, butaprost, sulprostone, enprostil, misoprostol, ONO-NT-012, ONO-AE—829 (Ono Pharm. Co. Ltd.), 17-phenyl trinor-PGE₂, 11-deoxy PGE₁ (Nacalai tesque, Kyoto, Japan), and omeprazole (Hessle, Mondale, Sweden). Omeprazole was suspended in 0.5% carboxymethylcellulose solution. PGE₂ and other EP receptor ligands were first dissolved in absolute ethanol and then diluted with saline to a desired concentration. Each agent was prepared immediately before use and given in a volume of 0.5 ml per 100 g body weight in cases of i.p. and s.c. administration or in a volume of 0.1 ml per 100 g body weight in case of i.v. administration. In mice, PGE₂ was topically applied to the mucosa in a volume of 0.2 ml for 10 min. Control animals received saline in place of active agent.

Statistics

Data are presented as the means ± SE from 4 ~ 8 animals per group. Statistical analyses were performed using a two-tailed Dunnett’s multiple comparison test, and values of P < 0.05 were regarded as significant.

RESULTS

Effect of Various EP Agonists on Gastroduodenal HCO₃⁻ Secretion in Rats

Under the present experimental conditions, the rat stomach secreted HCO₃⁻ secretion at a steady rate of 0.2 ~ 0.4 μEq/5 min in the animals given omeprazole (60 mg/kg, i.p.) to inhibit acid secretion. The HCO₃⁻ secretion in the
Fig. 1. Effects of various EP agonists on gastric HCO₃⁻ secretion in anesthetized rats. EP agonists were administered i.v. as a single injection after the secretion had well stabilized. The animals were pretreated with omeprazole (60 mg/kg, i.p.) to inhibit acid secretion completely. ○: PGE₂ (0.1 ~ 1 mg/kg); ●: butaprost (1 ~ 10 mg/kg); □: sulprostone (0.3 ~ 3 mg/kg); ◇: enprostil (0.001 ~ 0.03 mg/kg); ◆: 11-deoxy PGE₁ (0.001 ~ 0.03 mg/kg); ■: misoprostol (0.1 ~ 1 mg/kg); △: ONO-NT-012 (0.1 ~ 1 mg/kg); ▲: 17-phenyl PGE₂ (0.3 ~ 3 mg/kg). Data are presented as the means ± SE of total net HCO₃⁻ output obtained for 1 hr from 5 ~ 7 rats. In Figure B, bars indicate total net HCO₃⁻ output obtained for 1 hr after administration of the agents and are presented as the means ± SE from 5 ~ 7 rats.
stomach was increased dose-dependently when the animals were administered with PGE$_2$ i.v. in doses of 0.1 ~ 3 mg/kg; the total net HCO$_3^-$ at 3 mg/kg was 4.9±0.4 μEq/hr (Fig. 1A). Likewise, both sulprostone (0.3 ~ 3 mg/kg) and enprostil (0.001 ~ 0.03 mg/kg) also increased gastric HCO$_3^-$ secretion in a dose-dependent manner, and the total net HCO$_3^-$ output obtained by these agents at the highest doses used was 5.0±0.4 μEq/hr and 5.5±0.7 μEq/hr, respectively, which were approximately equivalent to that induced by PGE$_2$ at 3 mg/kg (Fig. 1B). Although neither misoprostol, butaprost, 11-deoxy PGE$_1$ nor ONO-NT012 exhibited any stimulatory effect on gastric HCO$_3^-$ secretion at any doses used in this study, 17-phenyl PGE$_2$ (0.3 ~ 3 mg/kg) dose-dependently stimulated HCO$_3^-$ secretion in the stomach, similar to PGE$_2$, the total net HCO$_3^-$ output being 2.8±0.4 μEq/hr at a dose of 3 mg/kg.

On the other hand, the rat duodenum also spontaneously secreted HCO$_3^-$ at a steady rate of 0.2 ~ 0.4 μEq/5 min during a 90-min test period. Intravenous administration of PGE$_2$ (0.1 ~ 3 mg/kg) as a single bolus injection produced a dose-dependent increase of HCO$_3^-$ secretion in the duodenum (Fig. 2A). After administration of PGE$_2$ at 1 mg/kg, the duodenal HCO$_3^-$ secretion was increased from 0.25±0.30 μEq/5 min to the maximal values of 0.78±0.12 μEq/5 min, about 3 times as great as basal levels, and remained elevated for at least 60 min. The HCO$_3^-$ output also increased dose-dependently in response to i.v. administration of other prostanoids such as sulprostone (0.3 ~ 3 mg/kg), and ONO-NT-012 (0.1 ~ 1 mg/kg); the total net HCO$_3^-$ output at the highest doses used was 4.1±0.3 μEq/hr, 6.9±0.6 μEq/hr, 4.2±0.3 μEq/hr, 7.9±0.3 μEq/hr and 4.7±1.2 μEq/hr, respectively (Fig. 2B). However, both butaprost (1 ~ 10 mg/kg) and 17-phenyl PGE$_2$ (0.3 ~ 3 mg/kg) did not cause either an increase or a decrease in duodenal HCO$_3^-$ secretion at any doses used in this study.

To further clarify which EP receptor subtype is involved in the stimulation of HCO$_3^-$ secretion, we examined the effect of ONO-AE-829 (EP$_1$ antagonist) on the HCO$_3^-$ response induced by PGE$_2$. Intravenous administration of PGE$_2$ (0.3 mg/kg) increased gastric HCO$_3^-$ secretion over control levels, from 0.40±0.06 μEq/5 min to the maximal values of 0.98±0.07 μEq/5 min; the total net HCO$_3^-$ output was 4.9±0.5 μEq/hr. ONO-AE-829 (3 and 10 mg/kg) administered s.c. dose-dependently antagonized the HCO$_3^-$ stimulatory action of PGE$_2$ in the stomach, and a significant effect was observed at 10 mg/kg, the inhibition being 80%. In contrast, ONO-AE-829 at 10 mg/kg, however, did not have any effect on the HCO$_3^-$ response to PGE$_2$ in the duodenum; the total net HCO$_3^-$ output was 4.0±0.3 μEq/hr and 4.3±0.3 μEq/hr, respectively, the absence or presence of ONO-AE-829.
Fig. 2. Effects of various EP agonists on duodenal HCO$_3^-$ secretion in anesthetized rats. EP agonists were administered i.v. as a single injection after the secretion had well stabilized. O: PGE$_2$ (0.1 ~ 3 mg/kg); ●: butaprost (1 ~ 10 mg/kg); □: sulprostone (0.3 ~ 3 mg/kg); ◊: enprostil (0.003 ~ 0.03 mg/kg); ♦: 11-deoxy PGE$_1$ (0.001 ~ 0.03 mg/kg); ■: misoprostol (0.1 ~ 1 mg/kg); △: ONO-NT-012 (0.1 ~ 1 mg/kg); ▲: 17-phenyl PGE$_1$ (0.3 ~ 3 mg/kg). Data presented as the means ± SE of total net HCO$_3^-$ output obtained for 1 hr from 5 ~ 8 rats. In Figure B, values indicate total net HCO$_3^-$ output obtained for 1 hr after administration of the agents and are presented as the means ± SE from 5 ~ 8 rats.
Fig. 3. Gastric HCO$_3^-$ secretory responses induced by PGE$_2$ in wild type- and EP receptor knockout mice. The animals were pretreated with omeprazole (60 mg/kg, i.p.) to inhibit acid secretion completely. PGE$_2$ (0.1 mg/ml) was applied luminally to the stomach for 10 min. ○: wild type (saline); △: wild-type (PGE$_2$); ▲: EP$_1$ (-/-); □: EP$_3$ (-/-). Data are presented as the means±SE of values determined every 10 min from 4 ~ 6 mice. * Statistically significant difference from values observed immediately before PGE$_2$ treatment, at P < 0.05. In Figure B, values indicate total HCO$_3^-$ output obtained for 1 hr before and after PGE$_2$ treatment and are presented as the means±SE from 4 ~ 6 mice per group. * Statistically significant difference from values in before, at P < 0.05.
HCO\textsubscript{3}^- Secretory Response to PGE\textsubscript{2} in Knockout Mice

Under urethane anesthesia, the mouse stomach or duodenum spontaneously secreted HCO\textsubscript{3}^- at a steady rate of 0.3 \( \sim \) 0.5 \( \mu \)Eq/hr or 1.9 \( \sim \) 2.4 \( \mu \)Eq/hr, respectively, during a 90 min test period. Gastric HCO\textsubscript{3}^- secretion was increased in response to luminal exposure to PGE\textsubscript{2} (0.3 mg/ml) in wild-type mice, from 0.45 \( \pm \) 0.05 \( \mu \)Eq/hr to a maximal value of 1.10 \( \pm \) 0.03 \( \mu \)Eq/hr; the total HCO\textsubscript{3}^- output (1.02 \( \pm \) 0.06 \( \mu \)Eq/hr) was about two fold greater than basal values (0.38 \( \pm \) 0.02 \( \mu \)Eq/hr) (Fig. 3A, 3B). This process was, however, almost absent in EP\textsubscript{1}-receptor knockout mice, and the total HCO\textsubscript{3}^- output remained in the same ranges (0.4 \( \sim \) 0.5 \( \mu \)Eq/hr) before and after the mucosal exposure to PGE\textsubscript{2}. On the other hand, the perfusion of the duodenum with PGE\textsubscript{2} (0.1 mg/ml) also caused a significant increase of the HCO\textsubscript{3}^- secretion in wild-type mice; the secretion increased from 2.0 \( \pm \) 0.2 \( \mu \)Eq/hr to a maximal value of 5.9 \( \pm \) 0.5 \( \mu \)Eq/hr, the total HCO\textsubscript{3}^- output being 5.5 \( \pm \) 0.2 \( \mu \)Eq/hr (Fig. 4A, 4B). The HCO\textsubscript{3}^- stimulatory effect of PGE\textsubscript{2} was similarly observed in EP\textsubscript{1}-but not EP\textsubscript{3}-receptor knockout mice. In the latter animals lacking EP\textsubscript{3} receptors, PGE\textsubscript{2} failed to stimulate HCO\textsubscript{3}^- secretion in the duodenum, the total HCO\textsubscript{3}^- output being 2.1 \( \pm \) 0.5 \( \mu \)Eq/hr which is not significantly different from basal values (2.6 \( \pm \) 0.7 \( \mu \)Eq/hr).

The mucosal perfusion of PGE\textsubscript{2} (0.01 \( \sim \) 0.3 mg/ml) caused a dose-dependent increase of HCO\textsubscript{3}^- secretion in both the stomach and duodenum of wild-type mice, although the dose required to stimulate HCO\textsubscript{3}^- secretion was higher in the stomach as compared to the duodenum (Fig. 5A, 5B). Likewise, a dose-dependent HCO\textsubscript{3}^- response to PGE\textsubscript{2} was observed in the stomach of EP\textsubscript{3}-receptor knockout mice or in the duodenum of EP\textsubscript{1}-receptor knockout mice, and the degrees of HCO\textsubscript{3}^- response to PGE\textsubscript{2} in such animals were similar to those observed in wild-type mice.

**DISCUSSION**

PG\textsubscript{n}, either generated endogenously or administered exogenously, act on multiple receptors (9). In the previous study using subtype-specific various EP\textsubscript{1} agonist in rats, we demonstrated that different EP receptors are involved in the HCO\textsubscript{3}^- response to PGE\textsubscript{2} in the gastroduodenal mucosa; EP\textsubscript{1}-receptors in the stomach and EP\textsubscript{3}-receptors in the duodenum (13). In the present study, we further demonstrated using EP-receptor knockout mice that the HCO\textsubscript{3}^- response in the stomach and duodenum was totally absent in the animals with EP\textsubscript{1}-or EP\textsubscript{3}-receptor gene disruption, respectively, confirming the findings in rats obtained with subtype-specific various EP agonists.
Fig. 4. Duodenal HCO₃⁻ secretory responses induced by PGE₂ in wild type- and EP receptor knockout mice. PGE₂ (0.1 mg/ml) was applied luminally to the duodenal loop for 10 min. ○: wild type (saline); △: wild-type (PGE₂); △: EP₁ (−/−); □: EP₁ (−/−). Data are presented as the means ± SE of values determined every 10 min from 4 ~ 6 mice. * Statistically significant difference from values observed immediately before PGE₂ treatment, at P < 0.05. In Figure B, values indicate total HCO₃⁻ output obtained for 1 hr before and after PGE₂ treatment and are presented as the means ± SE from 4 ~ 6 mice per group. * Statistically significant difference from values in before, at P < 0.05.
Fig. 5. Dose-response effects of PGE$_2$ on gastric (A) and duodenal (B) HCO$_3^-$ secretion in wild-type and EP receptor knockout mice. PGE$_2$ (0.03 ~ 0.3 mg/ml) was applied lumina1ly to the stomach or the duodenal loop for 10 min. In case of determination of gastric HCO$_3^-$ secretion, the animals were pretreated with omeprazole (60 mg/kg, i.p.) to inhibit acid secretion completely. Values indicate total HCO$_3^-$ output obtained for 1 hr after PGE treatment and are presented as the means ± SE from 4 ~ 6 mice per group. * Statistically significant difference from basal secretion, at P < 0.05.
First, we confirmed that PGE₂ stimulates HCO₃⁻ secretion in the stomach as well as in the duodenum of rats, in agreement with other observations (1, 3, 7, 13). The present study also confirmed that this effect on HCO₃⁻ secretion is mediated by different EP receptor subtypes in the stomach and duodenum. Duodenal HCO₃⁻ secretion is stimulated by either sulprostone (EP₁/EP₃ agonist), enprostil, 11-deoxy PGE₁ (EP₃/EP₄ agonist) or misoprostol (EP₂/EP₃ agonist). However, neither 17-phenyl PGE₂ (EP₁ agonist) nor butaprost (EP₂ agonist) was effective in stimulating this secretion, while ONO-NT-012 (EP₃ agonist) dose-dependently increased HCO₃⁻ secretion in the duodenum. In contrast, gastric HCO₃⁻ secretion was stimulated by 17-phenyl PGE₂, sulprostone and enprostil, whereas neither butaprost, ONO-NT-012, misoprostol nor 11-deoxy PGE₁ effectively altered HCO₃⁻ secretion in the stomach. These results strongly suggest that PGE-stimulated HCO₃⁻ secretion in the duodenum is brought about by activation of EP₃ receptors, while that in the stomach occurs via EP₁ receptors. The latter is supported by the fact that ONO-AE-829, an EP₁ antagonist, significantly attenuated the HCO₃⁻ stimulatory action of PGE₂ in the stomach but not in the duodenum. Hassen et al (18) reported that gastric mucus secretion is stimulated by PGs through EP₄ receptors. However, 11-deoxy PGE₁, a potent EP₄ agonist, did not show any effect on gastric HCO₃⁻, suggesting the different mechanisms involved in mucus and HCO₃⁻ secretions in the stomach.

Of most important is the finding that the HCO₃⁻ stimulatory action of PGE₂ in the stomach is absent in EP₁-receptor knockout mice, while that in the duodenum is not observed in knockout mice lacking EP₃ receptors. These results confirmed the finding in rats and strongly indicate that PGE₂ stimulates HCO₃⁻ secretion in the stomach via EP₁-receptors and in the duodenum via EP₃-receptors. It should be noted that the effective dose of PGE₂ in stimulating HCO₃⁻ secretion was different between the stomach and duodenum; the ED₅₀ was much lower in the latter. This phenomenon remains to be explained but might be related with a low rate of acid secretion in the stomach was about 1/5 of that in the duodenum. Since the surface area of the tissue used or the route of PGE₂ administration was different in the experiments for rats and mice, it is difficult to compare the rate of HCO₃⁻ secretion between these two species. Yet, considering only the surface of the tissue, it was revealed that the rate of basal HCO₃⁻ secretion in the duodenum was almost same in both rats and mice, about 5 ~ 6 μEq/cm²/hr.

Morimoto et al (19) recently examined the cellular distribution of the mRNAs for the EP receptors in the mouse gastrointestinal tract by in situ hybridization. They showed that strong signals for EP₁ transcripts was observed in the smooth muscle cells in the muscularis mucosa throughout the tract, while moderate EP₃ mRNA expression was detected in the epithelial cells and also in the neurons of the myenteric ganglia throughout the gastrointes-
tinal tract. These results are not necessarily compatible with the present finding that HCO$_3^-$ secretion, one of the epithelial functions, is mediated by EP$_1$ receptors in the stomach and by EP$_3$ receptors in the duodenum. On the other hand, the EP receptor subtypes are coupled with different signal transduction systems; activation of EP$_1$ receptors are coupled with G$_q$ protein, resulting in an increase of intracellular Ca$^{2+}$, while the activation of EP$_3$ receptors are coupled with Gi protein, leading to an inhibition of adenylyl cyclase activity (9, 20). We previously reported that the HCO$_3^-$ response to PGE$_2$ in the stomach was mitigated by verapamil the Ca$^{2+}$ antagonist but was not affected by isobutylmethyl xanthine the inhibitor of phosphodiesterase, suggesting a mediator role of Ca$^{2+}$ in this response. On the other hand, duodenal HCO$_3^-$ secretion results from an elevation of intracellular 3'5'-cyclic adenosine monophosphate levels (21, 22) and can be elicited also by receptor-independent adenylyl cyclase activator like forskolin (23). Since duodenal HCO$_3^-$ secretion is stimulated by activation of EP$_3$ receptors, there seems to be some controversy in the signal transduction coupled with EP$_3$ receptors. However, a recent study showed the existence of four isoforms in the EP$_3$ receptors (24). EP$_{3A}$ is linked to activation of Gi protein, while that of EP$_{3B}$ and EP$_{3C}$ is coupled with activation of Gs protein, resulting in stimulation of adenylyl cyclase activity.

The present results taken together indicate that the presence of EP$_1$- or EP$_3$-receptors is essential for maintaining the HCO$_3^-$ secretion in the stomach or the duodenum, respectively. Further studies should certainly be needed regarding signal transduction of the HCO$_3^-$ secretion mediated by these different EP receptors as well as the cellular distribution of EP receptor subtypes.

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Received: September 11, 1998
Accepted: April 13, 1999

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