Role of Prostaglandin E Receptor Subtypes in Gastroduodenal HCO_3^- Secretion

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Abstract: Gastroduodenal HCO_3^- secretion is a key process that aids in preventing acid-peptic injury. Endogenous prostaglandins (PGs) play a particularly important role in the local control of this secretion. The secretion of HCO_3^- in both the stomach and duodenum was increased in response to PGE_2 as well as mucosal acidification, the latter occurring with concomitant enhancement of mucosal PG generation. These HCO_3^- responses in the duodenum were markedly reduced by prior administration of the EP4 antagonist in rats, and profoundly decreased in the animals lacking EP3 receptors but not EP1 receptors. In contrast, gastric HCO_3^- responses induced by PGE_2 and mucosal acidification were prevented by the EP1 antagonist and disappeared in EP1, but not EP3-knockout mice. Consistent with these findings, duodenal HCO_3^- secretion was stimulated by both EP3 and EP4 agonists but not EP1 or EP2 agonists, while gastric HCO_3^- secretion was increased by the EP1 agonist but not EP2, EP3 or EP4 agonists. In addition, the HCO_3^- stimulatory action of sulprostone (EP1/EP3 agonist) in the stomach was inhibited by the Ca^{2+} antagonist verapamil but not affected by IBMX, the inhibitor of phosphodiesterase, while that in the duodenum was inhibited by verapamil and enhanced by IBMX. Forskolin, the stimulator of adenlate cyclase, increased HCO_3^- secretion in the duodenum but not the stomach. Thus, the HCO_3^- stimulatory action of PGE_2 in the duodenum is mediated by both EP3 and EP4 receptors being coupled intracellularly with both Ca^{2+} and cAMP, while that in the stomach is mediated by EP1 receptors, coupled with Ca^{2+}.

Key Words: HCO_3^- secretion, stomach, duodenum, prostaglandin, EP receptor subtypes, mucosal protection.

INTRODUCTION

It is known that HCO_3^- secretion occurs from the surface epithelial cells in the gastroduodenal mucosa and plays an important role in the mucosal defensive ability of these tissues against acid [1-4]. The effectiveness of this defensive ability would be considerably enhanced if the neutralization of HCO_3^- with H^+ (luminal acid) occurs in the mucus gel layer with low turbulence. Indeed, the secretion of HCO_3^- maintains neutral pH in the mucus gel adherent to the surface, despite that the acidity is as high as pH 1.5 to 3 in the lumen, resulting in a pH gradient across the mucus gel at the surface of the mucosa [5-7]. It is thus considered that the secretion of HCO_3^-, together with the mucus gel, provides a first line of mucosal defense in the stomach and may be the most important mechanism in the duodenum.

The process of HCO_3^- secretion is mediated by endogenous prostaglandins (PGs) and nitric oxide (NO) as well as neuronal pathways, yet PGs, mainly E type of PGs, play a particularly important role in the local regulation of this secretion. Indeed, various analogues of PGs or agents that enhance the biosynthesis of endogenous PGs stimulate HCO_3^- secretion, while nonsteroidal anti-inflammatory agents, such as indomethacin and aspirin, decrease the secretion of HCO_3^- by inhibiting PG generation [8-11]. On the other hand, the receptors activated by PGE_2 are pharmacologically subdivided into 4 subtypes, EP1~EP4, and their complementary DNAs have been cloned [12,13]. These messenger RNAs (mRNAs) of EP receptors distribute throughout the gastrointestinal tract, including the stomach and duodenum. Morimoto *et al*. [14] demonstrated using by *in situ* hybridization that strong signals for EP1 transcripts were observed in the smooth muscle cells in the muscularis mucosa throughout the tract, while EP3 mRNAs were expressed in the neurons of the myenteric ganglia and also in the fundic gland epithelial cells, but not the surface mucous cells in the stomach. They also showed that the expression of EP4 mRNA was observed in the surface epithelial cells throughout the mouse intestine [14]. So far, only a few studies have dealt with the relationship between EP receptor subtypes and various functions in the gastrointestinal tract, i.e., the EP1 receptors in smooth muscle contraction [15], the EP2/EP3 receptors in acid secretion [16], and the EP4 receptors in mucus secretion [17]. We have investigated the relationship between EP receptor subtypes and the secretion of HCO_3^- in both the stomach and duodenum, and found that this secretion has a distinctive mechanism in these two tissues, concerning the EP receptor subtypes involved in this process.

In this article, we reviewed the importance of endogenous PGE_2 in the local regulation of gastroduodenal HCO_3^- secretion and the EP receptor subtypes responsible for this action of PGE_2, mainly based on our data obtained using various subtype selective EP agonists and antagonists as well as EP receptor knockout mice [18-22] (Fig. **1**).

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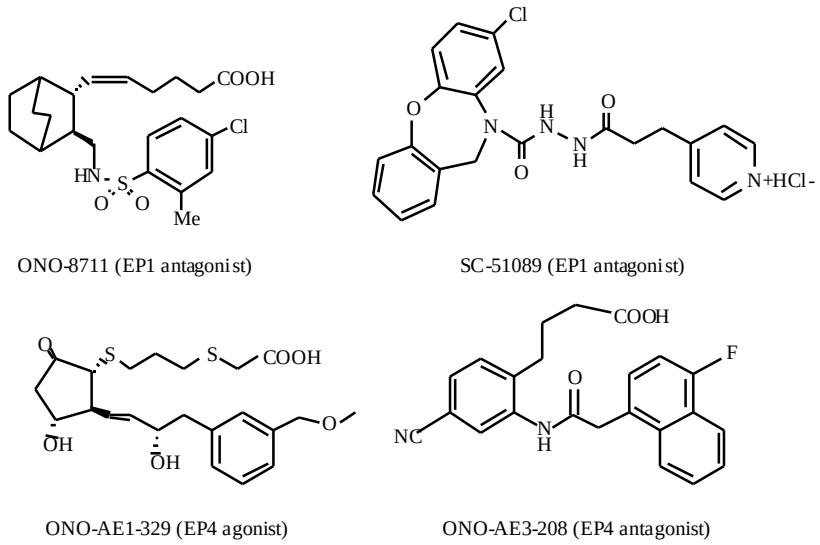

ONO-8711 (EP1 antagonist) SC-51089 (EP1 antagonist)

ONO-AE1-329 (EP4 agonist) ONO-AE3-208 (EP4 antagonist)

Fig. (1). Chemical structures of ONO-8711 (EP1 antagonist), SC-51089 (EP1 antagonist), ONO-AE1-329 (EP4 agonist) and ONO-AE3-208 (EP4 antagonist).

EXPERIMENTAL SYSTEM

The data presented in this article were obtained in both the stomachs and duodenums of rats and mice under urethane anesthesia. A chambered stomach was perfused with saline that was gassed with 100 %O_2, heated at 37°C and kept in a reservoir, and the HCO_3^- secretion was measured at pH 7.0 by using a pH-stat method and by adding 2 mM HCl to the reservoir [23]. To unmask HCO_3^- in the stomach, acid secretion was completely inhibited by omeprazole given i.p. at a dose of 60 mg/kg [24,25]. On the other hand, a duodenal loop was made between the pyloric ring and the area just above the outlet of the common bile duct, to exclude the influences of bile and pancreatic juice. Then, the loop was perfused with saline as described for the gastric preparation, and HCO_3^- secretion was measured at pH 7.0 by using the pH-stat system and by adding 2 mM HCl.

STIMULATION OF HCO_3^- SECRETION BY E TYPE PROSTAGLANDINS

Table **1** shows the EP receptor subtype preference of PGE_2 and other EP agonists and antagonists used in our study. Intravenous administration of PGE_2 as a single bolus injection produced a dose-dependent increase of HCO_3^- secretion in the duodenum [18,20,26]. After administration of PGE_2 at a dose of 1 mg/kg, duodenal HCO_3^- secretion increased approximately three times greater than basal levels (Fig. **2**). The HCO_3^- secretion was also increased dose-dependently in response to intravenous administration of other prostanoids, subtype selective EP agonists such as sulprostone (EP1/EP3 agonist), misoprostol (EP2/EP3), and ONO-NT012 (EP3 agonist) (Figs. **2** and **3**). However, both butaprost (EP2 agonist) and 17-phenyl PGE_2 (EP1 agonist) did not affect duodenal HCO_3^- secretion even if we used high dose of these agents [18,26].

Similar to the findings in the duodenum, HCO_3^- secretion in the stomach was also increased dose-dependently by PGE_2 given intravenously. Likewise, both 17-phenyl PGE_2 and sulprostone increased gastric HCO_3^- secretion in a dose-dependent manner. Neither misoprostol, butaprost nor ONO-NT012 exhibited any stimulatory effect on gastric HCO_3^- secretion at any doses used [18,26] (Fig. **4**).

Table 1. Various Prostanoids, Subtype-Specific EP Receptor Agonists and Antagonists

Prostanoids	EP Subtype Selectivity
PGE_2	EP1/EP2/EP3/EP4 agonist
17-phenyl PGE_2	EP1 agonist
Sulprostone	EP1/EP3 agonist
Misoprostol	EP2/EP3 agonist
Butaprost	EP2 agonist
ONO-NT-012	EP3 agonist
ONO-AE1-329	EP4 agonist
ONO-AE-829	EP1 antagonist
ONO-AE3-208	EP4 antagonist
SC-51089	EP1 antagonist

PARTICIPATION OF EP4 RECEPTORS IN HCO_3^- SECRETION

Recently, a highly specific EP4 agonist (ONO-AE1-329) and antagonist (ONO-AE3-208) developed in Japan have become available [27,28]. The EP4 agonist ONO-AE1-329 given intravenously caused a dose-dependent increase of HCO_3^- secretion in the duodenum (Fig. **2**), and this action was completely attenuated by prior administration of ONO-AE3-208 [22]. This EP4 antagonist also significantly inhibited the HCO_3^- stimulatory action of PGE_2, but not sulprostone (EP1/EP3 agonist) in the duodenum. These

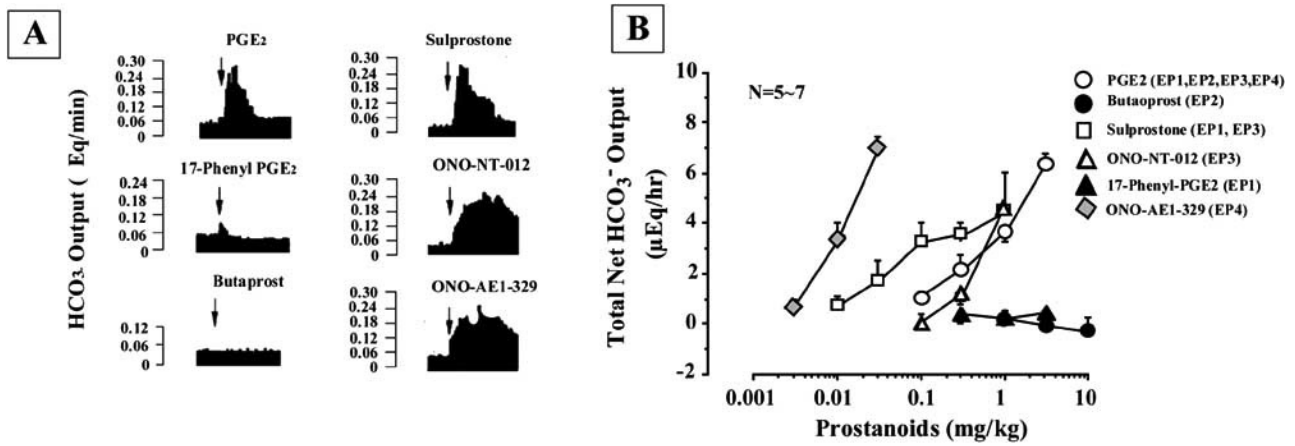

Fig. (2). A: Representative figures showing the effect of various EP agonists on duodenal HCO_3^- secretion in anesthetized rats. The recording of pulses of titrant was performed every 1 min using a Zero Suppression Adapter. **B:** Dose-response relationships for various EP agonists in stimulation of duodenal HCO_3^- secretion in anesthetized rats. Each agent was administered i.v. as a single bolus injection. Values indicate total net HCO_3^- secretion obtained for 1 hr after administration of the agent at the respective dose and are presented as the mean±SE from 5~7 rats.

results suggest that the HCO_3^- stimulatory effect of PGE_2 in the duodenum is substantially reduced when either EP3 or EP4 receptor is inhibited. It is assumed that PGE_2 stimulates duodenal HCO_3^- secretion by activating either of these EP receptor subtypes, EP3 and EP4, and the activation of both subtypes is required for full stimulation. This idea was supported by the finding that co-administration of sulprostone with ONO-AE1-329, at the doses that do not by themselves affect the HCO_3^- secretion, produced a marked increase in duodenal HCO_3^- secretion (Fig. **5**). Furthermore, the stimulatory action of ONO-AE1-329 on duodenal HCO_3^- secretion was significantly enhanced by IBMX, similar to sulprostone, although verapamil had no effect. We also observed that the EP4 agonist ONO-AE1-329, had no effect on the HCO_3^- secretion in the stomach.

On the other hand, the stimulatory effect of PGE_2 on gastric HCO_3^- secretion was significantly inhibited by ONO-8711 the EP1 antagonist, but not ONO-AE3-208 the EP4 antagonist [22]. All these data confirmed the involvement of EP1 receptors in the stimulatory action of PGE_2 on gastric HCO_3^- secretion and clearly showed that EP4 receptors do not play any role in the stimulation of HCO_3^- secretion induced in the stomach by PGE_2.

INTRACELLULAR MEDIATORS IN HCO_3^- SECRETION

Sulprostone the EP1/EP3 agonist, stimulated HCO_3^- secretion in both the stomach and duodenum [18]. This increase of HCO_3^- secretion induced by sulprostone was

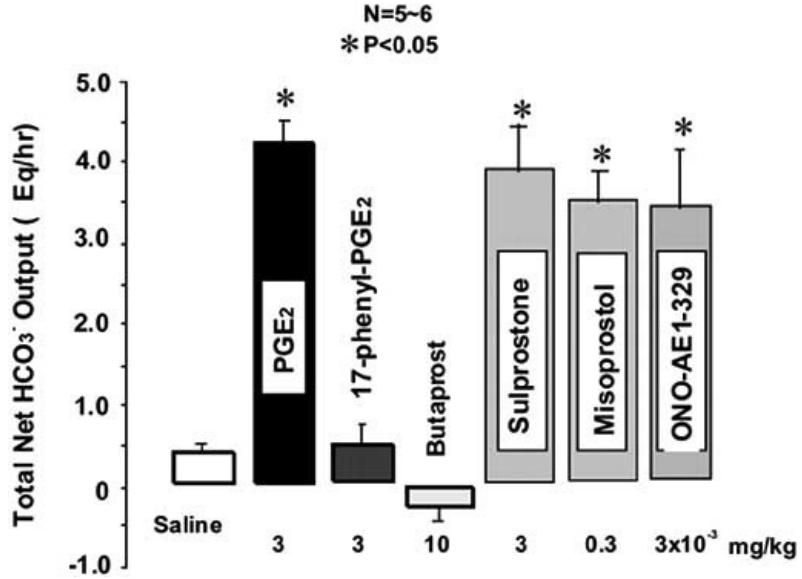

Fig. (3). Effects of various prostanodis, subtype selective EP agonists, on gastric HCO_3^- secretion in rats. Each agent was administered i.v. as a single bolus injection. Values indicate total HCO_3^- output obtained for 1 hr after treatment and are presented as the mean±SE from 4~7 rats. *Significant difference from saline, at $P<0.05$.

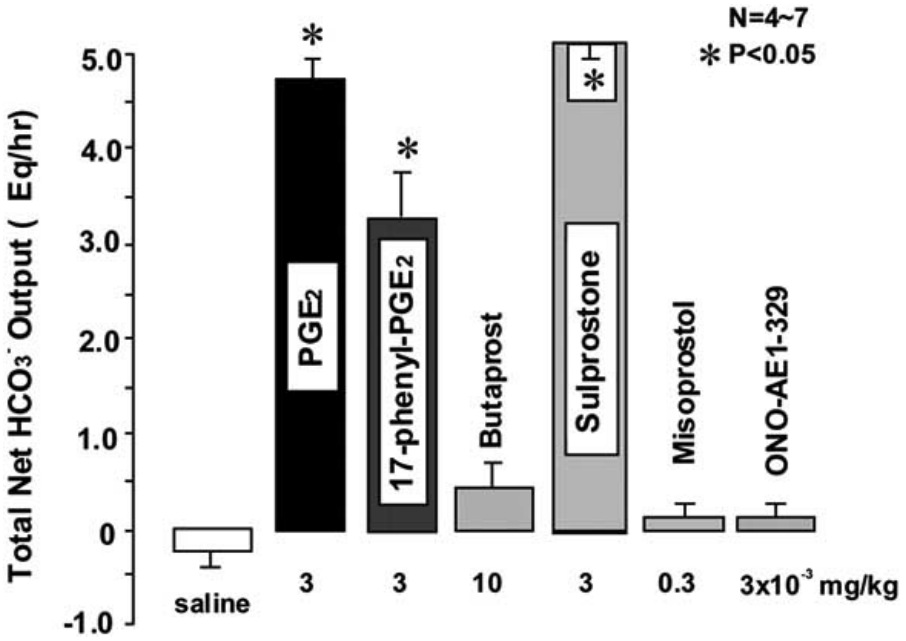

Fig. (4). Effects of various prostanodis, subtype selective EP agonists, on duodenal HCO_3^- secretion in rats. Each agent was administered i.v. as a single bolus injection. Values indicate total HCO_3^- output obtained for 1 hr after treatment and are presented as the mean±SE from 4~7 rats. *Significant difference from saline, at P<0.05.

attenuated by SC-51089 (EP1 antagonist)[29] in the stomach, although the HCO_3^- stimulatory effect in the duodenum was not affected by pretreatment of this agent [26]. In addition, duodenal HCO_3^- secretion was inhibited by ONO-AE3-208 the EP4 antagonist and stimulated by ONO-AE1-329 the EP4 agonist [22]. These results strongly suggest that gastric HCO_3^- secretion mediated by EP1 receptors, while duodenal HCO_3^- secretion was stimulated *via* both EP3 and EP4 receptors. In general, activation of EP1 receptor causes an elevation of intracellular Ca^{2+} levels [13]. Concerning the EP3 receptors, 4 splicing variants exist, each coupled to different signaling pathways; EP3A receptor is linked to activation Gi protein, both EP3B and EP3C are coupled with activation of Gs protein resulting in stimulation of adenylate cyclase (AC) activity, and the activation of EP3D receptor causes an elevation of intracellular Ca^{2+} by stimulating phosphatidyl inositol (PI) turnover *via* Gq protein [16,29,30]. In addition, EP4 receptor is associated with activation of Gs protein, leading to cAMP formation through stimulation of AC. Although the phosphodiesterase inhibitor isobutylmethyl xanthine (IBMX) had no effect on the HCO_3^- secretion induced in the stomach by sulprostone [18], this treatment significantly potentiated the HCO_3^- response to sulprostone or ONO-AE1-329 in the duodenum. By contrast, the Ca^{2+} antagonist verapamil significantly mitigated the stimulatory action of sulprostone in both the stomach and duodenum. These findings suggest that PGE_2 stimulates HCO_3^- secretion *via* EP1 receptors linked to elevation of intracellular Ca^{2+} in the stomach, while the HCO_3^- response to PGE_2 in the duodenum is mediated by EP3/EP4 receptors, coupled with intracellular accumulation of both cAMP and Ca^{2+}. It is also speculated that the HCO_3^- stimulatory action of PGE_2 in the duodenum is mediated by both EP3B, EP3C and EP3D in addition to EP4 receptors [18,22].

EP RECEPTOR SUBTYPES INVOLVED IN HCO_3^- RESPONSE INDUCED BY MUCOSAL ACIDIFICATION

It is known that briefly exposing the gastroduodenal mucosa to acid results in a sustained rise in the HCO_3^- secretion [3,6,31-34]. This process is mediated by multiple mechanisms, including endogenous PGs and nitric oxide (NO), as well as capsaicin-sensitive afferent neurons [35]. Notwithstanding, this response is almost totally inhibited by indomethacin, the cyclooxygenase inhibitor, in both the stomach and duodenum. This is understandable because the mediation of this HCO_3^- response depends somehow on endogenous PGs [18,36,37] and because NO stimulates HCO_3^- secretion, at least partly mediated by endogenous PGs [21,38]. We also observed that the acid-induced HCO_3^- secretion in the duodenum was significantly inhibited by the EP4 antagonist, confirming the participation of EP4 receptors in this process, in addition to EP3 receptors. The HCO_3^- secretion in the stomach was certainly abolished by the EP1 antagonist. On the other hand, the acid-induced HCO_3^- secretion in both the stomach and duodenum was significantly attenuated by chemical ablation of capsaicin-sensitive afferent neurons. Stimulation by capsaicin of these afferent neurons also increased the HCO_3^- secretion, and this response was also inhibited by indomethacin [36,39]. It is known that these afferent neurons are activated by H^+ or heat, through the interaction with vanilloid receptor type 1, in addition to capsaicin [40,41]. Thus, it is speculated that the afferent side of this reflex pathway in the acid-induced HCO_3^- response might be influenced by endogenous PGs, probably by facilitating the neuronal excitation in response to H^+. Since acidification of the mucosa caused an increase of PG production, endogenous PGs might stimulate the reflex pathway on the afferent side, in addition to direct

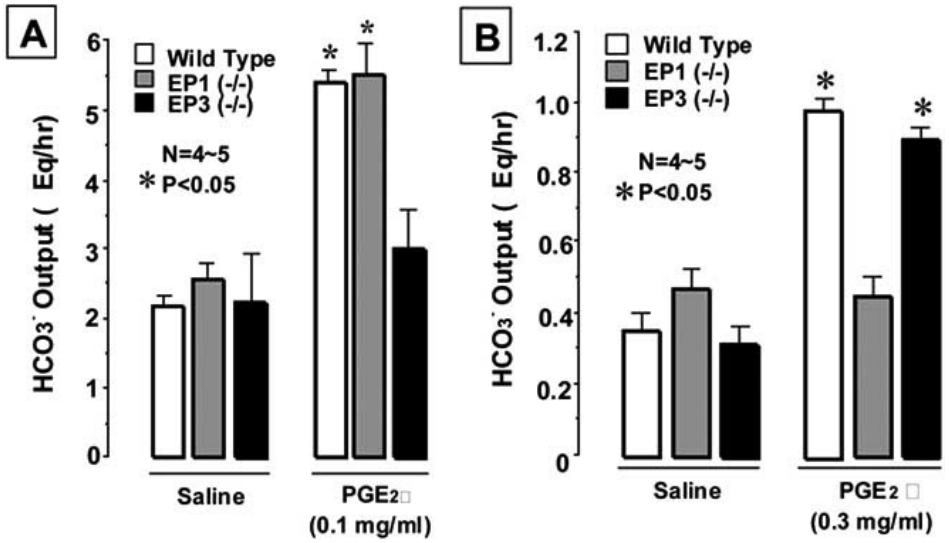

Fig. (5). Effect of sulprostone and ONO-AE1-329, either alone or in combination, on duodenal HCO_3^- secretion in anesthetized rats. Sulprostone (0.03 mg/kg) or ONO-AE1-329 (3 μg/kg) was given i.v. as a single injection. In the combined administration, sulprostone was first given i.v., immediately followed by i.v. administration of ONO-AE1-329. Data are presented as the mean±SE of values determined every 10 minutes from 5 rats. Fig. B shows the net HCO_3^- output for 1 hr after each treatment. The data are presented as the mean±SE for 5 rats. *Significant difference from control, at $P<0.05$.

stimulation of the epithelial cells, both resulting in an increase in the HCO_3^- secretion. However, the HCO_3^- stimulatory action of PGE_2, sulprostone (EP1/EP3 agonist) or ONO-AE1-329 (EP4 agonist) in the duodenum, was not significantly affected by capsaicin pretreatment [22]. Thus, it is assumed that PGE_2 stimulates HCO_3^- secretion mainly through a direct action on the epithelial cells. Certainly, when capsaicin-sensitive afferent neurons are stimulated by some means, then PGE_2 may enhance the activation of these neurons to result in enhancement of the HCO_3^- response. Further study should be needed to clarify this point.

STUDIES USING MICE LACKING EP1 AND EP3 RECEPTORS

An animal model lacking various receptors for prostanoids has recently been established [42,43]. Using these knockout mice, the roles of specific PG receptors in various biological actions of prostanoids have been demonstrated; for example, FP receptors in parturition [42] or EP3 receptor subtype in febrile response [43]. We investigated the relationship between EP receptor subtypes and gastroduodenal HCO_3^- secretion using various EP receptor knockout mice. Perfusion of the duodenum with PGE_2 caused a dose-dependent HCO_3^- secretion in wild-type mice. Likewise, a dose-dependent HCO_3^- response to PGE_2 was observed in the duodenum of EP1-receptor knockout mice (Fig. **6A**). In the animals lacking EP3 receptors, however, PGE_2 at all doses failed to stimulate HCO_3^- secretion. On the other hand, duodenal HCO_3^- secretion was stimulated by forskolin, the receptor-independent adenylate cyclase activator [44], in all groups of mice, irrespective of whether EP1 or EP3 receptors were knocked out. Furthermore, the acidification of the duodenal mucosa in wild-type animals increased HCO_3^- secretion, and this process was almost totally attenuated by indomethacin, again confirming a major mediator role of endogenous PGs in this mechanism [19]. A marked increase of HCO_3^- secretion in response to acidification was also observed in EP1-receptor knockout mice, similar to wild-type mice, but not in the animals lacking EP3 receptors. Acidification of the duodenal mucosa stimulated PG biosynthesis to increase the mucosal PGE_2 contents in all groups of mice, including EP3 receptor knockout mice. These data in EP receptor knockout mice are consistent with the data obtained in rats, and suggest that EP3 receptors are involved in the HCO_3^- stimulatory action of PGE_2 and mucosal acidification in the duodenum.

On the other hand, the secretion of HCO_3^- in the mouse stomach was also increased by luminal perfusion of PGE_2 or mucosal acidification, similar to the duodenum. These responses were observed in the stomach of EP3 receptor knockout mice, but disappeared in the animals lacking EP1 receptors (Fig. **6B**). These findings confirmed the data obtained in rats showing that the presence of EP1 receptors is essential for the HCO_3^- secretion in response to PGE_2 or mucosal acidification [19,22].

ROLE OF HCO_3^- SECRETION IN MUCOSAL PROTECTION

The concept that HCO_3^- secretion plays an important role in protection of gastric epithelial cells in collaboration with mucus has been discussed for many years. The mucus gel adherent to the luminal surface of the mucosa provides a zone of low turbulence, allowing the development of a pH gradient within the mucus gel [4,7]. Hence, small amounts of HCO_3^- protect the mucosa against large amounts of acid by neutralizing H^+ ion that diffuses back into the mucus layer. Indeed, the impairment of HCO_3^- secretion plays an important role in the pathogenesis of experimental and clinical duodenal ulcers [3,33,45]. For example, perfusion of

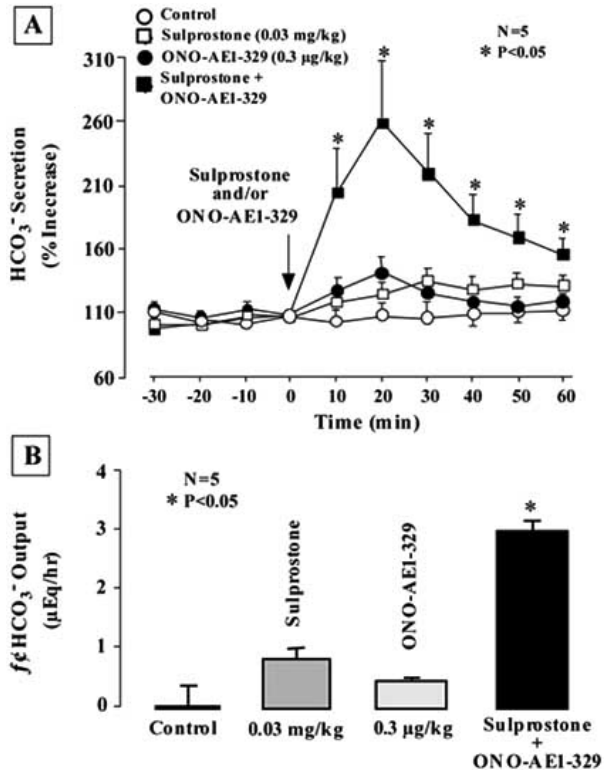

Fig. (6). The HCO_3^- secretory responses induced by PGE_2 in the duodenum **(A)** and the stomach **(B)** of wild-type and EP1- or EP3-receptor knockout mice. PGE_2 (0.3 mg/ml) was applied luminally in the duodenum or the stomach for 10 min. Values indicate total net HCO_3^- secretion obtained for 1 hr after administration of the agent at the respective dose and are presented as the mean±SE from 5~7 rats.

the proximal duodenum with 20 mM HCl for 4 hr in wild-type littermates caused only slight damage in the duodenum [19], yet the same treatment produced extensive hemorrhagic damage in the animals lacking EP3 receptors. Severe damage in the duodenum was also induced in the presence of indomethacin at the dose that inhibits the secretion of HCO_3^- induced by mucosal acidification [3,19,38], confirming a close relationship between the mucosal ulcerogenic response and the impairment of HCO_3^- secretion. Certainly, many factors such as background stress [45,46] or infection of *Helicobacter pylori* [47] are involved in the mechanisms of the impaired HCO_3^- secretion in patients with duodenal ulcers, in addition to the dysfunction of EP receptor system.

COMMENTARY

The HCO_3^- secretion from surface epithelial cells is one of the mucosal defensive mechanisms and plays an important role in protecting the gastroduodenal mucosa against acid [1,2,46]. It is known that the secretion of HCO_3^- is markedly elevated in response to luminal acid (pH<2.0)[3,6,31-33]. Endogenous PGs particularly play an important role in the local regulation of this secretion. Indeed, PGE_2 showed a potent stimulation of HCO_3^- secretion in both stomach and duodenum, and this effect was observed even in *in vitro* conditions, devoid of neuronal innervations, suggesting that this action is peripheral in origin [21].

Our studies, using various EP receptor subtype-specific agonists and antagonists [18-20,22], clearly showed that the secretion of HCO_3^- in the stomach is stimulated by only EP1 agonists while that in the duodenum is stimulated by both EP3 and EP4 agonists. In addition, the HCO_3^- stimulatory action of PGE_2 in the stomach was inhibited by the EP1 but not EP4 antagonist, but in the duodenum was prevented by the EP4 but not EP1 antagonist. These data strongly suggest a distinct mechanism involved in the HCO_3^- stimulatory action of PGs in the stomach and duodenum; the secretion of HCO_3^- in the stomach is exclusively mediated by activation of EP1 receptors, whereas that in the duodenum is mediated by activation of both EP3 and EP4 receptors. Certainly, this contention was supported by the data in EP receptor knockout mice, ie., gastric HCO_3^- response to PGE_2 was not observed in EP1 receptor knockout mice, while that in the duodenum mostly disappeared in EP3 receptor knockout mice. Furthermore, the duodenal HCO_3^- response to PGE_2 was markedly reduced by the EP4 antagonist and also impaired in the animals lacking EP3 receptors. It is assumed that PGE_2 stimulates the secretion of HCO_3^- by activating either receptor subtype EP3 or EP4, but the activation of both subtypes is required for full stimulation [22].

Stimulation of duodenal HCO_3^- secretion is associated with an elevation of intracellular cAMP levels [48,49]. Indeed, this secretion can be elicited by forskolin, the receptor-independent AC activator, as well as by cAMP analogues [45,50,51]. Ca^{2+} also functions as an intracellular mediator in duodenal HCO_3^- secretion, since this secretion is stimulated by A-23187, the Ca^{2+} ionophore, and inhibited by the removal of Ca^{2+} from the serosal solution [1,52]. The EP receptor subtypes are coupled with different signal transduction systems; activation of EP1 receptors are coupled with Gq protein, resulting in an increase of intracellular Ca^{2+} levels *via* a Ca^{2+} channel, independent of PI turnover, whereas that of EP2 and EP4 receptors are coupled with Gs protein which stimulates AC activity, resulting in an elevation of intracellular cAMP levels [13]. Although EP3 receptors are thought to be coupled with Gi protein, leading to inhibition of AC activity [16,29], there are four splicing variants of EP3 receptors, coupled to different signaling pathways [30]. The EP3A receptor is linked to activation of Gi protein, while EP3B and EP3C are coupled with activation of Gs protein. In addition, the activation of EP3D causes an elevation of intracellular Ca^{2+} levels by stimulating PI turnover *via* Gq protein [30]. The secretion of HCO_3^- induced by the EP1/EP3 agonist sulprostone was mitigated by verapamil in both the stomach and duodenum, while that in the duodenum but not in the stomach was markedly potentiated by pretreatment with IBMX. In addition, IBMX enhanced the HCO_3^- secretion in response to the EP4 agonist ONO-AE1-329. These results suggest that the HCO_3^- stimulatory action of PGE_2 is mediated by Ca^{2+} in the stomach and by both Ca^{2+} and cAMP in the duodenum. In general, a synergetic response to pharmacological actions is produced by the activation of two different signaling pathways. For example, stimulation of acid secretion involves an initial elevation of intracellular Ca^{2+} and/or cAMP,

followed by activation of a cAMP-dependent protein kinase cascade that triggers the translocation and insertion of the proton pump enzyme into the apical membrane of parietal cells [53]. Full response of acid secretion requires both cAMP and Ca^{2+}, and the lack of either factor results in substantial decrease in the response. The same is observed in regulation of duodenal HCO_3^- secretion, although the exact mechanism for a synergetic response or the effectors that are activated by Ca^{2+} and cAMP remain to be fully identified. At present, it remains unknown whether the EP3 agonist activates the Ca^{2+} and cAMP pathways at a similar time or dose, despite the activation of EP3 receptors being coupled with these two pathways. As mentioned above, it seems, however, that co-stimulation of these pathways by both EP3 and EP4 agonists produces a synergetic increase in duodenal HCO_3^- secretion. This idea may also apply to the secretion of HCO_3^- induced by acidification of the mucosa, and a malfunction of either the EP3 or EP4 receptor system results in a substantial loss of this response. Certainly, one would predict partial agonist and antagonist effects of these subtype specific EP agonists. However, this possibility may be excluded, because ONO-AE1-329 or ONO-AE3-208 is a highly specific and full EP4 agonist or antagonist, respectively [27,28].

Many investigators reported that mucosal acidification increases HCO_3^- secretion in these tissues, with concomitant rise in mucosal PGE_2 contents, suggesting an involvement of endogenous PGs in this mechanism [1,4,11,19,34,45]. Capsaicin also stimulated the secretion of HCO_3^- in both the stomach and duodenum, in an indomethacin-inhibitable manner, suggesting an involvement of endogenous PGs in this action [36,39]. However, capsaicin was reported to increase PGE_2 production in the duodenum but not in the stomach [37,54]. We previously reported that the gastroprotective action of capsaicin against HCl/ethanol was significantly attenuated by indomethacin in wild-type mice, but totally disappeared in animals lacking IP receptors [54]. In addition, Boku *et al*. [55] reported the lack of calcitonin gene-related peptide release in the stomach in response to mild injury in prostacyclin IP receptor knockout mice. Capsaicin increased gastric HCO_3^- secretion in EP1- and EP3-receptor knockout mice, similar to wild-type mice, but not in the animals lacking IP receptors (unpublished data). Consistent with previous findings [59,60], these results strongly suggest that endogenous PGI_2 plays a supportive role in the action of capsaicin in the stomach, probably by sensitizing the sensory neurons through IP receptors. We also reported that duodenal HCO_3^- secretion induced by mucosal acidification was unaltered in IP receptor knockout mice and disappeared in the animals lacking EP1-receptors [37]. These data further support the idea that the HCO_3^- response induced in the stomach by acidification and capsaicin, though both depending on the sensory neurons, are mediated by different mechanisms concerning PG dependency; the former is mainly mediated by PGE_2 through EP1 receptors, while the latter depends on PGI_2/IP receptors. Similar results were obtained for gastric hyperemic response induced by acid or capsaicin [54]. Thus, it is not unreasonable that the presence of different prostanoid receptors is required for gastric HCO_3^- secretion in response to acid or capsaicin.

There is controversy about the ionic transporters responsible for the secretion of HCO_3^- into the lumen. The HCO_3^- transporters, such as down regulated in adenoma (DRA), anion exchanger isoform 1 or 3, putative anion transporter (PAT-1) and cystic fibrosis transmembrane conductance regulator (CFTR), were expressed in the apical membrane of the duodenal epithelium [56,57]. In particular, it is considered that CFTR, regulated by cAMP-dependent protein kinase A, plays an important role in the secretion of HCO_3^- in the duodenum. Indeed, neither PGE_2, vasoactive intestinal polypeptide or mucosal acidification had any stimulatory effect on duodenal HCO_3^- secretion in CFTR-deficient mice [58]. Thus, one possible pathway responsible for duodenal HCO_3^- secretion induced by PGE_2 is the activation of CFTR by accumulation of cAMP through activation of EP3/EP4 receptors. The lack of CFTR expression in the gastric epithelium further differentiates the mechanism of gastric HCO_3^- secretion from that of duodenum. At present, no reliable information is available about the HCO_3^- transporters in the stomach.

SUMMARY AND FUTURE PROSPECTS

Endogenous PGs play a central role in the mucosal defensive mechanism of the gastrointestinal tract, and among them, PGE_2 is most important in their actions, including HCO_3^- secretion. As reviewed in this chapter, PGE_2 is a potent stimulator of HCO_3^- secretion in both the stomach and duodenum, and this effect in the stomach is mediated by activation of EP1 receptors coupled with elevation of intracellular Ca^{2+}, while that in the duodenum is associated with intracellular accumulation of both Ca^{2+} and cAMP caused by activation of EP3/EP4 receptors (Fig. **7**). Furthermore, the HCO_3^- stimulatory action of PGE_2 is limited in peripheral tissues, since this action was not affected by ablation of intrinsic or extrinsic nerves. However, the HCO_3^- secretion induced by mucosal acidification depends on both afferent neurons and endogenous PGs, and the latter supports the HCO_3^- secretion by sensitizing the afferent neurons. Thus, PGs, especially PGE_2, play an important role in the local regulation of HCO_3^- secretion and modulate the integrity of the gastroduodenal mucosa. Since the results introduced in this chapter were obtained in rats using subtype specific EP agonists and were further confirmed in EP-receptor knockout mice, they would be reliable and have a high reproducibility when compared to those obtained in either rats or knockout mice alone. Anyway, it is interesting to note that the EP receptor subtypes involved in the HCO_3^- stimulatory action of PGE_2 are different, depending upon the tissues. These approaches would contribute to further understanding of the regulatory mechanism of HCO_3^- secretion in the gastroduodenal mucosa and to future development of the mucosal protective drugs against acid injury.

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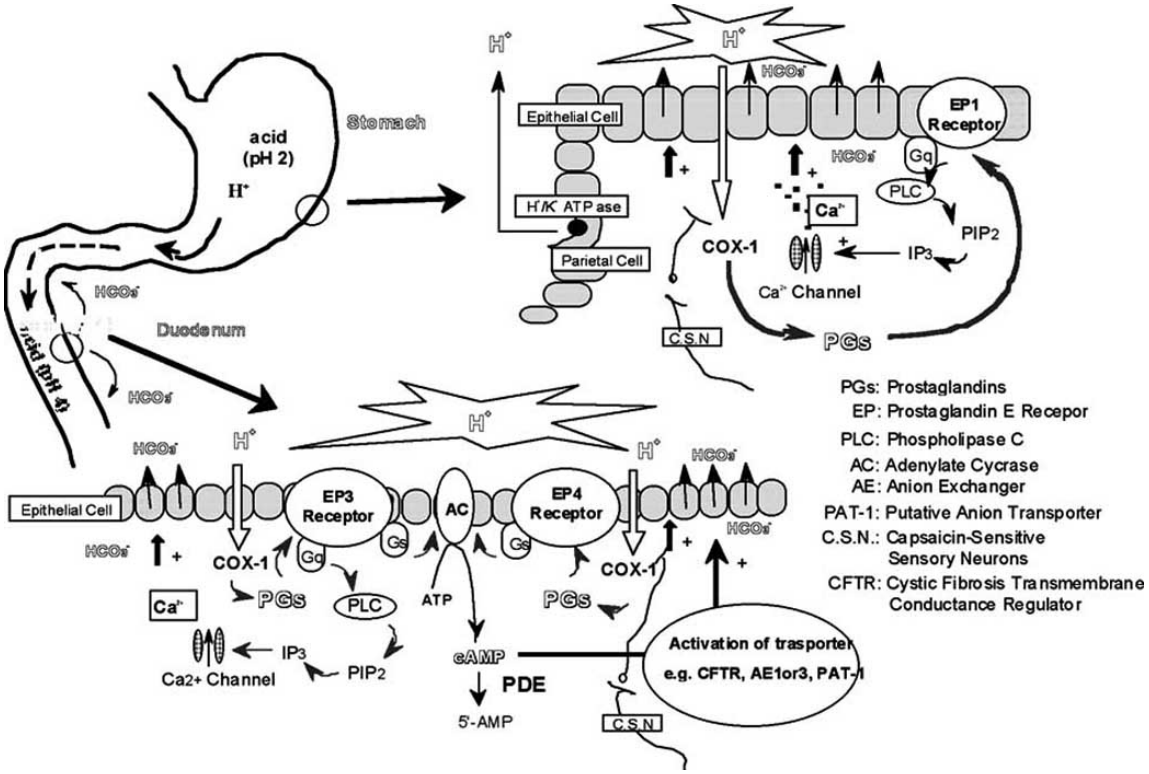

Fig. (7). Roles of PGE_2 and EP receptor subtypes in the regulatory mechanisms of gastroduodenal HCO_3^- secretion and mucosal defense against acid injury.

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