J Pharmacol Sci **114**, 248 – 261 (2010)

Journal of Pharmacological Sciences

Critical Review

Prostaglandin EP Receptors Involved in Modulating Gastrointestinal Mucosal Integrity

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Received August 24, 2010; Accepted September 22, 2010

Abstract. Endogenous prostaglandins (PGs) play an important role in modulating the mucosal integrity and various functions of the gastrointestinal tract, and E type PGs are most effective in these actions. PGE_2 protected against acid-reflux esophagitis and prevented the development of gastric damage induced by ethanol or indomethacin, the effects mimicked by EP1 agonists and attenuated by an EP1 antagonist. Adaptive cytoprotection induced by mild irritants was also attenuated by the EP1 antagonist. On the other hand, the acid-induced duodenal damage was prevented by EP3/EP4 agonists and worsened by EP3/EP4 antagonists. Similarly, the protective effect of PGE_2 on indomethacin-induced small intestinal damage or DSS-induced colitis was mimicked by EP3/EP4 agonists or EP4 agonists, respectively. The mechanisms underlying these actions of PGE_2 are related to inhibition of stomach contraction (EP1), stimulation of duodenal HCO_3^- secretion (EP3/EP4), inhibition of small intestinal contraction (EP4), and stimulation of mucus secretion (EP3/EP4) or down-regulation of cytokine secretion in the colon (EP4), respectively. PGE_2 also showed a healing-promoting effect on gastric ulcers and intestinal lesions through the activation of EP4 receptors, the effect associated with stimulation of angiogenesis via an increase in VEGF expression. These findings should aid the development of new strategies for treatment of gastrointestinal diseases.

Keywords: prostaglandin E, EP receptor subtype, mucosal protection, function, gastrointestinal tract

Contents

1. Introduction

Prostaglandins (PGs), produced from arachidonic acid by two isoforms of cyclo-oxygenase (COX), are present throughout the gastrointestinal tract and known to bring about a variety of actions in the gut, including the control of acid secretion, bicarbonate secretion, mucus production, and mucosal blood flow, and maintenance of mucosal integrity (1). Indeed, the administration of PGs protects the gastrointestinal mucosa against ulcerogenic stimuli such as stress, necrotizing agents, and nonsteroidal antiinflammatory drugs (NSAIDs). Robert et al. (2) were the first to demonstrate that PGs protect the stomach

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Published online in J-STAGE on October 28, 2010 (in advance)
doi: 10.1254/jphs.10R06CR

Invited article

against necrotizing agents, a phenomenon called "gastric cytoprotection". PGE_2 is particularly effective in this regard.

Pharmacological studies have classified PGE_2 receptors into four specific G protein–coupled subtypes, EP1 to EP4 (3), and the distribution of these receptors is considered to explain the multiple effects of PGE_2 in various tissues including the alimentary tract. In addition, mice lacking receptors for prostanoids have been established (4 – 6), and by using these "knockout mice", the roles of specific PG receptors in the various biological actions of PGs have been demonstrated (6 – 8). We have performed a series of experiments to determine the EP-receptor subtypes mediating the gastrointestinal protection as well as healing afforded by PGE_2, using various models in both rats and EP-receptor–knockout mice (8 – 12). We also used prostanoids, subtype-specific EP-receptor agonists, and antagonists, as a tool to characterize the EP-receptor subtypes involved in gastrointestinal protection (Table 1).

Table 1. Various subtype-specific EP-receptor agonists and antagonists used

Prostanoids	EP subtype selectivity
17-Phenyl PGE_2	EP1 agonist
Sulprostone	EP1/EP3 agonist
Butaprost	EP2 agonist
ONO-NT-012	EP3 agonist
11-Deoxy PGE_2	EP3/EP4 agonist
ONO-AE1-329	EP4 agonist
ONO-AE1-734	EP4 agonist
ONO-8711	EP1 antagonist
ONO-AE-829	EP1 antagonist
ONO-AE5-599	EP3 antagonist
ONO-AE3-208	EP4 antagonist
CJ42794	EP4 antagonist

We herein review our publications on the relation between EP-receptor subtypes and the protective as well as the healing-promoting action in the gastrointestinal tract afforded by endogenous or exogenous PGE_2 and discuss possible functional alterations responsible for these actions of PGE_2 in the esophagus, stomach, duodenum, and small and large intestines (Tables 2 and 3).

2. Esophageal protection

Reflux esophagitis is caused mainly by exposure of the gastric contents due to dysfunction of the mechanisms that prevent reflux into the esophagus and resist against refluxate (13). We investigated the effect of PGs, especially PGE_2, on acid reflux esophagitis, using subtype-selective EP-receptor agonists and antagonists, in relation to their influences on gastric acid and pepsin in rats (14). Acid reflux esophagitis was induced in rats by ligating both the pylorus and the transitional region between the forestomach and glandular portion under ether anesthesia, and the animals were killed 4 h later (15). The esophageal lesions in this model were markedly aggravated by prior administration of indomethacin. PGE_2 prevented these esophageal lesions at doses of 0.1 and 0.3 mg/kg, yet the protective effect disappeared totally when the dose was increased further to 1 mg/kg. These biphasic effects were mimicked by 17-phenyl PGE_2 and significantly antagonized by the EP1 antagonist ONO-8711, while other PGE derivatives, including EP2, EP3, and EP4 agonists, had no effect. PGE_2 and 17-phenyl PGE_2 had no effect on acid secretion but significantly increased pepsin secretion, in an EP1 antagonist–sensitive manner (16). These results indicate that PGE_2 has a biphasic effect on acid reflux esophagitis depending on the dose: a protective effect at lower doses and an aggravating effect at high doses, both mediated by EP1 receptors. Since the latter effect is brought about by increasing pepsin secretion, it is assumed that pepsin plays a primary role in the pathogenesis of acid reflux esophagitis, prior to acid insult. At present, the functional mechanism by

Table 2. EP-receptor subtype(s) responsible for protective effects of PGE_2 in various models of lesions in the gastrointestinal tract

Tissue	Model	ER-receptor subtype	References
Esophagus	Acid-reflux esophagitis	EP1 receptor	16
Stomach	HCl/ethanol-induced damage	EP1 receptor	8, 10
	Indomethacin-induced lesion	EP1 receptor	11
Duodenum	Acid-induced damage	EP3/EP4 receptors	7, 62
Small intestine	Indomethacin-induced damage	EP3/EP4 receptors	12, 64, 87 – 89
Large intestine	Dextan sulfate–induced ulcerative colitis	EP4 receptor	75, 76

Table 3. EP-receptor subtype(s) responsible for functional effects of PGE_2 in the gastrointestinal tract

Function	Action	ER-receptor subtype	References
Pepsin secretion	Increase	EP1 receptor	16
Acid secretion	Decrease	EP3 receptor	8, 33
	Increase	EP4 receptor	37
Bicarbonate secretion			
Stomach	Increase	EP1 receptor	9, 36
Duodenum	Increase	EP3/EP4 receptors	7, 9, 58, 60, 62
Mucus secretion			
Stomach	Increase	EP4 receptor	34
Small intestine	Increase	EP3/EP4 receptors	12
Large intestine	Increase	EP4 receptor	68
Gastric mucosal blood flow			
Normal stomach	Increase	EP2/EP4 receptors	8, 35
Damaged stomach	Increase	EP1 receptor	51, 52
Motility (circular smooth muscle contraction)			
Stomach	Decrease	EP1 receptor	11
Small intestine	Decrease	EP4 receptor	12
Th1 cytokine secretion			
Large intestine	Decrease	EP4 receptor	76

which PGE_2 protects against acid reflux esophagitis remains unkown, although this action is known to be brought about by the activation of EP1 receptors.

3. Gastric protection

A variety of models have been used to assess antiulcer drugs, and PGE_2 is shown to be effective in most (1, 2). Among them, gastric lesions produced by necrotizing agents (HCl/ethanol, etc.) and NSAIDs are considered the most suitable for examining the protective action of PGE_2 in the stomach (8, 10, 11). By contrast, it is known that prostacyclin (PGI_2) compared to PGE_2 is more effective in preventing the occurrence of gastric lesions under stressed conditions such as cold-restraint or ischemia/reperfusion (I/R) (17, 18). We introduce herein the protective effect of PGE_2 against gastric lesions produced by HCl/ethanol and indomethacin as well as stress.

3.1. HCl/ethanol-induced gastric damage

3.1.1. Direct cytoprotection

Oral administration of HCl/ethanol (1 ml, 60% ethanol in 150 mM HCl) produced multiple band-like lesions in the glandular mucosa, along the long axis of the stomach. PGE_2 given prior to HCl/ethanol dose-dependently prevented the development of these lesions. This action of PGE_2 was mimicked by a prostanoid, such as 17-phenyl PGE_2 or sulprostone, specific to the EP1 receptor, and was significantly attenuated by ONO-AE-829, a selective EP1 antagonist (8). Neither butaprost, ONO-NT-012, nor 11-deoxy PGE_1 had any effect on the gastric ulcerogenic response to HCl/ethanol. Of interest, the EP4 agonist ONO-AE1-329 dose-dependently reduced the severity of HCl/ethanol-induced gastric lesions, but the effect was only partially mitigated by the EP4 antagonist ONO-AE3-208, suggesting that the effect may be largely due to an action unrelated to the activation of EP4 receptors (19). Certainly, the protective effect of PGE_2 was attenuated by the EP1 antagonist but not the EP4 antagonist (8, 19). It is thus assumed that the protective action of PGE_2 against HCl/ethanol is mediated by activation of the EP1 receptors. These results obtained in rats were confirmed using EP-receptor–knockout mice. Oral administration of HCl/ethanol produced similar band-like lesions in the stomachs of wild-type mice and those lacking EP1 or EP3 receptors. The development of these lesions was prevented by prior administration of PGE_2 in both wild-type and EP3-receptor–knockout mice but not in the animals lacking EP1 receptors (8).

3.1.2. Adaptive cytoprotection

When the stomach is pre-exposed to a mild irritant such as taurocholate (TC), the resistance of the mucosa to subsequently applied necrotizing agents increases, a phenomenon called “adaptive cytoprotection” (20). Since this effect disappears in the presence of indomethacin, a COX inhibitor, it is assumed to be mediated through the enhanced production of endogenous PGs. Indeed, 20 mM TC given p.o. increased the PGE_2 content in the stomach and prevented the formation of gastric lesions

induced by a subsequent challenge with HCl/ethanol (10). This effect of TC was also antagonized by ONO-AE-829, the EP1 antagonist, but not affected by AE3-208, the EP4 antagonist, as well as NS-398, a selective COX-2 inhibitor, suggesting that the adaptive gastric cytoprotection is mediated mainly by endogenous COX-1/PGE_2 through EP1 receptors. Likewise, TC acted as a mild irritant in the mouse stomach to increase production of PGE_2, which resulted in prevention of HCl/ethanol-induced damage. This effect of TC was significantly mitigated by pretreatment with indomethacin as well as ONO-AE-829. In addition, the protective action of TC was observed in EP3-receptor–knockout mice but totally disappeared in EP1-receptor–knockout animals (10, 21). These results strongly suggest that EP1 receptors are essential for the cytoprotective action of PGE_2, either generated endogenously or administered exogenously, in the stomach against necrotizing agents.

3.1.3. Capsaicin-induced cytoprotection

Endogenous PGs play a role in the gastric cytoprotection induced by capsaicin and some antiulcer drugs. Capsaicin in particular is unique in that it causes a selective stimulation of capsaicin-sensitive afferent neurons through interaction with vaniloid type 1 receptors (22). The protective action of capsaicin was totally blocked by chemical ablation of these afferent neurons and significantly attenuated by the antagonist of calcitonin gene-related peptide (CGRP) as well as nitric oxide (NO) synthase inhibitors. Thus, it is considered that capsaicin exhibits gastroprotective action through capsaicin-sensitive afferent neurons mediated by both CGRP and NO. Interestingly, the protective action of capsaicin was also significantly mitigated in the presence of indomethacin, suggesting an involvement of endogenous PGs, similar to the case of adaptive cytoprotection induced by a mild irritant (23, 24). However, this effect of capsaicin was not affected by the selective EP1 antagonist, in contrast to that of TC as a mild irritant (18). It should also be noted that neither stimulation of sensory neurons by capsaicin nor sensory deafferentation affected mucosal PGE_2 levels in the stomach. These results suggest that although endogenous PGs are involved in the gastric protection induced by both mild irritants and capsaicin, the mode of action seems to be different in these two cases (10, 21). It is assumed that the stimulation of afferent neurons by capsaicin does not increase production of PG in the stomach, yet it exerts a gastroprotective action partly dependent on endogenous PGs. We found that the protective action of capsaicin was significantly restored even in the presence of indomethacin by prior administration of the EP2 agonist butaprost, but not an EP3 or EP4 agonist. Since the capsaicin-induced gastric protection was not affected by the EP1 antagonist, it is unlikely that EP1 receptors are involved in the facilitation by endogenous PGs of this action. Indeed, significant protection by capsaicin was observed even in the knockout mice lacking EP1 and EP3 receptors, confirming that the capsaicin-induced gastric protection has nothing to do with the EP1 and EP3 receptors. However, we found that capsaicin did not provide gastric cytoprotection against HCl/ethanol in IP-receptor–knockout animals (21). These findings in knockout mice suggest that IP receptors are also involved in the protective action of capsaicin in the stomach, in addition to EP2 receptors. At present, the exact mechanism by which endogenous PGs contribute to the protective action of capsaicin remains unknown. Boku et al. (25) reported a lack of release of CGRP in response to mild injury in the stomach of IP-receptor–knockout mice. Thus, it is assumed that endogenous PGI_2 plays a supportive role in the mechanism of capsaicin-induced gastric cytoprotection, probably by sensitizing capsaicin-sensitive afferent neurons.

3.2. Indomethacin-induced gastric damage

NSAIDs such as indomethacin damage the stomach of experimental animals and humans through adverse reactions. Since these drugs induce a depletion of endogenous PGs by inhibiting COX activity, it is considered that a deficiency of PG is a major pathogenic factor in this model. Indeed, gastric ulceration induced by indomethacin was effectively and dose-dependently prevented by the administration of PGE_2 (11, 26). This effect of PGE_2 was mimicked by sulprostone and 17-phenyl PGE_2, both having a strong affinity for EP1 receptors, and significantly attenuated by the EP1 antagonist ONO-AE-829, the result being similar to the protective action against HCl/ethanol (11). Neither butaprost, ONO-NT-012, nor 11-deoxy PGE_1 afforded significant protection against indomethacin-generated gastric lesions. In addition, indomethacin caused gastric damage similarly in both wild-type and knockout mice lacking EP1 or EP3 receptors, yet the protective action of PGE_2 was observed in wild-type and EP3-receptor–knockout mice but not in mice lacking EP1 receptors. Given the above findings, it is assumed that PGE_2 prevents indomethacin-induced gastric ulceration through the activation of EP1 receptors.

3.3. Stress-induced gastric damage

Ischemia followed by reperfusion (I/R) leads to tissue injury (27, 28). The development of gastric lesions in response to I/R was significantly aggravated by the selective COX-2 inhibitor rofecoxib, similar to indomethacin, confirming the involvement of COX-2/PGs in mucosal defense during I/R (17, 29). In addition, the severity of

I/R-induced gastric damage was markedly increased in IP-receptor–knockout mice but not in the animals lacking EP1- or EP3-receptors. These results suggest that the type of prostanoid responsible for mucosal defense during I/R is PGI_2 not PGE_2. These results are understandable because the expression of COX-2 in the gastric mucosa following I/R was observed mainly in the endothelial cells (30) and because PGI_2 is a major prostanoid produced in the endothelial cells (31). Indeed, we observed that iloprost, a stable analogue of PGI_2, significantly prevented the I/R-induced gastric damage, in the absence or presence of COX inhibitors, supporting the involvement of endogenous PGI_2 in mucosal defense during I/R. This PGI_2 analogue has an affinity for not only IP receptors but also EP receptors as well (32). However, iloprost had no effect on the development of I/R-generated gastric lesions in IP-receptor–knockout mice, excluding the involvement of EP receptors in the protective action of this agent. Thus, it is assumed that endogenous PGs derived from COX-2 play a crucial role in gastric mucosal defense during I/R, and this action is mainly mediated by PGI_2 through the activation of IP receptors.

Cold-restraint stress (10ºC, 90 min) induced multiple hemorrhagic lesions in wild-type mice, and the severity of these lesions was significantly aggravated by pretreatment with indomethacin or SC-560 but not rofecoxib (18). Furthermore, the gastric ulcerogenic response to cold-restraint stress was similar in EP1- or EP3-receptor–knockout animals, as compared to wild-type mice, but significantly worsened in the animals lacking IP-receptors. Pretreatment of wild-type animals with iloprost significantly prevented the stress-induced gastric damage in the absence or presence of indomethacin. The expression of COX-2 mRNA was not detected in the stomach following stress while COX-1 expression was observed under normal and stressed conditions. These results suggest that endogenous PGs derived from COX-1 play a crucial role in protecting the gastric mucosa against cold-restraint stress, and this action is mainly mediated by PGI_2 through the activation of IP receptors.

Although the COX isozyme responsible for PG production is different in these two stress models, the importance of PGI_2/IP receptors in the gastric mucosal defense is common to both models.

3.4. Functional alterations related to gastric protection

Endogenous PGs play a role in the regulation of various gastric functions, such as acid secretion, mucus/bicarbonate secretion, mucosal blood flow, and motility, that may contribute to gastric cytoprotection. According to previous studies including our own (7, 9, 33 – 36), PGE_2 inhibits acid secretion through EP3 receptors and increases mucus and bicarbonate secretion in the stomach through EP4 and EP1 receptors, respectively. Recently, we also found that PGE_2 has an acid stimulatory effect mediated by histamine released from enterochromaffin-like (ECL) cells through EP4 receptors (37). In addition, the acid inhibitory action of PGE_2 is mediated by EP3 receptors in two ways, directly by inhibiting acid secretion at the parietal cells and indirectly through inhibition of histamine release at ECL cells. In a preliminary study, we observed that gastric mucosal blood flow was increased by EP2, EP3, and EP4 agonists but not EP1 agonists (8). Of interest, prostanoids exhibiting a preference for only EP1 receptors affected gastric motility and provided mucosal protection against gastric lesions produced by HCl/ethanol or indomethacin (8, 11). These effects were both antagonized by ONO-AE-892, an EP1 antagonist, suggesting that the motility effect of PGE_2 is paralleled by a reduction in gastric mucosal damage.

We reported that a variety of compounds afforded gastric cytoprotection at doses that inhibit gastric motility (23, 38, 39). The inhibition of gastric motility may lead to a flattening of the mucosal foldings and a decrease in mucosal vulnerability to irritants, resulting in prevention of the fold-related band-like lesions, as observed following the administration of HCl/ethanol. A role for muscle elements in the pathogenic mechanism of indomethacin-induced gastric ulceration has also been demonstrated (26, 38, 40). Mersereau and Hinchey (38) were the first to show the importance of stomach hypermotility and mucosal foldings in the genesis of gastric lesions in response to NSAIDs. We also reported that indomethacin at an ulcerogenic dose enhances gastric motility and induces microcirculatory disturbances due to abnormal mucosal compression of the gastric wall (40, 41). Since neither butaprost, ONO-NT-012, nor 11-deoxy PGE_1 provided any gastric protection against HCl/ethanol or indomethacin, despite causing an increase in gastric mucosal blood flow, it is unlikely that the gastric cytoprotection afforded by PGE_2 is functionally associated with an increase of gastric mucosal blood flow (8). Certainly, because inhibition of gastric motility may lead to attenuation of microvascular disturbances due to stomach contraction, it is possible that prostanoids through EP1 receptors help to maintain mucosal blood flow during exposure to noxious agents.

The mechanism by which PGE_2 inhibits gastric motility through EP1 receptors remains unknown. Milenov and Golenhofen (42) reported that PGE_2 relaxed the circular muscle but contracted the longitudinal muscle of the canine stomach. Narumiya and his group reported the distribution of mRNA of the EP receptors along the gastrointestinal tract (43, 44). They showed that strong

signals for EP1 transcripts occurred in the smooth muscle cells in the muscularis mucosa throughout the tract. Since EP1 receptors are coupled to phosphatidyl inositol (PI) turnover (6), it is assumed that contraction of longitudinal smooth muscle by PGE_2 is associated with an increase of cytosolic calcium. Contraction of circular smooth muscle leads to the appearance of mucosal folds, which have been implicated in the pathogenesis of ulcers including indomethacin-generated gastric lesions (26, 38, 40, 41). At present, the mechanism by which PGE_2 relaxes circular smooth muscle through activation of EP1 receptors is unknown.

Neutrophils have been implicated in the damage associated with NSAIDs (45). It is known that PGE_2 has an inhibitory effect on neutrophil functions, including chemotaxis (46). We confirmed that PGE_2 exhibited an inhibitory effect on the migration of neutrophils caused by formyl-methionyl-leucyl-phenylalanine in vitro (11). The same inhibitory action was shown by both butaprost and 11-deoxy PGE_1, but not by 17-phenyl PGE_2, sulprostone, or ONO-NT-012, clearly indicating that the anti-neutrophil chemotaxis action of PGE_2 is mediated by activation of EP2 and EP4 receptors. Thus, it is assumed that the inhibition of neutrophil migration by itself is not sufficient to reduce the overall expression of gastric lesions in response to indomethacin. Since the increase in myeloperoxidase activity as well as ulceration induced by indomethacin was prevented when the enhanced gastric motility was inhibited by atropine (39), it is likely that the neutrophil infiltration is secondary to the event associated with gastric hypermotility following indomethacin treatment. Melange et al. (47) even showed that NSAID-induced gastric injury is neutrophil-independent in the neutropenic rat.

Endogenous PGE_2 also plays a role in the gastric hyperemic and protective responses following barrier disruption in the stomach as induced by bile acids. We reported that the COX-1 isozyme is involved in gastric functional responses, such as an increase of gastric mucosal blood flow and a decrease in acid secretion, observed acutely after barrier disruption in the stomach (48 – 51). These functional alterations following barrier disruption are adaptive responses of the stomach and play an important role in protecting the mucosa against acid injury by disposing of H^+ and maintaining a microclimate for cellular restitution. This hyperemic response in the damaged stomach is attenuated by the EP1 antagonist ONO-8711 and disappears in EP1-receptor–knockout mice, strongly suggesting mediation by the activation of EP1 receptors (52). PGI_2/IP receptors do not play a role in this phenomenon (53).

4. Duodenal protection and HCO_3^- stimulation

Duodenal mucosal HCO_3^- secretion is a key process that aids in preventing acid-peptic injury. This is most exemplified by the finding that the tissues respond to acid by secreting more HCO_3^- (54). Although this process has been shown to involve both humoral and neural factors as well as PGs (55), it is thought that endogenous PGs are particularly important in the local control of this secretion. Indeed, PGE_2 and its analogues, whether applied luminally or vascularly, stimulate duodenal HCO_3^- secretion in vivo and in vitro, in a variety of species and in this way may contribute to protection of the mucosal epithelium against acid-induced injury (56). We have recently shown that COX-1 but not COX-2 is a key enzyme in regulating this process and maintaining the mucosal integrity against acid in the duodenum (57).

PGE_2 increased HCO_3^- secretion by the rat duodenal mucosa; this action was verapamil-sensitive and potentiated by an inhibitor of phosphodiesterase, isobutylmethylxanthine (IBMX) (9). This effect was mimicked by sulprostone, ONO-NT012, 11-deoxy PGE_1, and ONO-AE1-329 but not by butaprost or 17-phenyl PGE_2 (9, 58). These results strongly suggest that PGE_2 stimulates duodenal HCO_3^- secretion via both EP3 and EP4 receptors, and this action is coupled with Ca^{2+} and adenosine 3′,5′-cyclic monophosphate (cAMP). Concerning the EP3 receptors, 4 splicing variants exist, each coupled to different signaling pathways; the EP3A receptor is linked to the activation of Gi protein, the EP3B and EP3C receptors are coupled with the activation of Gs protein resulting in stimulation of adenylate cyclase (AC) activity, and activation of the EP3D receptor causes an increase in intracellular Ca^{2+} by stimulating PI turnover via Gq protein (6). Thus, it is possible that EP3B, EP3C, and EP3D receptors are involved in stimulating the secretion of HCO_3^- in the duodenum. On the other hand, the duodenal response to the EP4 agonist ONO-AE1-329 was significantly augmented by pretreatment with IBMX but not affected by verapamil, confirming the mediation by cAMP of the action of the EP4 agonist (59). In general, a synergetic response to pharmacological actions is produced by the activation of two different signaling pathways. It remains unknown whether or not the Ca^{2+} and cAMP pathways are activated by EP3 agonists at a similar time or dose, yet it seems 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. Morimoto et al. (43) demonstrated by Northern blot analysis the significant expression of

EP3 and EP4 receptors in the gastroduodenal mucosal layer containing epithelial cells and also in the neurons of the myenteric ganglia throughout the gastrointestinal tract. These results are compatible with the present observation that HCO_3^- secretion, an epithelial function, is mediated by EP3 and EP4 receptors in the duodenum.

In the duodenum of wild-type mice, secretion of HCO_3^- increased in response to luminal perfusion of PGE_2 and forskolin as well as mucosal acidification (7). The latter effect was significantly inhibited by prior administration of indomethacin. The increase in HCO_3^- secretion in response to acid was observed in EP1-receptor–knockout mice but disappeared in the animals lacking EP3 receptors, although the acidification increased mucosal PGE_2 levels to a similar degree in all groups. Consistent with the results obtained with rats, the stimulatory effect of PGE_2 on HCO_3^- was markedly reduced in EP3-receptor–knockout but not EP1-receptor–knockout mice, but forskolin's effect was observed in both groups of animals, similar to wild-type mice. It is believed that the acid-induced HCO_3^- secretion is mediated via an axonal reflex pathway, in addition to endogenous PGs, and the mediator on the efferent side of this reflex pathway may be vasoactive intestinal peptide (59). Since this response is substantially inhibited by indomethacin, it is also speculated that the afferent side of this pathway is influenced by PGs, probably by facilitating neuronal excitation in response to H^+. We have previously reported that acid-induced HCO_3^- secretion was significantly attenuated by chemical ablation of capsaicin-sensitive afferent neurons and that the stimulatory effect of capsaicin on HCO_3^- is also suppressed by indomethacin (60). EP3 receptors, which are a prerequisite for acid-induced duodenal HCO_3^- secretion, might be present on cells on the afferent side of the reflex pathway. It is assumed that the local release of PGE_2 would stimulate the reflex pathway on the afferent side and may also directly stimulate the epithelial cells, both resulting in an increase in HCO_3^- secretion.

As mentioned above, the secretion of HCO_3^- plays an important role in protection of the duodenal mucosa against luminal acid (54, 61). Indeed, perfusion of the proximal duodenum with 20 mM HCl for 4 h produced only a few hemorrhagic lesions in wild-type mice. Gene disruption of EP1 receptors did not affect the duodenal ulcerogenic response to acid perfusion, and the lesion score was not different from that of wild-type mice. In EP3-receptor–knockout mice, however, acid perfusion for 4 h generated severe lesions over almost the entire proximal duodenum, the lesion score being about 6 times greater than that of wild-type littermates (7). Certainly, increased duodenal ulcerogenecity to acid perfusion was also observed in wild-type mice after indomethacin pretreatment. It is assumed that a decrease of HCO_3^- secretion in EP3-receptor–knockout mice leads to a progressive breakdown of the mucosal defensive response to acid and increases the mucosal susceptibility to acid injury. Thus, the presence of EP3 receptors is essential for maintaining duodenal HCO_3^- secretion and mucosal integrity against luminal acid. We also demonstrated in rats that duodenal damage caused by mucosal perfusion with 150 mM HCl for 4 h was worsened by pretreatment with AE5-599 (EP3 antagonist) and AE3-208 (EP4 antagonist) as well as indomethacin and further aggravated by co-administration of these antagonists (62). These results suggest that both EP3 and EP4 receptors are involved in maintaining the duodenal mucosal integrity against acid.

5. Small intestinal protection

NSAIDs such as indomethacin are known to cause intestinal damage, including ulcers complicated by bleeding and perforation, in experimental animals and in humans. Although several factors have been postulated as pathogenic elements of intestinal ulceration induced by indomethacin, including a deficiency of PGs, bile acid, bacterial flora, and nitric oxide (NO) (63), the exact mechanisms remain unexplored. It is, however, certain that a deficiency of PGs plays a critical role in the pathogenesis of these lesions. Indeed, all these events caused by indomethacin are effectively prevented by supplementation with exogenous PGE_2 (12, 64).

5.1. Indomethacin-induced small intestinal damage

Indomethacin caused hemorrhagic lesions in the rat small intestine, mainly in the jejunum and ileum, accompanied by an increase in enterobacterial translocation. The development of these lesions was prevented by pretreatment of the animals with 16,16-dimethyl PGE_2 in a dose-dependent manner (12). Other prostanoids such as ONO-NT-012 and ONO-AE1-329 also provided dose-dependent protection against indomethacin-induced intestinal damage, while neither 17-phenyl PGE_2 nor butaprost had any effect on these lesions. These results strongly suggest that the intestinal protection by dmPGE_2 against indomethacin is brought about by activation of EP3 and EP4 receptors, similar to the protective action in the duodenum. We confirmed this using EP-receptor–knockout mice and showed that 16,16-dimethyl PGE_2 provided less protection against indomethacin-induced intestinal damage in the animals lacking EP3 receptors, although the agent exhibited marked inhibition in both wild-type and EP1-receptor–knockout mice (64). The fact that even in EP3-receoptor–knockout mice 16,16-dimethyl PGE_2 provided partial protection against these

lesions, supports the involvement of another EP-receptor subtype, EP4, in the protective action of 16,16-dimethyl PGE_2.

5.2. Functional alterations related to small intestinal protection

Although multiple factors are implicated in the pathogenesis of indomethacin-induced intestinal damage, enterobacteria and NO play a key pathogenic role in this model; the release of bacterial products such as endotoxin contributes to the development of intestinal damage through overproduction of NO by up-regulating the expression of inducible NO synthase (iNOS) in the mucosa (65). Indeed, the prevention of these lesions was observed on the blockade of NO production through inhibition of the iNOS activity by an NO synthase inhibitor or iNOS expression by dexamethasone (66, 67). It was also suggested that NO interacts with the superoxide radicals to produce a cytotoxic peroxynitrite, which has a deleterious influence on the intestinal mucosal integrity. Certainly, the development of intestinal lesions as well as bacterial translocation and the up-regulation of iNOS activity following treatment with indomethacin were both markedly prevented by supplementation with 16,16-dimethyl PGE_2, suggesting a pathogenic role for PG deficiency in this model (12). These effects of 16,16-dimethyl PGE_2 were reproduced by ONO-NT-012 and ONO-AE1-329 but not by 17-phenyl PGE_2 or butaprost, confirming a close relationship between intestinal protection and prevention of bacterial translocation as well as iNOS activity.

It is known that mucin plays an important part in the innate host defense against intestinal pathogens and irritants. We found that $dmPGE_2$, ONO-NT-012, and ONO-AE1-329 all increased the amount of mucus secreted in the small intestine, suggesting the involvement of EP3/EP4 receptors in the stimulatory action of PGE_2 (12). Belly and Chadee (68) demonstrated that PGE_2 coupled to the EP4 receptor stimulates cAMP-dependent mucin exocytosis in the rat colon. Although the reason for these different results remains unknown, experimental conditions such as the tissues used may be a factor. In any case, it is possible that PGE_2, by stimulating the secretion of mucus and by increasing the mucus gel's thickness, hampers bacterial invasion in the mucosa, which is responsible for excessive NO production through the induction of iNOS expression. In addition, secretion of intestinal fluid may prevent the process of bacterial translocation, by washing out these microorganisms. The enteropooling was increased by $dmPGE_2$, ONO-NT-012, and ONO-AE1-329, suggesting stimulation of this process by EP3 and EP4 receptors (12). Since the amount of fluid accumulated in the intestine can be affected by changes in secretion, absorption, transit, and the volume of upper gastrointestinal secretions, the interpretation of these results is limited. Yet, this event is largely influenced by intestinal fluid (Cl^-) secretion. Several studies have examined the effect of PGE_2 on Cl^- secretion in the gastrointestinal tract. Gastrointestinal Cl^- secretion was reportedly stimulated by PGE_2 through activation of both EP3 and EP4 receptors (69). Since prostanoids exhibiting a preference for EP3 and EP4 receptors stimulated the secretion of mucus and fluid and provided intestinal protection against indomethacin, it is likely that these processes contribute to the intestinal protection afforded by PGE_2, through suppression of bacterial translocation. Interestingly, indomethacin caused a marked increase of intestinal motility, resulting in an increase in both the amplitude and frequency of contractions (12, 65, 70, 71). Because the spasmodic nature of the intestinal motility results in a disruption of the unstirred mucus layer over the epithelium, leading to an increase in mucosal susceptibility to pathogens and irritants, the enhanced intestinal contractions may also be part of the pathogenic mechanism for indomethacin-induced small intestinal damage. The enhanced intestinal motility caused by indomethacin was antagonized by both $dmPGE_2$ and another prostanoid specific to EP4 receptors. Since EP4 receptors are coupled to AC, it is speculated that the relaxation of circular smooth muscle by PGE_2 is associated with an increase of intracellular cAMP.

Thus, intestinal protection by PGE_2 may be functionally associated with the stimulation of mucus and fluid secretion as well as inhibition of intestinal hypermotility, the former two processes being mediated by both EP3 and EP4 receptors and the latter mediated by EP4 receptors (12). These functional changes strengthen the barrier against intestinal pathogens and irritants, resulting in prevention of bacterial invasion and inhibition of iNOS up-regulation and by so doing prevent the development of small intestinal lesions.

6. Large intestinal protection

Ulcerative colitis is a chronic inflammatory disease of unknown etiology affecting the rectum and colon (72). Experimental colitis induced by dextran sodium sulfate (DSS) is accompanied by erosion and ulceration as well as inflammatory cell infiltration, characteristics resembling those of human ulcerative colitis (73). Studies demonstrated that the occurrence of DSS-induced colitis in mice or rats was prevented by ONO-AE1-329, an EP4 agonist, and worsened by ONO-AE3-208, an EP4 antagonist, suggesting a protective effect of endogenous PGs on DSS-induced colitis (74 – 76). Kabashima et al. (76) examined the roles of prostanoids in DSS-induced colitis,

using mice deficient in various prostanoid receptors and found that only EP4-deficient mice developed severe colitis with DSS treatment. They also showed that this phenotype was mimicked in wild-type mice by administration of AE3-208 (EP4 antagonist), while AE1-734 (EP4 agonist) ameliorated severe colitis in wild-type mice. Furthermore, Nitta et al. (75) reported that expression of EP4 receptors was up-regulated during DSS treatment. It is thus assumed that endogenous PGE_2 suppresses DSS-induced colitis, mainly via the activation of EP4 receptors. This idea is supported by the findings that DSS-induced colitis was aggravated by NSAID due to suppression of PG production (77, 78).

It is known that PGE_2 inhibits inflammatory cytokines and stimulates mucus secretion in the gastrointestinal mucosa through activation of EP4 receptors (68, 74, 76). Kabashima et al. (76) reported that ONO-AE3-208 enhanced and ONO-AE1-734 suppressed Th1 cytokine production of lamina propria mononuclear cells from the colon. On the other hand, several studies support a pathogenic role for enterobacteria in experimental colitis and inflammatory bowel diseases (72, 79). Indeed, the antibiotic metronidazole did prevent the occurrence of DSS-induced colitis (80, 81). Since the mucus layer is a barrier to bacterial infiltration, it is possible that PGE_2 hampers bacterial invasion in the mucosa by strengthening the mucus barrier through stimulation of the secretion of mucus. Tanaka et al. (78) showed that expression of mucin proteins was involved in the exacerbation by NSAIDs of DSS-induced colitis and its suppression by PGE_2. A cause–effect relationship between the increased mucus secretion and the prevention of bacterial infiltration has also been demonstrated in the rat small intestine using 16,16-dimethyl PGE_2 or several mucosal protective drugs (12, 82). Although further studies are needed to elucidate the mechanism underlying the PGE_2-induced colonic protection, it is assumed that EP4 receptors play a role in maintaining intestinal homeostasis by keeping mucosal integrity and down-regulating the immune response.

7. Healing-promoting action

The healing of gastric ulcers was significantly delayed in both rats and mice by indomethacin and rofecoxib but not SC-560, given for 7 – 14 days after the ulceration, indicating the importance of COX-2/PGs (83 – 85). The impaired healing was also observed in COX-2 knockout mice (85, 86). Mucosal PGE_2 content increased after the ulceration, and this response was significantly suppressed by indomethacin and rofecoxib but not SC-560. The delayed healing caused by indomethacin was significantly reversed by the co-administration of 11-deoxy PGE_1 (EP3/EP4 agonist), but not other prostanoids including the EP1, EP2, and EP3 agonists. By contrast, CJ42794 (selective EP4 antagonist) significantly delayed the healing process in rats and mice (85, 87). Vascular endothelial-derived growth factor (VEGF) expression and angiogenesis were both up-regulated in the ulcerated mucosa, and these responses were suppressed by indomethacin, rofecoxib, and CJ42794. The expression of VEGF in primary rat gastric fibroblasts was increased by PGE_2 or ONO-AE1-329 (EP4 agonist), and these responses were both attenuated by co-administration of CJ42794. These results confirmed the importance of COX-2/PGE_2 in the healing of gastric ulcers and further suggested that the healing-promoting action of PGE_2 is mediated by the activation of EP4 receptors and associated with VEGF expression.

Essentially similar results were obtained in the healing of small intestinal lesions produced by indomethacin (88, 89). Indomethacin (10 mg/kg) caused severe damage in the small intestine, but the lesions healed rapidly, decreasing to about 1/5 of their initial size within 7 days. The healing process was significantly impaired by indomethacin (2 mg/kg) given once daily for 6 days after the ulceration. This effect of indomethacin was mimicked by the EP4 antagonist ONO-AE3-208 and reversed by co-administration of ONO-AE1-329. Mucosal VEGF expression was up-regulated after the ulceration, reaching a peak on day 3 and then decreasing. The changes in VEGF expression paralleled those in mucosal COX-2 expression as well as PGE_2 content. Indomethacin (2 mg/kg) reduced both VEGF expression and angiogenesis in the mucosa during the healing process, and these effects were significantly reversed by co-treatment with the EP4 agonist. These results suggest that endogenous PGE_2 promotes the healing of small intestinal lesions by stimulating angiogenesis via the up-regulation of VEGF expression mediated by the activation of EP4 receptors.

8. Proulcerogenic action

Since PGs act as mediators in the inflammatory responses of various tissues (90, 91), it is possible that they have a deleterious influence on the gastric mucosa when administered together with other inflammatory mediators such as histamine. Indeed, Wedgewood et al. (92) reported a pathogenic role of PGs in a model of pancreatitis in cats and suggested that an increase in microvascular permeability in the pancreas caused by 16,16-dimethyl PGE_2 converted edematous pancreatitis to hemorrhagic pancreatitis. Likewise, pretreatment with PGE_2 worsened gastric mucosal injury induced by histamine (80 mg/kg dissolved in 10% gelatin) in rats, and this effect was associated with potentiation of the increased vascular

permeability caused by histamine through stimulation of H_1-receptors (93, 94). This effect of PGE_2 was mimicked by 17-phenyl PGE_2 and sulprostone, but not other EP agonists, including EP2, EP3, and EP4 agonists. The mucosal vascular permeability was slightly increased by histamine, and this response was markedly enhanced by co-administration of 17-phenyl PGE_2 as well as PGE_2. Both the mucosal ulcerogenic and vascular responses to histamine plus PGE_2 were suppressed by pretreatment with ONO-AE829 (EP1 antagonist), suggesting that the aggravation by PGE_2 of these responses is mediated by EP1 receptors and functionally associated with potentiation of the increased vascular permeability caused by histamine.

It is interesting that PGE_2, on the one hand, exhibits protective action in the stomach against necrotizing agents through EP1 receptors, yet on the other hand, aggravates histamine-induced gastric ulceration mediated by the same receptor subtype. Szabo et al. (95) proposed a "histodilution barrier" as one of the mechanisms for PGE_2-induced gastric cytoprotection. The hypothesis is based on the accumulation of fluid at the extracellular site, leading to the dilution of toxic substances. Since edema is an accumulation of fluid at an extracellular site resulting from increased vascular permeability, it does not seem unreasonable that PGE_2 induces both protective and proulcerogenic actions through activation of the same EP receptor subtype. However, it remains unknown whether PGE_2 potentiates histamine-induced vascular permeability by acting directly on the vascular smooth muscle or by interacting with histamine at H_1-receptors through the activation of EP1 receptors.

9. 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. This paradigm is largely based on the finding of "gastric cytoprotection" by Robert et al. (2). Since then, a number

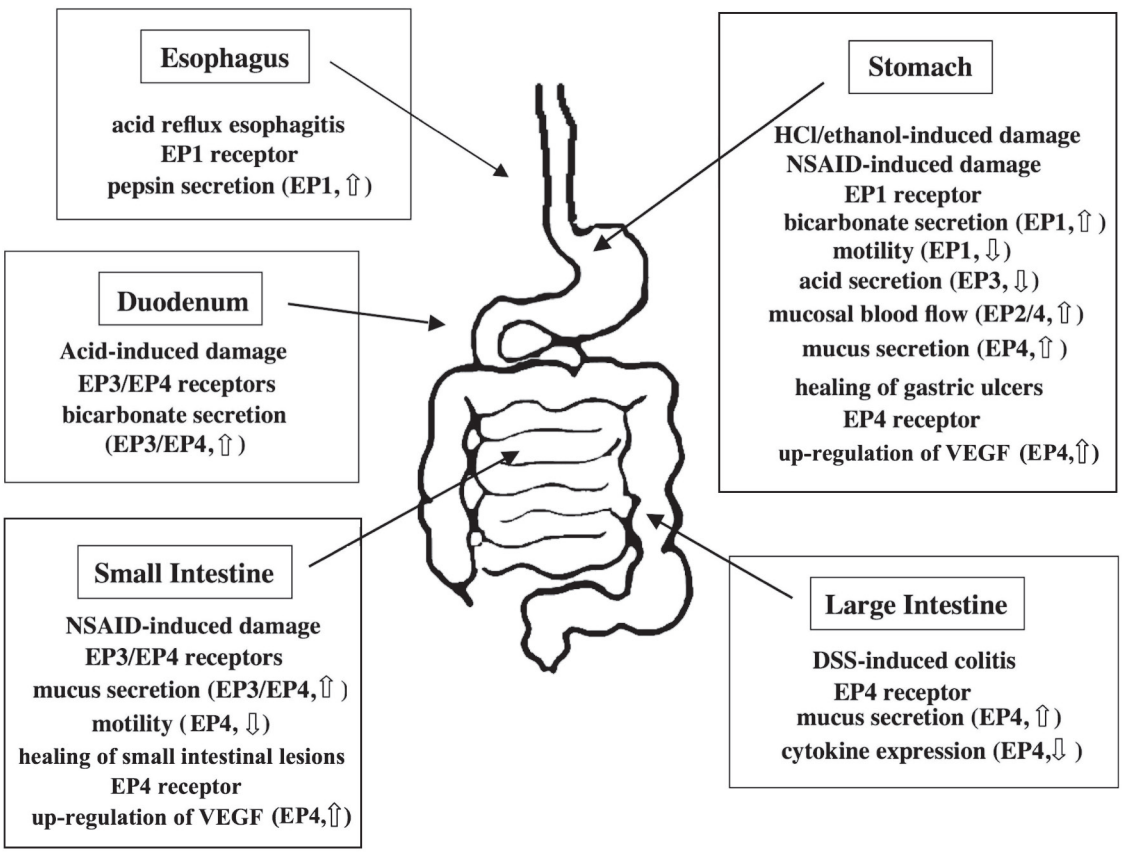

Fig. 1. EP-receptor subtypes involved in the protective and healing-promoting actions of PGE_2 in the gastrointestinal tract. PGE_2 exhibits protective effects in various organs, including the esophagus, stomach, duodenum, and small and large intestines. However, the EP receptor subtypes involved in these actions differ depending on the tissue, for example, the protective effect in the stomach is mediated by EP1 receptors, while that in the duodenum is mediated by both EP3 and EP4 receptors. In addition, PGE_2 promotes healing of gastric ulcers or small intestinal lesions via the activation of EP4 receptors. Certainly, the functional changes responsible for these actions also differ depending on the tissues and are mediated by different EP-receptor subtypes.

of studies have been conducted to elucidate the factors involved in this phenomenon, yet the true mechanism underlying this action remains unexplored. As reviewed in this paper, exogenous PGE_2 prevents acid-reflux esophagitis and affords protection of the stomach against ulcerogenic stimuli, irrespective of whether it is necrotizing agent (HCl/ethanol) or NSAID (indomethacin), mainly through the activation of EP1 receptors (Fig. 1). As observed in the adaptive cytoprotection induced by a mild irritant, endogenous PGE_2 also exhibits gastric protection mediated by EP1 receptors. On the other hand, PGE_2 affords protection of the intestinal mucosa, including the duodenum and small intestine, through the activation of both EP3 and EP4 receptors. The underlying mechanism related to these actions of PGE_2 in the esophagus, stomach, duodenum, or small intestine may be related to inhibition of pepsin secretion (EP1), gastric contraction (EP1), stimulation of duodenal alkaline secretion (EP3/EP4), or suppression of bacterial translocation due to inhibition of intestinal contraction (EP4) as well as stimulation of mucus secretion (EP3/EP4), respectively. This prostanoid also protects the colon from ulceration through the activation of EP4 receptors, probably by keeping mucosal integrity and down-regulating the immune response (75, 76). Furthermore, PGE_2 shows a healing-promoting effect on gastric ulcers or intestinal lesions, through the up-regulation of VEGF expression and stimulation of angiogenic responses via the activation of EP4 receptors. Since the results introduced in this paper were obtained in rats using subtype-specific EP agonists and were further confirmed in EP-receptor–knockout mice, they would be reliable and have high reproducibility compared to those obtained in either rats or knockout mice alone. Anyway, it is worth noting that the EP-receptor subtypes responsible for cytoprotection are different depending upon the tissues and that the functional alterations responsible for the protective action also differ depending on the tissues. Even PGE_2 has biphasic effects in the stomach through the activation of the same receptor subtype, EP1; a protective action against necrotizing agents; and a proulcerogenic action in the presence of histamine (8, 10, 94), although the physiological relevance of this remains unknown. Notwithstanding, these approaches should contribute to further understanding of the mechanism of "cytoprotection" as well as "healing-promoting action" of PGs in the gastrointestinal tract and also to the future development of new strategies for the treatment of gastrointestinal diseases.

Acknowledgments

This research was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. We thank Professor Shu Narumiya, Kyoto University Faculty of Medicine for kindly supplying EP-receptor–knockout mice. We also thank Ono Pharmaceutical Co., Ltd. for generously supplying various EP agonists and antagonists.

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