INTRODUCTION

Prostaglandins (PGs), produced from the membrane phospholipid arachidonic acid by two isozymes of cyclooxygenase (COX), are present throughout the gastrointestinal tract and have been implicated in the modulation of mucosal integrity and regulation of various functions, including acid secretion, bicarbonate secretion, mucus production, and mucosal blood flow (1). The administration of PGs was shown to protect the gastrointestinal mucosa against ulcerogenic stimuli such as stress, necrotizing agents, and non-steroidal antiinflammatory drugs (NSAIDs). Robert et al. (2) were the first to demonstrate that PGs protected the stomach against necrotizing agents. Similar results were obtained using mice lacking various EP receptor subtypes; i.e., PGE<sub>2</sub> failed to provide both direct and adaptive cytoprotection in EP1 (-/-) mice, while capsaicin-induced protection was observed in EP1 (-/-) mice, but disappeared in IP (-/-) mice.

Various models have been used to assess antiulcer drugs, and among them gastric lesions produced by necrotizing agents, including ethanol, and NSAIDs are most frequently used as suitable model to examine the protective action of PGE<sub>2</sub> in the stomach (2, 7, 8). In contrast, prostacyclin (PGI<sub>2</sub>) was shown to protect the stomach against necrotizing agents (4).

Endogenous prostaglandins (PGs) play a role in modulating mucosal integrity and have various functions in the stomach, with E type PGs being the most effective. PGE<sub>2</sub> provides gastric cytoprotection against damage induced in rats by HCl/ethanol, indomethacin, or acid back-diffusion after barrier disruption. These effects were mimicked by EP1 agonists and/or attenuated by an EP1 antagonist, and disappeared in EP1 (-/-) mice. Furthermore, the adaptive cytoprotection induced by a mild irritant was attenuated by the EP1 antagonist and indomethacin. Capsaicin also provides gastric protection against HCl/ethanol, and its action was mitigated by indomethacin and sensory deafferentation, but not by the EP1 antagonist. Similar results were obtained using mice lacking various EP receptor subtypes; i.e., PGE<sub>2</sub> failed to provide both direct and adaptive cytoprotection in EP1 (-/-) mice, while capsaicin-induced protection was observed in EP1 (-/-) mice, but disappeared in IP (-/-) mice. The effects of PGE<sub>2</sub> on various gastric functions are mediated by different EP receptor subtypes; inhibition of acid secretion (EP3) and motility (EP1), stimulation of mucus secretion (EP4) and HCO<sub>3</sub><sup>-</sup> secretion (EP1), and an increase in mucosal blood flow (EP2/EP4). In conclusion, the presence of EP1 receptors is essential to the protective action of PGE<sub>2</sub>, either generated endogenously or administered exogenously, against HCl/ethanol or indomethacin, and this action is functionally associated with the inhibition of gastric motility. Endogenous PGs also contribute to maintaining mucosal integrity after barrier disruption through an increase in mucosal blood flow, which occurs via sensory neurons influenced by activation of the EP1 receptor.

Key words: prostaglandin E, prostacyclin, capsaicin, EP receptor subtype, cytoprotection, cyclooxygenase, gastrointestinal mucosa, hydrochloric acid, alkaline secretion
be involved in modulating the gastric cytoprotection afforded by capsaicin, a selective stimulant of capsaicin-sensitive afferent neurons (14). In addition, endogenous PGs are known to contribute to the maintenance of mucosal integrity after barrier disruption via an increase in mucosal blood flow (4).

We herein reviewed our publications on the relationship between EP receptor subtypes and the gastric cytoprotection afforded by endogenous or exogenous PGE2 against gastric lesions produced by HCl/ethanol, indomethacin, and barrier disruption as well as the modulating role of PGI2 in the protective effect of capsaicin against these lesions, and discussed the possible functional alterations responsible for gastric cytoprotection.

GASTRIC DAMAGE INDUCED BY NECROTIZING AGENTS

Direct cytoprotection

Oral administration of necrotizing agents such as 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. PGE2 given prior to HCl/ethanol prevented the development of these lesions in a dose-dependent manner. In addition, the mucosal ulcerogenic response to HCl/ethanol was dose-dependently reduced by pretreating the animals with 17-phenylPGE2 (EP1 agonist) and sulprostone (EP1/EP3 agonist) (8). However, neither butaprost (EP2 agonist), ONO-N1012 (EP3 agonist), nor 11-deoxy PGE1 (EP3/EP4 agonist) had any effect on the development of gastric lesions in response to HCl/ethanol. Previous report showed the importance of EP4 receptors in the gastric cytoprotective action of PGE2 (15). However, the protective effect of PGE2 was significantly abrogated by the EP1 antagonist (AE-829), but not by the EP3 (AE5-599) or EP4 antagonist (AE3-208), which excluded the involvement of EP4 receptors in this action of PGE2 (7, 16) (Fig. 1). We showed that the selective EP4 agonist AE1-329 dose-dependently reduced the severity of HCl/ethanol-induced gastric lesions; however, this effect was not influenced by the EP4 antagonist AE3-208 at a dose that totally antagonized the stimulatory effect of PGE2 on duodenal HCO3− secretion or NSAID-induced enteropathy (16-18). Even though some EP4 agonists may cause gastric cytoprotection, this effect may not be mediated by the activation of EP4 receptors and has been attributed to other actions unrelated with EP4 receptors. It should be noted that the cytoprotective action of PGE2 was not affected by chemical deafferentation (14). Thus, it is assumed that the protective action of PGE2 against HCl/ethanol is mediated by the activation of EP1 receptors.

The importance of EP1 receptors in PGE2-induced gastric protective action was confirmed using various EP receptor

Table 1. PGE2 EP receptor subtypes, their coupled G proteins, and signal messengers.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Isoform</th>
<th>G Protein</th>
<th>Second Messenger</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP1</td>
<td>Gq</td>
<td>Ca2+ ↑</td>
<td></td>
</tr>
<tr>
<td>EP2</td>
<td>Gs</td>
<td>cAMP ↑</td>
<td></td>
</tr>
<tr>
<td>EP3</td>
<td>EP3A</td>
<td>Gi</td>
<td>cAMP ↓</td>
</tr>
<tr>
<td></td>
<td>EP3B</td>
<td>Gs</td>
<td>cAMP ↑</td>
</tr>
<tr>
<td></td>
<td>EP3C</td>
<td>Gs</td>
<td>cAMP ↑</td>
</tr>
<tr>
<td></td>
<td>EP3D</td>
<td>Gi, Gs, Gq</td>
<td>cAMP ↓, cAMP ↑, Ca2+↑</td>
</tr>
<tr>
<td>EP4</td>
<td>Gs</td>
<td>cAMP ↑</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Various prostanoids, subtype-specific EP receptor agonists, and antagonists.

<table>
<thead>
<tr>
<th>Prostanoids</th>
<th>EP Subtype Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-Phenyl PGE2</td>
<td>EP1 agonist</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>EP1/EP3 agonist</td>
</tr>
<tr>
<td>Butaprost</td>
<td>EP2 agonist</td>
</tr>
<tr>
<td>ONO-N1012</td>
<td>EP3 agonist</td>
</tr>
<tr>
<td>AE1-329</td>
<td>EP4 agonist</td>
</tr>
<tr>
<td>ONO-8711</td>
<td>EP1 antagonist</td>
</tr>
<tr>
<td>AE-829</td>
<td>EP1 antagonist</td>
</tr>
<tr>
<td>AE5-599</td>
<td>EP3 antagonist</td>
</tr>
<tr>
<td>At3-208</td>
<td>EP4 antagonist</td>
</tr>
</tbody>
</table>
knockout mice (7). 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 severity of these lesions was significantly reduced by prior administration of PGE₂ in both wild-type and EP3 (-/-) mice, but not in animals lacking EP1 receptors.

Adaptive cytoprotection

Various mild irritants are also known to cause gastric protection by a phenomenon, referred to as adaptive cytoprotection, which is the response in the stomach induced by mild irritants to increase mucosal resistance to injury (3). Since this effect was shown to disappear in the presence of indomethacin, a COX inhibitor, it is assumed to be mediated through the enhanced production of endogenous PGs (3, 9). Indeed, 20 mM taurocholate (TC) given p.o. increased the PGE₂ content in the stomach and prevented the formation of gastric lesions induced by a subsequent challenge with HCl/ethanol (9) (Fig. 2). This effect was also antagonized by AE-829, the EP1 antagonist, but was not affected by AE-5-599, the EP3 antagonist, AE-3-208, the EP4 antagonist, or NS-398, a selective COX-2 inhibitor.

Fig. 1. Effect of PGE₂ on HCl/ethanol-induced lesions in rat stomachs, in the absence or presence of the EP1, EP3, or EP4 antagonist. (A): Animals were given 1 ml of HCl/ethanol (60% ethanol in 150 mM HCl) p.o. and were killed 1 hour later. PGE₂ (0.003–0.03 mg/kg) was given i.v. 10 min before the HCl/ethanol challenge. AE-829 (EP1 antagonist: 10 mg/kg), AE5-599 (EP3 antagonist, 3 mg/kg), or AE3-208 (EP4 antagonist, 1 mg/kg) was given p.o. 30 min before PGE₂. Data are presented as the means±S.E. from 6–8 rats. Significant difference at P<0.05; * from control; # from PGE₂ (0.03 mg/kg). (B) shows the gross appearances of gastric lesions induced in rats by HCl/ethanol. Left: Control, Middle: PGE₂ pretreatment; Right: PGE₂ pretreatment in the presence of AE-829 (EP1 antagonist). Part of data from Ref. 7 after modification.

Fig. 2. (A): Effect of TC on HCl/ethanol-induced gastric lesions in rats, in the absence or presence of indomethacin or the EP1, EP3, or EP4 antagonist. Animals were given 1 ml of HCl/ethanol (60% ethanol in 150 mM HCl) p.o. and were killed 1 hour later. TC (1 ml: 5–20 mM) was given p.o. 30 min before and HCl/ethanol. Indomethacin (5 mg/kg), AE-829 (EP1 antagonist: 10 mg/kg), AE5-599 (EP3 antagonist, 3 mg/kg), or AE3-208 (EP4 antagonist, 1 mg/kg) was given p.o. 30 min before TC. Data are presented as the means±S.E. from 6–8 rats. Significant difference at P<0.05; * from control; # from TC at 20 mM. (B): Effect of 20 mM TC on mucosal PGE₂ content in rat stomachs, in the absence or presence of indomethacin or AE-829. TC (1 ml: 5–20 mM) was given p.o., and indomethacin (5 mg/kg) or AE-829 (10 mg/kg) was given p.o. 30 min before TC. Data are presented as the means±S.E. from 6–8 rats. Significant difference at P<0.05; * from control; ′from vehicle (20 mM TC alone). Part of data from Ref. 9 after modification.
inhibitor, which suggested that adaptive gastric cytoprotection is mediated mainly by endogenous COX-1/PGE\(_2\) through EP1 receptors (16). The protective effect of TC was also not affected by sensory deafferentation.

TC acted as a mild irritant in the mouse stomach to increase the production of PGE\(_2\), which resulted in the prevention of HCl/ethanol-induced damage (9). The protective action of TC against HCl/ethanol in wild-type mice was significantly attenuated by pretreatment with either indomethacin or AE-829, the EP1 antagonist. In addition, the protective action of TC was observed in EP3 (−/−) mice, but totally disappeared in animals lacking EP1-receptors (9). These results strongly suggest that EP1 receptors are essential for the cytoprotective action of PGE\(_2\), either generated endogenously or administered exogenously, in the mouse stomach against necrotizing agents. The increased PGE\(_2\) response induced in wild-type mice by TC was significantly attenuated by prior administration of indomethacin, but not by NS-398 or AE-829.

Yamamoto et al. (19) reported that TC-induced adaptive cytoprotection in the rat stomach lasted for over 5 hours, and the underlying mechanism differed depending on the period after the irritation; the early phase was shown to be mainly mediated by COX-1/PGs, while the later phase was mediated by inducible nitric oxide (NO) synthase (iNOS)/NO, in addition to PGs produced by both COX-1 and COX-2. The expression of mRNA for both COX-2 and iNOS was observed in the stomach from 3 hours after the TC treatment, which supported the involvement of both COX-2/PGs and iNOS/NO in the later mechanism of adaptive cytoprotection. Further studies are needed to verify this point.

**Capsaicin-induced cytoprotection**

Endogenous PGs play a role in the gastric cytoprotection induced by capsaicin and some antulcer drugs. Capsaicin in particular is unique in that it causes the selective stimulation of capsaicin-sensitive afferent neurons through an interaction with transient receptor potential vanilloid type 1 receptor (TRPV1) (20). The protective action of capsaicin was totally blocked by chemical ablation of these afferent neurons and was significantly attenuated by the antagonist of calcitonin gene-related peptide (CGRP) and NOS inhibitors, in addition to capsazepine the TRPV1 antagonist (20-22). Therefore, capsaicin is considered to exhibit gastric cytoprotection through capsaicin-sensitive afferent neurons mediated by both CGRP and NO. Interestingly, the cytoprotective action of capsaicin was significantly mitigated in the presence of indomethacin, which suggested the involvement of endogenous PGs, similar to adaptive cytoprotection induced by a mild irritant (14, 23, 24) (Fig. 4).

However, this effect of capsaicin was not affected by the selective EP1 antagonist, in contrast to that of TC as a mild irritant (14). 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 (9, 14). It is assumed that the stimulation of afferent neurons by capsaicin does not increase the production of PG in the stomach; however, it exerts a cytoprotective action in the stomach, which is partly dependent on endogenous PGs.

We demonstrated 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 by an EP3 or EP4 agonist (14). Since 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. Significant protection by capsaicin was observed even in knockout mice lacking EP1 and EP3 receptors, which confirmed that capsaicin-induced gastric protection has nothing to do with EP1 or EP3 receptors (14). However, we found that capsaicin did not provide gastric cytoprotection against HCl/ethanol in IP (-/-) mice (14, 25). 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. (26) reported a lack of release of CGRP in response to mild injury in the stomachs of IP (-/-) mice.

Capsaicin conferred gastric cytoprotection, essentially through the stimulation of sensory neurons mediated by TRPV1 and depending on endogenous PGs and NO (14, 20-22). The action of PGs is mediated by mainly IP-receptors and partly by EP2 receptors, despite that capsaicin increased PGI2 but not PGE2 generation in the stomach (14). It is assumed that endogenous PGI2 plays a supportive role in the mechanism of capsaicin-induced gastric cytoprotection, maybe by sensitizing capsaicin-sensitive afferent neurons. Interestingly, mucosal acid exhibits gastric hyperemic response as well as mucosal protection through sensory neurons mediated by both PGs and NO, similar to capsaicin (9, 27), yet their modes of action differ in terms of the TRPV1-sensitivity and the prostanoid receptor-dependency (Fig. 5). Although luminal acid plays a modulator role in the physiological response mediated by capsaicin-sensitive afferent neurons in the stomach, it is unlikely that this action results from the interaction of H+ with the capsazipine-sensitive site of TRPV1. Concerning this point, a recent study suggests the involvement of acid-sensing ion channel 3 (ASIC3) in the activation of sensory neurons by mucosal acidification (28). This idea was supported by the findings that acid-induced duodenal HCO3− response was greater in female than male rats, the different response being parallel with the intensity of the expression of ASIC3, and that ovariectomy suppressed the expression of ASIC3 in the duodenum and abolished such a gender difference in HCO3− response. Certainly, further studies are needed to verify this point.

NONSTEROIDAL ANTIINFLAMMATORY DRUGS-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, a deficiency in PG is considered to be a major pathogenic factor in this model. Gastric ulceration induced by indomethacin was effectively and dose-dependently prevented by the administration of PGE2 (8, 29, 30). These lesions were also prevented by anti-secretory drugs such as cimetidine, omeprazole, and atropine, which confirmed the importance of luminal acid in the pathogenesis of these lesions (29). In addition, anti-neutrophil antisera reduced the severity of these lesions, but less effectively than other agents (31). Pretreatment with PGE2 significantly inhibited the development of gastric lesions at all time points during a 4-hours test period following the administration of indomethacin. In contrast, the anti-neutrophil antiserum did not affect the onset, but significantly reduced the severity of lesions 4 hours after the indomethacin treatment. It is assumed that neutrophils do not play a role in the onset of these lesions, but may be involved in the later extension of the damage. On the other hand, the protective effect of PGE2 was mimicked by sulprostone and 17-phenyl PGE2, both having potent affinity for EP1 receptors, and was significantly attenuated by the EP1 antagonist ONO-AE-829, the result being similar to the protective action of PGE2 against HCl/ethanol (7, 8) (Fig. 6).

Neither butaprost, ONO-NT-012, nor 11-deoxy PGE2 afforded protective action of PGE2 against HCl/ethanol (7, 8) (Fig. 6). In wild-type and knockout mice, butaprost did not affect the onset, but significantly reduced the severity of lesions 4 hours after the indomethacin treatment. It is assumed that neutrophils do not play a role in the onset of these lesions, but may be involved in the later extension of the damage. On the other hand, the protective effect of PGE2 was mimicked by sulprostone and 17-phenyl PGE2, both having potent affinity for EP1 receptors, and was significantly attenuated by the EP1 antagonist ONO-AE-829, the result being similar to the protective action of PGE2 against HCl/ethanol (7, 8) (Fig. 6).

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Recent studies showed that the risk of gastrointestinal bleeding was increased due to the concomitant use of antiplatelet drugs with low-dose aspirin (32, 33). We confirmed in rats that gastric bleeding caused by the luminal perfusion of aspirin with exogenous HCl or under the stimulation of acid secretion was markedly increased by pretreatment with clopidogrel (34, 35). As expected, the aggravation of gastric bleeding and ulcerogenic response to aspirin in the presence of clopidogrel was significantly attenuated by pretreatment with PGE₂, although it remains undetermined which EP receptor subtype is responsible for this action.

GASTRIC DAMAGE ASSOCIATED WITH ACID-BACK DIFFUSION FOLLOWING BARRIER DISRUPTION

Mucosal exposure to TC in the rat stomach caused a marked decrease in potential difference (PD), followed by an increase in

![Figure 6](image_url)

**Fig. 6.** Effects of various EP agonists on gastric lesions induced by indomethacin in rats. (A): The dose-response relationship; (B): the effect at the highest dose of each EP agonist. Animals were given indomethacin s.c. in a dose of 35 mg/kg and were killed 4 hours later. PGE₂ (0.3 mg/kg), 17-phenyl PGE₂ (0.3 mg/kg), sulprostone (0.3 mg/kg), butaprost (10 mg/kg), ONO-NT-012 (10 mg/kg) or 11-deoxy PGE₁ (3 mg/kg) was given i.v. 10 min before indomethacin. Data are presented as the means ± S.E. from 4–6 rats. Significant difference at P<0.05; * from controls. Part of data from Ref. 8 after modification.

![Figure 7](image_url)

**Fig. 7.** Effects of the EP₁ antagonist (ONO-8711) on gastric functional and ulcerogenic responses to TC in wild-type mice. The stomach was exposed to 20 mM HCl every 10 min before and after exposure to 20 mM TC (in 20 mM HCl) for 20 min. ONO-8711 was given s.c. at a dose of 10 mg/kg 30 min before the TC treatment. Data are presented as the means of values determined every 10 min from 5 mice per group. Significantly different at P<0.05; * from wile-type group. Data from Ref. 29 after modification.
acid loss and gastric mucosal blood flow (GMBF) (4). The decreased PD was gradually normalized after removal of TC from the chamber, with minimal damage to the mucosa 1 hour after TC treatment. This hyperemic response was inhibited by indomethacin, resulting in severe lesions in the mucosa without any change in PD or acid loss. Similar results were obtained in the stomachs of wild-type and EP3 (-/-) mice (36). However, the TC treatment did not increase GMBF in mice lacking EP1 receptors, despite causing PD reduction and acid loss, and resulted in severe damage in the mucosa. Mucosal PGE2 content was significantly increased after TC in all groups of mice. On the other hand, pretreatment with ONO-8711, the EP1 antagonist, did not affect the degree of PD reduction and luminal acid loss after exposure to TC, but totally attenuated the increase in GMBF in association with acid back-diffusion, resulting in a marked aggravation of gastric damage (Fig. 7). It is unlikely that the impaired GMBF response in EP1-receptor knockout mice is due to an inadequate mucosal PG biosynthetic response to TC because PGE2 generation was increased in response to TC in EP1 (-/-) mice similar to that in wild-type litter mates. These findings strongly support the presence of EP1-receptors being essential for the gastric hyperemic response and mucosal integrity against luminal acid following barrier disruption.

The gastric hyperemic response associated with acid back-diffusion was previously shown to be partly mediated by NO and capsaicin-sensitive afferent neurons, in addition to PGs (20). Since these factors interact with each other to maintain gastric function and modulate mucosal integrity, the lack of any one factor may...
lead to the failure to fully express these functions. The GMBF response induced by acid back-diffusion following TC returned to the pre-exposure level much earlier in IP-receptor knockout mice than in wild-type animals (14, 36). Thus, the GMBF response following barrier disruption may be mediated by different mechanisms depending on the period; mainly mediated by PGE2 through EP1 receptors during the early phase and by PGI2/IP receptors during the later phase, and the process in both periods may collaborate with sensory neurons. The increase in GMBF induced by acid or capsaicin was mitigated by the desensitization of capsaicin-sensitive afferent neurons (14, 36); however, the former response induced by acid required the presence of EP1 receptors (14) while the latter by capsaicin required the presence of IP receptors (36). Recent studies have shown that both capsaicin and acid increased GMBF and duodenal HCO3- secretion through capsaicin-sensitive afferent neurons, but the mode of action differs in terms of the sensitivity of capsaicin, the antagonist of TRPV1 (37). As mentioned before, capsaicin activates these afferent neurons via TRPV1, while acid stimulates these neurons through sites other than TRPV1, probably via the activation of ASIC3 (Fig. 5).

FUNCTIONAL ALTERATIONS RELATED TO GASTRIC CYTOPROTECTION

Although a number of studies have been conducted to clarify the mechanisms involved in gastric cytoprotection afforded by PGE2, the exact mechanism remains unknown. Endogenous PGs play a role in regulating various gastric functions, such as acid secretion, mucus/bicarbonate secretion, mucosal blood flow, and motility, and these functional changes may contribute to gastric cytoprotection. However, since this effect of PGE2 is mediated by the activation of EP1 receptors, any functional mechanism responsible for cytoprotection should also be mediated by EP1 receptors.

According to previous studies including our own (7-9, 12, 38-43), PGE2 affected various gastric functions, such as inhibition of acid secretion and motility, and an increase in mucus and HCO3- secretion as well as GMBF. We also recently found that PGE2 had an acid stimulatory effect mediated by histamine released from enterochromaffin-like (ECL) cells through EP4 receptors (41). The acid inhibitory action of PGE2 was mediated by EP3 receptors in two ways, directly by inhibiting acid secretion at the parietal cells and indirectly by inhibiting histamine release at ECL cells. On the other hand, PGE2 was shown to stimulate HCO3- secretion in the stomach, and this action was mediated by the activation of EP1 receptors (12, 42, 43). However, the effect on acid or HCO3- secretion can be excluded in the possible mechanism for the cytoprotective action of PGE2 because this effect has been observed in the stomach against damage induced by a strong acid (0.6 M HCl) or base (0.2 M NaOH), in which changes in endogenous acid or HCO3- secretion were masked (2). Furthermore, we showed that PGE2 inhibited gastric motility and increased mucosal blood flow by EP4 receptors and EP2/IP receptors, respectively (7, 8, 38, 40) (Fig. 8). Since PGE2-induced gastric cytoprotection was attenuated by the EP1 antagonist and this phenomenon disappeared in mice lacking EP1 receptors (7-9), it is assumed that this action of PGE2 may be functionally associated with the inhibition of gastric motility, but not with changes in other gastric functions. Indeed, neither butaprost, ONO-NT-012, nor 11-deoxy PGE1 provided any gastric protection against HCl/ethanol or indomethacin, despite causing an increase in gastric mucosal blood flow, it is likely that an increase in gastric

Fig. 10. Effects of various EP agonists on the neutrophil chemotaxis stimulated by fMLP. Neutrophils were pretreated for 45 min with atropine or various EP agonists such as PGE2, 17-phenyl PGE2, butaprost, ONO-NT-012, and 11-deoxy PGE1 at the indicated concentrations, and cells were then stimulated by incubation with fMLP (1 × 10-7 M) for another 45 min. Data are expressed as % of the stimulated values (control) observed in the presence of fMLP and represent the means ± S.E. from 4 experiments. Significant difference at P<0.05; * from normal; # from control. Data from Ref. 8 after modification.
PGE2 is paralleled by a reduction in gastric mucosal damage. We hypothesized that the motility effect of PGE2 is paralleled by a reduction in gastric mucosal damage. We previously reported that various compounds afforded gastric cytoprotection at doses that inhibited gastric motility (7, 22, 44, 45). The inhibition of gastric motility was observed by PGE2 and capsaicin at doses that exhibited protection against HCl/ethanol in the stomach (7, 22). The inhibition of gastric motility may lead to a flattening of the mucosal folds and decrease in mucosal vulnerability to irritants, resulting in the prevention of fold-related band-like lesions induced by HCl/ethanol (44). A role for muscle elements in the pathogenic mechanism of indomethacin-induced gastric ulceration has also been demonstrated (33, 44, 46). Mersereau and Hinchey (37) 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 ulcerogenic doses enhanced gastric motility and induced microcirculatory disturbances due to abnormal mucosal compression of the gastric wall (39, 40). Because the inhibition of gastric motility may lead to an attenuation in 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 PGE2 inhibits gastric motility through EP1 receptors remains unknown. Milenov and Golenhofen (41) reported that PGE2 relaxed the circular muscle and contracted the longitudinal muscle of the canine stomach. Narumiya and his group showed the distribution of mRNA of the EP receptors along the gastrointestinal tract (42, 43). They reported 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 (5, 6), it is assumed that the contraction of longitudinal smooth muscle by PGE2 is associated with an increase in cytosolic calcium. Contraction of the circular smooth muscle leads to the appearance of mucosal folds, which have been implicated in the pathogenesis of ulcers including indomethacin-generated gastric lesions (22, 37, 39, 40). At present, the mechanism by which PGE2 relaxes circular smooth muscle by activating EP1 receptors remains unknown.

Neutrophils have been implicated in the damage associated with NSAIDs (44). PGE2 is known to have an inhibitory effect on neutrophil functions, including chemotaxis (45). We confirmed that PGE2 exhibited an inhibitory effect on the migration of neutrophils caused by formyl-methionyl-leucyl-phenylalanine in vitro (8) (Fig. 10). The same inhibitory action was shown by both butaprost and 11-deoxy PGE2, but not by 17-phenyl PGE2, sulprostone, or ONO-NT-012, which suggested that the anti-neutrophil chemotaxis action of PGE2 is mediated by activating EP2 and EP4 receptors. Thus, the inhibition of neutrophil migration by itself is assumed to be 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 enhanced gastric motility was inhibited by atropine (39), it is likely that neutrophil infiltration is secondary to the event associated with gastric hypermotility following indomethacin treatment. Melange et al. (46) showed that NSAID-induced gastric injury was neutrophil-independent in the neutropenic rat.

Endogenous PGE2 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 was involved in gastric functional responses, such as an increase in gastric mucosal blood flow and a decrease in acid secretion, observed acutely after barrier disruption in the stomach (47, 48). These functional alterations following barrier disruption are adaptive responses in 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 was shown to be attenuated by the EP1 antagonist ONO-8711 and disappeared in EP1 (-/-) mice, which strongly suggested mediation by the activation of EP1 receptors (29). PGF2α/IP receptors did not play a role in this phenomenon (49).

We demonstrated that the increase in GMBF caused by PGE2 in rat stomachs is mediated by the activation of EP2 and EP4 receptors (7). It is unlikely that endogenous PGE2 directly acts on the vascular smooth muscle through the activation of EP1 receptors. However, since PGE2 causes the dissolution of the mucosal foldings through EP1 receptors (8), and since the contraction of stomach smooth muscle may restitute mucosal blood flow, the possibility that PGE2 contributes to the increased GMBF response after TC treatment by relaxing the stomach smooth muscle through EP1 receptors cannot be excluded. Haupt et al. (50) reported that PGE2 had complex effects on intestinal afferent discharge, acting by direct and indirect mechanisms, and was mediated by different receptor subtypes including EP1 and EP2 receptors. Thus, PGE2 may interact with afferent neurons through EP1 receptors, resulting in an increase in GMBF after exposure of the stomach to TC. Further studies are needed to clarify the interaction of PGE2 and sensory afferent neurons in relation to EP receptor subtypes (Fig. 5).

It is interesting that PGE2 exhibited a protective action on the stomach against necrotizing agents through EP1 receptors, and also aggravated histamine-induced gastric ulceration mediated by the same receptor subtype (51). Szabo et al. (52) proposed a "histodilution barrier" as one of the mechanisms for PGE2-induced gastric cytoprotection. This 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 PGE2 induces both protective and proulcerogenic actions by activating the same EP receptor subtype. However, whether PGE2 potentiates histamine-induced vascular permeability by acting directly on the vascular smooth muscle or by interacting with histamine at H1 receptors by activating EP1 receptors remains unknown.

SUMMARY AND FUTURE PROSPECTS

Endogenous PGs play a central role in the mucosal defensive mechanism of the gastrointestinal tract, with PGE2 being the most important. This paradigm is largely based on the finding of "gastric cytoprotection" by Robert et al. (2) in 1979. Since then, a number of studies have been conducted to elucidate the factors involved in this phenomenon; however, the true mechanism underlying this action remains unexplored. As reviewed in this chapter, exogenous PGE2 confers protection on the stomach against ulcerogenic stimuli, irrespective of whether it is a necrotizing agent (HCl/ethanol) or NSAID (indomethacin), mainly by activating EP1 receptors. As observed in the adaptive cytoprotection induced by a mild irritant, endogenous PGE2 also exhibits gastroprotection mediated by EP1 receptors. Because a functional mechanism responsible for gastric cytoprotection should be mediated by EP1 receptors and because among the various functional alterations...
induced by PGE\(_2\), only the inhibition of gastric motility was mediated by the activation of EP1 receptors, it may assumed that the gastric cytoprotection of PGE\(_2\) is functionally associated with the inhibition of gastric motility. It is also assumed that endogenous PGI\(_2\) plays a supportive role in the mechanism of capsaicin-induced gastric cytoprotection, maybe by sensitizing capsaicin-sensitive afferent neurons. In addition, endogenous PGE\(_2\) plays a mediator role in the gastric hyperemic response following barrier disruption and contributes to the maintenance of mucosal integrity under such conditions, by activating the EP1 receptor subtype, in addition to capsaicin-sensitive afferent neurons. Since the results introduced in this review were obtained in rats using subtype-specific EP agonists and were further confirmed in EP receptor or IP receptor knockout mice, they would be reliable and have a high reproducibility when compared to those obtained in either rats or knockout mice alone. Although this review does not cover the action of PGs in the intestinal tract, many studies reported the protective effect of PGE\(_2\); in various pathological conditions such as NSAID-induced enteropathy and dextran sulfate Na-induced colitis, and in some cases identified the EP receptor subtype responsible for these actions (60-62). Anyhow, these approaches should contribute to further understanding of the mechanism of "cytoprotection" in the stomach and future development of mucosal protective drugs for the treatment of peptic ulcer diseases.

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