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Chapter 5

Modulation of Capsaicin-Induced Gastric Protection by Endogenous Prostaglandins through EP2/IP Receptors

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http://dx.doi.org/10.5772/58337

1. Introduction

Gastric mucosal integrity is maintained by multiple factors, including both paracrine and neuronal factors [1-4]. The former includes prostaglandins (PGs) [1] and nitric oxide (NO) [2], while capsaicin-sensitive afferent neurons play a central role in neuronal protection in the stomach [3]. Previous studies demonstrated that capsaicin, a selective stimulator of these afferent neurons, protected the gastric mucosa against various ulcerogenic stimuli such as nectrotizing agents [3]. The protective effects of capsaicin were shown to be mediated by these afferent neurons because they were completely attenuated by the chemical ablation of these neurons following a pretreatment with a large dose of capsaicin [3, 5]. The binding site of capsaicin has been cloned and named the transient receptor potential vaniloid type 1 receptor (TRPV1), a nonselective cationic channel [6]. Capsaicin is assumed to stimulate these afferent neurons by activating TRPV1, which results in the liberation of the neurotransmitter, calcitonin gene-related peptide (CGRP) and gastric protection.

Several studies, including our own, have showed that the protective effects of capsaicin were mitigated by the prior administration of indomethacin, which indicated the involvement of endogenous PGs in this action [6-9]. Endogenous PGs were previously shown to sensitize sensory neurons to nociceptive stimuli [10, 11]. Therefore, endogenous PGs are assumed to play a supportive role in the mechanism underlying capsaicin-induced gastric protection, possibly by sensitizing these afferent neurons, because capsaicin-induced gastric cytoprotection was shown to be attenuated by indomethacin.

On the other hand, recent pharmacological studies have classified PGE$_2$ receptors into four specific G protein-coupled subtypes, EP1 to EP4 [12]. The distribution of these receptors is
considered to explain the multiple effects of PGE\(_2\) in various tissues including the gastrointestinal tract. Mice lacking various receptors for prostanoids have been established \[13, 14\], and the roles of specific PG receptors in the various biological actions of PGs have been demonstrated using these "knockout mice" \[15\]. We performed a series of experiments to identify the EP receptor subtypes mediating the gastrointestinal protection afforded by PGE\(_2\) using various models in both rats and EP receptor knockout mice, and found that PGE\(_2\), administered exogenously or generated endogenously, provided gastric protection against HCl/ethanol mediated by EP1 receptors \[16, 17\]. However, the relationship between the EP receptor subtype and facilitation of capsaicin-induced gastric protection by PGs remains unknown.

We here investigated the role of endogenous PGs in the gastric protective action of capsaicin against HCl/ethanol-induced damage in rats, mainly in relation to PGE\(_2\) and EP receptors. Furthermore, because an animal model lacking various receptors for prostanoids is now available \[13, 15, 18\], we also evaluated the protective activity of capsaicin in knockout mice lacking EP1 or EP3 receptors and also in some cases IP receptors. In addition, we also examined the gastric hyperemic response to capsaicin in these knockout mice in order to provide functional evidence for the modulatory role of PGs in capsaicin-induced protective effects.

2. Methods

ANIMALS: Male Sprague-Dawley rats (200-220 g) and C57BL/6 mice (25-30 g) were used. Mice lacking the EP1, EP3, or IP receptors were generated as described previously \[13, 15, 19, 20\]. In brief, the mouse genes encoding the EP1, EP3, and IP receptors were individually disrupted, and chimaeric mice were generated. These animals were then back crossed with C57BL/6 mice, and the resulting heterozygous litter mates [EP1 (+/-), EP3 (+/-) or IP (+/-), ] were bred to produce homozygous EP1 (-/-), EP3 (-/-), or IP (-/-) mice. Homozygous mice were born at the predicted Mendelian frequency, grew normally, lived longer than 1 year and were fertile. The distribution of the EP1, EP3, and IP receptor genes was verified by northern blot hybridization, which failed to detect messenger RNAs encoding the respective receptors in EP1 (-/-), EP3 (-/-), and IP (-/-) mice. These knockout mice were deprived of food, but allowed free access to tap water for 18 hr before the experiments. All studies were performed using 4~8 animals per group.

INDUCTION OF GASTRIC LESIONS: Rats were administered 1 ml of HCl/ethanol (60% in 150 mM HCl) p.o. through esophageal intubation, and killed 1 hr later under deep ether anesthesia. The stomachs were removed, inflated by injecting 10 ml of 1% formalin for 10 min to fix the tissue walls, and opened along the greater curvature. The area (mm\(^2\)) of hemorrhagic lesions that developed in the stomach was measured under a dissecting microscope with a square grid (x10). Capsaicin (1-10 mg/kg) was given p.o. 30 min before the administration of HCl/ethanol. PGE\(_2\) (0.3 mg/kg) was given i.v. 10 min prior to the HCl/ethanol treatment. In some cases, indomethacin (5 mg/kg) or ONO-AE-829 (5 and 10 mg/kg), the EP1 receptor antagonist \[20\], was given s.c. 30 min before the administration of PGE\(_2\) or capsaicin. In
addition, the protective effects of PGE_{2} and capsaicin on HCl/ethanol were also examined in rats with the chemical ablation of capsaicin-sensitive sensory neurons. Chemical deafferentation was induced by s.c. injections of capsaicin once a day for three consecutive days (total dose: 100 mg/kg) 2 weeks before the experiment [5]. All capsaicin injections were performed under ether anesthesia, and rats were pretreated with terbutaline (0.1 mg/kg, i.m.) and aminophylline (10 mg/kg, i.m.) to counteract the respiratory impairment associated with capsaicin injections. The effectiveness of the treatment was tested by examining protective wiping movements of the eye. In a separate experiment, we examined the rescue effect of various subtype-specific EP agonists on the protective effects of capsaicin in indomethacin-treated rats. Animals were first administered indomethacin (5 mg/kg) s.c., followed by capsaicin p.o. 30 min later, and were then given HCl/ethanol p.o. 30 min following the capsaicin treatment. Butaprost (an EP2 agonist: 3 mg/kg), ONO-NT-012 (an EP3 agonist: 3 mg/kg) or 11-deoxy PGE_{1} (an EP3/EP4 agonist: 1 mg/kg) was administered i.v. 10 min before the capsaicin treatment. Animals were killed 1 hr after the administration of HCl/ethanol. In another experiment, wild-type mice and EP1-, EP3-, or IP-receptor knockout mice were administered HCl/ethanol p.o. in a volume of 0.3 ml, and killed 1 hr later [16]. The stomach was then removed and treated with formalin, and the mucosa was examined for hemorrhagic lesions under a dissecting microscope, as described previously. Capsaicin (10 mg/kg) was given p.o. to half of the animals in each group 30 min before the administration of HCl/ethanol. In addition, indomethacin (5 mg/kg) or ONO-AE-829 (10 mg/kg) was given s.c. 30 min before the administration of capsaicin in wild-type mice.

MEASUREMENT OF GASTRIC MUCOSAL BLOOD FLOW (GMBF): GMBF was measured in both wild type mice and EP1-, EP3-, or IP-receptor knockout mice according to methods described in our previous study [21, 22]. Under urethane anesthesia, the abdomen was opened through a midline incision, and the stomach exposed, mounted on an ex-vivo chamber (the exposed area: 0.7 mm²), and superfused at a rate of 0.5 ml/min. Gastric mucosal blood flow was measured by a laser Doppler flowmeter (ALF-21, Advance, Tokyo, Japan) and by placing a probe gently on the surface of the corpus mucosa using a balance (Medical Agent, Kyoto, Japan). Changes in mucosal blood flow were monitored on a recorder (U-228, Tokai-irika, Tokyo, Japan). After mucosal blood flow had stabilized, the solution in the chamber was withdrawn, and the mucosa was then exposed to 0.2 ml of cicaprost, a PGI_{2} analogue (5 µg/ml), or capsaicin (1 mg/ml) for 10 min. After the application of these agents, the mucosa was rinsed with saline, another 0.2 ml of saline was instilled, and perfusion was resumed. Indomethacin (5 mg/kg) was given s.c. to some of wild type mice 30 min before the application of capsaicin.

MEASUREMENT OF MUCOSAL PGE_{2} AND 6-keto PGF_{1α} LEVELS: PGE_{2} levels in the rat gastric mucosa and those of 6-keto PGF_{1α} in the mouse stomach were measured 30 min after the p.o. administration of capsaicin (10 mg/kg). Some of the rats used were pretreated with capsaicin for sensory deafferentation. In some cases, indomethacin (5 mg/kg) was administered s.c. 30 min before the capsaicin treatment. Under ether anesthesia, the stomachs were quickly removed, opened along the greater curvature, and rinsed with ice-cold saline. To separate the mucosal layer, the corpus mucosa was placed between two glass slides, squeezed
with a rubber band, and placed in hexane-frozen Dry Ice and acetone. These glasses were separated, and the mucosa was collected, weighed, and put in a tube containing 100% ethanol plus 0.1 M indomethacin [17, 23]. The samples were then homogenized, and centrifuged for 10 min at 12000 g at 4°C. The supernatant of each sample was used to determine PGE₂ and 6-keto PGF₁α levels by EIA using PGE₂- and 6-keto PGF₁α-kits, respectively (Cayman Chemical Co., Ann Arbor, MI).

**PREPARATION OF DRUGS:** The drugs used in the present study were capsaicin (Nakarai Tesque, Kyoto, Japan), prostaglandin E₂ (PGE₂), 11-deoxy prostaglandin E₁ (11-deoxy PGE₁) (Funakoshi, Tokyo, Japan), ONO-AE-829, butaprost, and ONO-NT-012 (Ono, Osaka, Japan), terbutaline (Fujisawa, Osaka, Japan), aminophylline (Eisai, Tokyo, Japan), and indomethacin (Sigma Chemicals, St. Louis, Mo). Capsaicin was dissolved in Tween 80-ethanol solution (10% ethanol, 10% Tween 80, 80% saline, w/w) for s.c. injections, while indomethacin was suspended in saline with a drop of Tween 80 (Wako, Osaka, Japan). ONO-AE-829 was dissolved in saline. PGE₂ and other EP receptor ligands were first dissolved in absolute ethanol and then diluted with saline to the desired concentration. Each agent was prepared immediately before use and given in a volume of 0.5 ml per 100 g body weight (rat) or 0.1 ml per 10 g body weight (mouse) for its p.o. and s.c. administration, respectively, and given i.v. in a volume of 0.1 ml per 100 g body weight (rat). Control animals received saline instead of the active agent.

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

### 3. Results

#### 3.1. Effect of capsaicin on HCl/ethanol-induced gastric lesions in rats

The oral administration of HCl/ethanol (60% in 150 mM HCl) produced multiple lesions in the glandular mucosa, along the long axis of the rat stomach. These lesions were dose-dependently prevented in animals pretreated with capsaicin (1-10 mg/kg) p.o. before the challenge with HCl/ethanol, and a significant effect was obtained at doses over 3 mg/kg, with the inhibition at 10 mg/kg being 81.6% (Figure 1A). The protective effects of capsaicin (10 mg/kg) were completely attenuated by the chemical ablation of sensory neurons as well as prior administration of indomethacin (5 mg/kg), but not by ONO-AE-829 (10 mg/kg)(Figure 1B). The severity of HCl/ethanol-induced gastric lesions was also significantly reduced by the prior i.v. administration of PGE₂ (0.03 mg/kg), with the inhibition being 82.1% (Figure 2). The protective effects of PGE₂ were significantly mitigated by the pretreatment with the EP1 antagonist, ONO-AE-829 (10 mg/kg), but not by chemical deafferentation. The degree of protection afforded by PGE₂ in the presence of ONO-AE-829 at 10 mg/kg was 19.8%, which was significantly less than that observed in vehicle-treated normal rats.
Figure 1. Dose-response relationship for the protective effects of capsaicin against HCl/ethanol-induced gastric lesions in rats (A), and the effects of indomethacin (5 mg/kg), ONO-AE-829 (10 mg/kg), or sensory deafferentation on the mucosal protective effects of capsaicin (B). Animals were administered 1 ml of HCl/ethanol (60% in 150 mM HCl), and killed 1 hr later. Capsaicin (1-10 mg/kg) was administered p.o. 30 min before HCl/ethanol. Indomethacin (5 mg/kg) or ONO-AE-829 (10 mg/kg) was given s.c. 30 min before capsaicin. Sensory deafferentation was achieved with 3 consecutive s.c. injections of capsaicin (total 100 mg/kg) 2 weeks before the experiment. Data are presented as means±SE from 4-8 rats. Significantly different at P<0.05: * from the control; # from the vehicle.
Figure 2. Effects of ONO-AE-829 or sensory deafferentation on the protective action of PGE$_2$ against HCl/ethanol in the rat stomach. Animals were administered 1 ml of HCl/ethanol (60% in 150 mM HCl), and killed 1 hr later. PGE$_2$ (0.3 mg/kg) was given i.v. 10 min before HCl/ethanol. ONO-AE-829 (10 mg/kg) was administered s.c. 30 min before PGE$_2$. Sensory deafferentation was induced with 3 consecutive s.c. injections of capsaicin (total 100 mg/kg) 2 weeks before the experiment. Data are presented as means±SE from 6-8 rats. Significantly different at P<0.05: *from the control; # from the vehicle.

The oral administration of capsaicin (10 mg/kg) did not affect the mucosal PGE$_2$ content when measured 0.5 hr after its administration (not shown). The prior administration of indomethacin (5 mg/kg, s.c.) markedly reduced PGE$_2$ levels in the presence of capsaicin. As in normal rats, capsaicin did not significantly affect mucosal PGE$_2$ levels in sensory deafferented animals.

3.2. Reversal of capsaicin-induced protection by EP agonists in indomethacin-pretreated rats

To investigate the roles of PGE$_2$ and EP receptors in capsaicin-induced gastric protection, we examined the rescue effects of various subtype-specific EP agonists on capsaicin in the presence of indomethacin. The oral administration of capsaicin (10 mg/kg) markedly protected against HCl/ethanol-induced gastric lesions (Figure 3A). This effect of capsaicin was significantly mitigated by the prior administration of indomethacin (5 mg/kg), and the degree of inhibition was reduced to 29.7%. When these animals were given various EP agonists i.v. 20 min after indomethacin, the protective effects of capsaicin were again observed in rats pretreated with butaprost (an EP2 agonist). Neither ONO-NT-012 (an EP3 agonist) nor 11-deoxy PGE$_2$ (an EP3/EP4 agonist) rescued the effects of capsaicin against HCl/ethanol in the presence of indomethacin. None of the EP agonists (i.v.) used, including butaprost, significantly protected against HCl/ethanol by themselves (Figure 3B).
Figure 3. A: Effects of various EP agonists on the mucosal protective action of capsaicin against HCl/ethanol-induced gastric lesions in the rat stomach in the presence of indomethacin. Animals were given 1 ml of HCl/ethanol (60% in 150 mM HCl) and killed 1 hr later. Capsaicin (10 mg/kg) was administered p.o. 30 min before HCl/ethanol. Indomethacin (5 mg/kg) was given s.c. 30 min before capsaicin. Various EP agonists were given i.v. 10 min before capsaicin. Data are presented as means±SE from 6-8 rats. Significantly different at P<0.05: * from the control; # from the saline; $ from vehicle.

B: Effects of various EP agonists on HCl/ethanol-induced gastric lesions in the rat stomach. Various EP agonists were given i.v. 10 min before HCl/ethanol. Data are presented as means±SE from 6 rats.
3.3. Gastric cytoprotection against HCl/ethanol by capsaicin in mice

To further investigate the relationship between capsaicin-induced gastric protection and EP receptor subtype, we examined the protective effects of capsaicin against HCl/ethanol in both wild-type and knockout mice lacking EP1 or EP3 receptors. In addition, since other study reported a role for PGI$_2$ in the release of CGRP in the stomach following capsaicin stimulation [19], the protective effect of capsaicin was also examined in IP receptor knockout mice. The intragastric administration of HCl/ethanol (0.3 ml) also caused hemorrhagic lesions in the mouse stomach (Figure 4). HCl/ethanol led to the development of gastric lesions in EP1, EP3, and IP receptor knockout mice, similar to wild-type mice, and the severity of these lesions was similar among these groups. The severity of these lesions in wild-type mice was also reduced by the prior p.o. administration of capsaicin (10 mg/kg). This agent significantly reduced the severity of these lesions in animals lacking either EP1 or EP3 receptors. However, capsaicin failed to protect the stomach against HCl/ethanol in IP receptor knockout mice, and the lesion score in these animals was not significantly different from that observed in IP receptor knockout animals without the capsaicin pretreatment.

Figure 4. Effects of capsaicin on HCl/ethanol-induced gastric lesions in wild-type and knockout mice lacking EP1, EP3 or IP receptors. Animals were administered 0.3 ml of HCl/ethanol (60% ethanol in 150 mM HCl) p.o. and killed 1 hr later. Capsaicin (10 mg/kg) was given p.o. 30 min before HCl/ethanol. In wild-type mice, indomethacin (5 mg/kg) was given s.c. 30 min before administration of capsaicin. Data are presented as means±SE from 4-6 mice. Significantly different at P<0.05; *from the vehicle in the corresponding group; # from saline.
The oral administration of capsaicin (10 mg/kg) did not significantly affect 6-keto PGF₁α levels, which was similar to the effects observed on PGE₂ content in the rat stomach (not shown). Indomethacin (5 mg/kg) markedly decreased 6-keto PGF₁α levels in the presence of capsaicin. Similarly, capsaicin had no effect on 6-keto PGF₁α levels in IP receptor knockout mice.

3.4. Effect of capsaicin on gastric mucosal blood flow in mice

Under urethane anesthesia, the chambered stomachs of both wild type mice and those lacking EP1, EP3, or IP receptors showed a relatively constant GMBF during a 2-hr test period. The mucosal application of capsaicin (1 mg/ml) for 10 min caused a marked increase in GMBF in wild type mice, and this effect was significantly attenuated by the prior administration of indomethacin (5 mg/ml) (Figure 5). A significant increase in GMBF by capsaicin was also observed in both EP1 and EP3 receptor knockout mice. However, the gastric hyperemic response to capsaicin was almost completely absent in animals lacking IP receptors, and GMBF values were significantly lower than those in control wild type mice, at most of the time points measured after the application of capsaicin.

Figure 5. Effects of capsaicin on GMBF in wild type mice and knockout mice lacking EP1, EP3 or IP receptors under urethane anesthetized conditions. Capsaicin was applied to the chamber at a concentration of 1 mg/ml for 10 min (100 µl), and GMBF was measured before and after its application. Indomethacin was administered s.c. at a dose of 5 mg/kg 30 min before capsaicin in wild-type mice. Data are expressed as an increase in GMBF (a % of basal values) and presented as means±SE of values determined every 10 min from 5-6 mice per group. Significantly different at P<0.05:

* from the corresponding basal values (time 0) in the corresponding group; # from wild type mice.
4. Commentary

PGs, either endogenous or exogenous derivatives, have been shown to act on multiple receptors [12]. Capsaicin affords gastric protection by stimulating afferent C-fibers [3], and this action is partly dependent on endogenous PGs [5, 8, 9, 24]. We previously examined the relationship between EP receptor subtypes and gastric protection against HCl/ethanol in rats using various EP agonists and found that the gastroprotective action afforded by endogenous or exogenous PGs was mediated by EP1 receptors [16, 17]. However, the EP receptor subtypes or other prostanoid receptors responsible for this phenomenon has yet to be established. The present study was conducted to determine the prostanoid receptor(s) involved in capsaicin-induced gastric protection.

First, we confirmed that PGE$_2$ prevented the development of HCl/ethanol-induced gastric lesions, and this action was attenuated by the EP1 antagonist, ONO-AE-829 [16]. This result was also verified in EP receptor knockout mice, with this protection being completely absent in mice lacking EP1 receptors [16, 17]. These results strongly suggest that the protective effects of exogenous PGE$_2$ in the stomach were mainly mediated by the activation of EP1 receptors. On the other hand, endogenous PGs play a role in the gastric cytoprotection induced by the oral administration of capsaicin [5, 8, 9]. As shown in this study, the protective effects of capsaicin against HCl/ethanol were dose-dependent, and were attenuated by the chemical ablation of capsaicin-sensitive sensory neurons. The protective effects of capsaicin were also significantly mitigated by the prior administration of indomethacin, which indicated the involvement of endogenous PGs in these effects. However, in contrast to the adaptive cytoprotection induced by a mild irritant [17], the effects of capsaicin were not affected by the selective EP1 antagonist, ONO-AE-829. This was confirmed by the stimulatory effects of capsaicin on gastric HCO$_3$- secretion which were attenuated by indomethacin and capsazepine, but not ONO-8711 (an EP1 antagonist) [25]. Furthermore, neither the stimulation of sensory neurons by capsaicin nor sensory deafferentation affected mucosal PGE$_2$ levels in the stomach. Many studies have shown that mild irritants increased the production of PGE$_2$ in the stomach [14, 17]. These findings suggest that although endogenous PGs are involved in the gastric cytoprotection induced by both mild irritants and capsaicin, the mode of action appears to be different in these two cases. The stimulation of afferent neurons by capsaicin was assumed to increase the production of PGs in the stomach, but exerted protective effects in the stomach, that were partly dependent on endogenous PGs.

In the present study, we administered various EP agonists to indomethacin-treated animals, to determine whether the inhibitory effect of indomethacin on capsaicin-induced gastric protection was reversed by exogenous PGE$_2$, and if so, which EP receptor subtype was responsible for this action. The protective effects of capsaicin were significantly restored, even in the presence of indomethacin, by the prior administration of butaprost, the EP2 agonist, but not by the EP3 or EP4 agonist. In addition, the protective effects of capsaicin were significantly enhanced in the presence of butaprost, which strongly suggested a supportive role for EP2 receptors in capsaicin-induced gastric protection. These results are supported by the findings of Haupt et al [26], who reported the involvement of the EP2 receptor in the potentiation of afferent neuronal discharges by PGE$_2$ in the rat jejunum. Jenkins et al [27] also demonstrated that the activation of DP, EP, and IP receptors could each cause the release of CGRP from
trigeminal neurons, and that the predominant EP receptor subtype involved may be the EP2 receptor. In the present study, neither of these EP agonists, including butaprost, offered any protection against HCl/ethanol-induced gastric damage by themselves. Furthermore, capsai-

cin-induced gastric protection was not affected by the EP1 antagonist, which excluded the involvement of EP1 receptors in the facilitation of this action by endogenous PGs. The significant level of protection afforded by capsaicin was also observed in knockout mice lacking EP1 and EP3 receptors, which confirmed that capsaicin-induced gastric protection did not involve EP1 or EP3 receptors. We could not confirm the involvement of EP2 receptors in this action because EP2 knockout mice were not available in our laboratory.

In contrast, we demonstrated that capsaicin failed to exhibit a cytoprotective effect against HCl/ethanol-induced gastric lesions in IP-receptor knockout mice. We previously showed that 20 mM taurocholate, as a mild irritant, protected the stomach against HCl/ethanol even in IP receptor knockout mice, which was similar to that observed in wild-type mice. These results suggested the absence of the involvement of PGI\textsubscript{2} in the mechanism responsible for adaptive cytoprotection [17]. We also reported that the adaptive cytoprotection induced by taurocholate was attenuated by ONO-AE-829, the EP1 antagonist, as well as indomethacin, and was not observed in EP1 receptor knockout mice [17]. The present results obtained in knockout mice suggest that IP receptors are also involved in the protective effects of capsaicin in the stomach, in addition to EP2 receptors. The exact mechanism by which endogenous PGs contributes to the protective action of capsaicin is currently unknown. Previous studies have suggested that endogenous PGs may sensitize sensory neurons to nociceptive stimuli [10, 11]. Boku et al. [19] reported the lack of CGRP release in response to mild injuries in the stomachs of IP-receptor knockout mice. Oishi et al. [10] demonstrated, using IP-receptor knockout mice, that PGI\textsubscript{2} was a major nociceptive mediator in the acetic acid-induced writhing reaction. Since capsaicin-

induced gastric cytoprotection was attenuated by indomethacin and was absent in IP-receptor knockout mice, endogenous PGI\textsubscript{2} may play a supportive role in the mechanism responsible for capsaicin-induced gastric cytoprotection, possibly by sensitizing sensory neurons [17, 28]. However, capsaicin did not have any effect on either PGE\textsubscript{2} production in the rat stomach or PGI\textsubscript{2} production in the mouse stomach. Thus, PGs generated constitutively may maintain the sensitivity of these neurons to the capsaicin stimulation.

Intragastric capsaicin has been shown to increase GMBF in the rat stomach, and this effect was attenuated by sensory deafferentation following a capsaicin pretreatment [9, 14, 29]. Although gastric hyperemia is not the exclusive mechanism responsible for gastric cytoprotection induced by PGE\textsubscript{2} or the stimulation of capsaicin-sensitive afferent neurons [16, 30], GMBF is considered to be a factor in capsaicin-induced gastric protection under certain experimental conditions [7, 29]. We previously reported that the gastric hyperemic response to capsaicin was also significantly mitigated by indomethacin, which suggested the involvement of endogenous PGs in this response [14]. In the present study, we confirmed that intragastric capsaicin markedly increased GMBF in wild type mice in an indomethacin-sensitive manner. This effect was also observed in EP1 or EP3 receptor knockout mice, but was completely absent in animals lacking IP receptors, similar to the gastroprotective effects of capsaicin. These results may provide functional evidence for the modulatory role of IP receptors in the facilitation of gastric protection mediated by capsaicin-sensitive afferent neurons by endogenous PGs.
The results of the present study suggest that capsaicin exhibits gastric cytoprotective effects as well as gastric hyperemic response, essentially by stimulating sensory neurons, and this partly depended on endogenous PG levels. The facilitative effects of endogenous PGs were mediated by EP2 and IP receptors, which may have sensitized the sensory neurons to capsaicin, even though capsaicin increased the production of PGI₂ but not PGE₂ in the gastric mucosa. However, whether endogenous PGs modulated the effects of capsaicin by interacting with TRPV1 remains unknown; however, capsaicin previously exhibited gastric protective effects by activating TRPV1 [31]. Endogenous phosphatidyl-inositol-4, 5-bisphosphate (PtdIns(4, 5)P₂) was shown to inhibit TRPV1, and this was alleviated by agents that activated phospholipase C [32, 33]. Therefore, PGs may sensitize these afferent neurons to capsaicin through EP2/IP receptors by somehow releasing TRPV1 from PtdIns(4, 5)P₂-mediated inhibition.

5. Summary

Capsaicin provided gastric cytoprotection essentially through the stimulation of sensory neurons, and this partly depended on endogenous PGs. PGs facilitated the protective effects of capsaicin, and this response was mediated by EP2 and IP receptors, possibly by sensitizing the sensory neurons to capsaicin, even though capsaicin increased the production of PGI₂ but not PGE₂ in the gastric mucosa (Figure 6). Although endogenous PGs are also known to be involved in the adaptive cytoprotection induced by mild irritants, this is different from that of capsaicin and is mediated by the activation of EP1 receptors with a concomitant increase in the production of mucosal PGE₂.

Figure 6. Mechanisms underlying the gastric cytoprotection induced by PGE₂, mild irritants, and capsaicin. Exogenous PGE₂ provides direct gastric cytoprotection, and this was mediated by the activation of EP1 receptors and completely blocked by the EP1 antagonist, ONO-AE-829. A mild irritant increased endogenous PGE₂ production in the stomach and exhibited adaptive gastric cytoprotection. This action was prevented by indomethacin as well as the EP1 antagonist. On the other hand, capsaicin exhibited gastric cytoprotective effects, essentially mediated by capsaicin-sensitive afferent neurons via the activation of TRPV1 receptors, and this effect was completely attenuated by the TRPV1 antagonist, capsazepine. Although capsaicin increased the production of PGI₂ but not PGE₂ in the gastric mucosa, this protective effect was facilitated by endogenous PGs through PGE₂/EP2 and PGI₂/IP receptors.
Acknowledgements

The authors are greatly indebted to Professor Shu Narumiya, Kyoto University Faculty of Medicine, for kindly supplying EP1, EP3, and IP receptor-knockout mice and Ono Pharmaceutical Company for supplying various EP agonists and antagonists. We also thank the students at the Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Kyoto, Japan, for their technical collaboration.

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