Facilitation by Endogenous Prostaglandins of Capsaicin-Induced Gastric Protection in Rodents through EP2 and IP Receptors

KOJI TAKEUCHI, SHINICHI KATO, MASANORI TAKEEDA, YOSHIHIRO OGAWA, MASATO NAKASHIMA, and MASAHIRO MATSUMOTO

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Kyoto, Japan

Received September 23, 2002; accepted November 19, 2002

ABSTRACT

We investigated the role that prostaglandins (PGs) and EP receptors play in facilitating the gastroprotective action of capsaicin against HCl/ethanol in rats and mice. Male Sprague-Dawley rats and C57BL/6 mice were used after 18 h of fasting. The animals were given HCl/ethanol (60% in 150 mM HCl) p.o. and killed 1 h later. Capsaicin or various EP agonists were given p.o. 30 min or i.v. 10 min before HCl/ethanol. In some cases, indomethacin or various EP agonists were given s.c. 30 min or i.v. 10 min before capsaicin, respectively. Gastric lesions induced by HCl/ethanol were significantly inhibited by PGE2 as well as capsaicin. The effect of PGE2 was antagonized by ONO-AE-829 (EP1 antagonist), whereas the capsaicin action was mitigated by indomethacin as well as sensory deafferentation but not by ONO-AE-829. The generation of mucosal PGE2 was not affected by either capsaicin or sensory deafferentation, but was significantly inhibited by indomethacin. Although neither butaprost (EP2), ONO-NT-012 (EP3), nor 11-deoxy PGE1 (EP4) alone had any effect on HCl/ethanol-induced gastric lesions, only butaprost restored the protective action of capsaicin in the presence of indomethacin. Capsaicin provided a protective action against HCl/ethanol-induced gastric lesions in wild-type (/H11001/) mice in an indomethacin-sensitive manner, and this action was similarly observed in EP1 (/H11002/) and EP3 (/H11002/) mice but not in the animals lacking IP receptors. These results suggest that capsaicin exhibits gastric cytoprotection, essentially by stimulating sensory neurons, and this action is facilitated by endogenous PGs through EP2/IP receptors, probably sensitizing the sensory neurons to capsaicin.

Gastric mucosal integrity is maintained by multiple factors both paracrine and neuronal (Robert et al., 1979, 1983; Holzer and Sametz, 1986; Whittle et al., 1990; Holzer, 1998). The former includes prostaglandins (PGs) (Robert et al., 1979, 1983; Miller, 1983) and nitric oxide (Whittle et al., 1990), whereas capsaicin-sensitive afferent neurons play a central role in the neuronal protection of the stomach (Holzer, 1998). Studies have demonstrated that capsaicin, a selective stimulator of these afferent neurons, protects the gastric mucosa against various ulcerogenic stimuli such as necrotizing agents (Holzer and Sametz, 1986). The protective action of capsaicin is mediated by these afferent neurons, because it is totally attenuated by chemical ablation of these neurons after pretreatment with a large dose of capsaicin (Holzer and Sametz, 1986; Takeuchi et al., 1991b). Recently, the binding site of capsaicin has been cloned and named the vanilloid type 1 receptor (VR1), a nonselective cationic channel (Caterina et al., 1997). It is assumed that capsaicin stimulates these afferent neurons through activation of VR1, resulting in the liberation of the neurotransmitter calcitonin gene-related peptide (CGRP) and gastric protection.

Several studies, including our own, have shown that the protective effect of capsaicin is mitigated by prior administration of indomethacin, suggesting an involvement of endogenous PGs in this action (Takeuchi et al., 1991b, 1993; Uchida et al., 1991; Brzozowski et al., 1993). It is known that endogenous PGs sensitize the sensory neurons to nociceptive stimulation (Ohishi et al., 1999; Ueno et al., 2000). Because capsaicin-induced gastric cytoprotection was attenuated by indomethacin, it is assumed that endogenous PGs play a supportive role in the mechanism of capsaicin-induced gastric protection, probably by sensitizing these afferent neurons.

On the other hand, recent pharmacological studies have classified PGE2 receptors into four specific G protein-coupled receptors: EP1, EP2, EP3, and EP4. This research was supported in part by the Bioventure Developing Program of, and grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org. DOI: 10.1124/jpet.102.044156.

ABBREVIATIONS: PG, prostaglandin; VR1, vanilloid type 1 receptor; CGRP, calcitonin gene-related peptide; GMBF, gastric mucosal blood flow; EIA, enzyme immunoassay; PtdIns(4,5)P2, phosphatidylinositol-4,5-bisphosphate.
of PGE₂ in various tissues, including the gastrointestinal tract. In addition, mice lacking various receptors for prostanoids have been established (Sugimoto et al., 1992; Morimoto et al., 1997), and by using these “knockout mice” the roles of specific PG receptors in various biological actions of PGs have been demonstrated (Ushikubi et al., 1998; Takeuchi et al., 1999). We have performed a series of experiments to determine the EP receptor subtypes mediating the gastrointestinal protection afforded by PGE₂, using various models in both rats and EP-receptor knockout mice, and found that PGE₂, administered exogenously or generated endogenously, provides gastric protection against HCl/ethanol mediated by EP1 receptors (Araki et al., 2000; Suzuki et al., 2001; Takeuchi et al., 2001a). However, the relationship between the EP receptor subtype and the facilitation by PGs of capsaicin-induced gastric protection remains unknown.

In the present study, we investigated the role of endogenous PGs in the gastric protective action of capsaicin against HCl/ethanol-induced damage in rats, mainly in relation to PGE₂ and EP receptors. Furthermore, because an animal model lacking various receptors for prostanoids has now been established (Oida et al., 1995; Sugimoto et al., 1992; Ushikubi et al., 1998), we also evaluated the protective activity of capsaicin in knockout mice lacking EP1 or EP3 receptors and also in some cases the receptors for prostacyclin (IP receptors). In addition, we also examined the gastric hyperemic response to capsaicin in these knockout mice to provide functional evidence for a modulatory role of PGs in capsaicin-induced action.

Materials and 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 (Sugimoto et al., 1997; Ushikubi et al., 1998; Boku et al., 2001). In brief, the mouse genes encoding the EP1, EP3, and IP receptors were individually disrupted, and chimeric mice were generated. These animals were then backcrossed with C57BL/6 mice, and the resulting heterozygous litters (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. 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 h before the experiments. All studies were performed using four to eight animals per group under unanesthetized conditions.

Induction of Gastric Lesions. The rats were given 1 ml of HCl/ethanol (60% in 150 mM HCl) p.o. through esophageal intubation and killed 1 h 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 (square millimeters) of hemorrhagic lesions developed in the stomach exposed, mounted on an ex vivo chamber (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 flow meter (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 the mucosal blood flow were monitored on a recorder (U-228; Tokai-irika, Tokyo, Japan). After the mucosal blood flow had stabilized, the solution in the chamber was withdrawn, and the mucosa was then exposed for 10 min to 0.2 ml of cicaprost, the PGI₂ analog (5 μg/ml), or capsaicin (1 mg/ml). After application of these agents, the mucosa was rinsed with saline, another 0.2 ml of saline was instilled, and the perfusion was resumed. In some of wild-type mice, indomethacin (5 mg/kg) was given s.c. 30 min before administration of HCl/ethanol.

In another experiment, wild-type mice and EP1, EP3, or IP receptor knockout mice were given HCl/ethanol p.o. in a volume of 0.3 ml and killed 1 h later (Araki et al., 2000). Then the stomach was removed and treated with formalin, and the mucosa was examined for hemorrhagic lesions under a dissecting microscope, as described previously. In half of the animals of each group, capsaicin (10 mg/kg) was given p.o. 30 min before administration of HCl/ethanol. In addition, indomethacin (5 mg/kg) or ONO-AE-829 (10 mg/kg) was given s.c. 30 min before administration of capsaicin in wild-type mice.

Measurement of Gastric Mucosal Flow. Gastric mucosal blood flow (GMBF) was measured in both wild-type mice and EP1, EP3, or IP receptor knockout mice, according to our previously published article (Takeuchi et al., 2002). Under urethane-anesthetized conditions, the abdomen was opened through a midline incision, and the stomach exposed, mounted on an ex vivo chamber (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 flow meter (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 the mucosal blood flow were monitored on a recorder (U-228; Tokai-irika, Tokyo, Japan). After the mucosal blood flow had stabilized, the solution in the chamber was withdrawn, and the mucosa was then exposed for 10 min to 0.2 ml of cicaprost, the PGI₂ analog (5 μg/ml), or capsaicin (1 mg/ml). After application of these agents, the mucosa was rinsed with saline, another 0.2 ml of saline was instilled, and the perfusion was resumed. In some of wild-type mice, indomethacin (5 mg/kg) was given s.c. 30 min before application of capsaicin.

Measurement of Mucosal PGE₂ and 6-Keto PGF₁α Levels. Levels of PGE₂ in the rat gastric mucosa and those of 6-keto PGF₁α in the mouse stomach were measured 30 min after 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 given s.c. 30 min before the capsaicin. 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 (Putaki et al., 1994; Takeuchi et al., 2001a). Then the samples were homogenized and centrifuged for 10 min at 12,000 rpm at 4°C. The supernatant of each sample was used for determination of PGE₂ and 6-keto PGF₁α by EIA using PGE₂ and 6-keto PGF₁α kits, respectively (Cayman Chemical, Ann Arbor, MI).

Preparation of Drugs. Drugs used were capsaicin (Nakarai Tesque, Kyoto, Japan); PGE₂, 11-deoxy PGE₁ (Funakoshi, Tokyo, Japan); ONO-AE-829, butaproest, ONO-NT-012 (Ono, Osaka, Japan); cicaprost (Searle, Tokyo, Japan); terbutaline (Bricanyl; Fujisawa,
Osaka, Japan); aminophylline (Neophyllin; Eisai, Tokyo, Japan); and indomethacin (Sigma-Aldrich, St. Louis, MO). Capsaicin was dissolved in Tween 80/ethanol solution (10% ethanol, 10% Tween 80, and 80% saline, w/w) for s.c. injection, whereas indomethacin was suspended in saline. 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 a desired concentration. Each agent was prepared immediately before use and given in a volume of 0.5 ml/100 g b.wt. (rat) or 0.1 ml/10 g b.wt. (mouse) for p.o. and s.c. administration, respectively, and given i.v. in a volume of 0.1 ml/100 g b.wt. (rat). Control animals received saline in place of the active agent.

**Statistics.** Data are presented as the mean ± S.E. for four to eight animals per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of P < 0.05 were regarded as significant.

**Results**

**Protection by PGE₂ and Capsaicin against HCl/Ethanol-Induced Gastric Lesions.** Oral administration of HCl/ethanol (60% in 150 mM HCl) produced multiple lesions in the glandular mucosa, along the long axis of the stomach. These lesions were potently prevented by prior i.v. administration of PGE₂ (0.3 mg/kg), the inhibition being 82.1% (Fig. 1). The protective effect of PGE₂ was significantly mitigated by 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 is significantly less than that observed in the vehicle-treated normal rats. Likewise, the severity of HCl/ethanol-induced gastric lesions was dose-dependently reduced in the animals pretreated with capsaicin (1–10 mg/kg) p.o. before challenge with HCl/ethanol, and a significant effect was obtained at over 3 mg/kg, the inhibition at 10 mg/kg being 81.6% (Fig. 2A). The protective action of capsaicin (10 mg/kg) was almost totally attenuated by chemical ablation of sensory neurons as well as prior administration of indomethacin (5 mg/kg) but not by ONO-AE-829 (10 mg/kg) (Fig. 2B).

**Effect of Capsaicin on PGE₂ Content in Rat Stomach.** Oral administration of capsaicin (10 mg/kg) did not significantly decrease the mucosal PGE₂ content when determined at 0.5 h after the administration (Fig. 3). Prior administration of indomethacin (5 mg/kg s.c.) markedly reduced the PGE₂ contents in the presence of capsaicin, the inhibition being 74.4%. On the other hand, gastric mucosal PGE₂ content was not significantly altered by chemical deafferentation after a large dose of capsaicin. As in normal rats, capsaicin did not significantly affect the mucosal PGE₂ content in sensory deafferented animals.

**Reversal by EP Agonists of Capsaicin-Induced Gastric Protection in Indomethacin-Pretreated Rats.** To investigate the roles of PGE₂ and EP receptors in capsaicin-induced gastric protection, we examined the rescue effect of various subtype-specific EP agonists on the capsaicin action in the presence of indomethacin. Oral administration of cap-
Capsaicin (10 mg/kg) provided a marked protection against HCl/ethanol-induced gastric lesions, the degree of inhibition being 83.4% (Fig. 4A). This effect of capsaicin was significantly mitigated by 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 action of capsaicin was again observed in the rats pretreated with butaprost (EP2 agonist). Neither ONO-NT-012 (EP3 agonist) nor 11-deoxy PGE1 (EP4 agonist) rescued the capsaicin action against HCl/ethanol in the presence of indomethacin. Either of EP agonists (i.v.) used, including butaprost, did not by itself provide a significant protection against HCl/ethanol (Fig. 4B). Furthermore, when butaprost (3 mg/kg) was given i.v. before capsaicin p.o. (1–10 mg/kg), the protective action of capsaicin was significantly enhanced at 1 and 3 mg/kg (Fig. 5). Under such conditions, capsaicin even at 1 mg/kg was effective in significantly reducing the severity of HCl/ethanol-induced gastric lesions.

Gastric Cytoprotection against HCl/Ethanol by Capsaicin in Mice. To further investigate the relation between capsaicin-induced gastric protection and EP receptor subtype, we examined the protective effect of capsaicin against HCl/ethanol in both wild-type and knockout mice lacking EP1 or EP3 receptors. In addition, because a recent study showed a role for PGI2 in the release of CGRP in the stomach after capsaicin stimulation (Boku et al., 2001), the protective effect of capsaicin was also examined in IP receptor knockout mice. Intragastric administration of HCl/ethanol (0.3 ml) also provoked hemorrhagic lesions in the mouse stomach (Fig. 6). HCl/ethanol caused gastric lesions in both EP1, EP3, and IP receptor knockout mice, similar to wild-type mice, and the severity of these lesions was about the same among these groups. Similar to the findings in rats, the severity of these lesions in wild-type mice was reduced by prior p.o. administration of capsaicin (10 mg/kg), the inhibition being 67.2%. Likewise, intragastric capsaicin significantly reduced the severity of these lesions in the animals lacking either EP1 or EP3 receptors, the degree of protection being 73.2 and 66.8%, respectively, in EP1 and EP3 receptor knockout mice. In contrast, 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 the IP receptor knockout animals without capsaicin pretreatment.

Effect of Capsaicin on 6-Keto PGF1α Content in Mouse Stomach. Because the protective action of capsaicin was not observed in IP receptor knockout mice, we measured the effect of capsaicin on gastric 6-keto PGF1α production in both wild-type and IP knockout mice. Oral administration of capsaicin (10 mg/kg) did not significantly affect 6-keto PGF1α content, just like the effect on PGE2 content in rat stomachs (Fig. 7). Indomethacin (5 mg/kg) markedly decreased 6-keto...
content in the presence of capsaicin, the reduction being 84.1%. Similarly, capsaicin had no effect on 6-keto PGF$_{1\alpha}$ production in IP receptor knockout mice.

**Effect of Capsaicin on Gastric Mucosal Blood Flow in Mice.** Because the gastroprotective action of capsaicin in certain experimental conditions is functionally related with the increase of GMBF (Holzer et al., 1991; Matsumoto et al., 1992; Brzozowski et al., 1996), we examined the gastric hyperemic response to capsaicin in mice and investigated the relation of this action with IP receptors. In addition, we also examined the effect of cicaprost, the PGI$_2$ agonist on GMBF, to check the absence of IP receptors in IP receptor knockout mice used in the present study.

**Effect of Capsaicin.** Chambered stomachs of both wild-type mice and those lacking EP1, EP3, or IP receptors showed a relatively constant GMBF of about 80 to 100 mV (arbitrary unit) during a 2-h test period. Mucosal application of capsaicin (1 mg/ml) for 10 min caused a marked increase of GMBF in wild-type mice, and this effect was significantly attenuated by prior administration of indomethacin (5 mg/ml) (Fig. 8). Likewise, a significant increase of GMBF by capsaicin was similarly observed in both EP1 and EP3 receptors of wild-type mice. In contrast, the GMBF in IP receptor knockout mice lacking EP1, EP3, or IP receptors was not increased by capsaicin, indicating the absence of IP receptors in these mice.

**Prostaglandin and Capsaicin-Induced Gastroprotection.** The gastroprotective action of capsaicin was also investigated in the presence of capsaicin, the reduction being 84.1%. Similarly, capsaicin had no effect on 6-keto PGF$_{1\alpha}$ production in IP receptor knockout mice.

**Effect of Capsaicin on Gastric Mucosal Blood Flow in Mice.** The gastroprotective action of capsaicin in certain experimental conditions is functionally related with the increase of GMBF (Holzer et al., 1991; Matsumoto et al., 1992; Brzozowski et al., 1996), we examined the gastric hyperemic response to capsaicin in mice and investigated the relation of this action with IP receptors. In addition, we also examined the effect of cicaprost, the PGI$_2$ agonist on GMBF, to check the absence of IP receptors in IP receptor knockout mice used in the present study.
tor knockout mice. However, the gastric hyperemic response to capsaicin almost totally disappeared in the animals lacking IP receptors, and the values in GMBF were significantly lower than those in control wild-type mice, at most of time points after application of capsaicin.

**Effect of Cicaprost.** In wild type mice, cicaprost (5 μg/ml) applied to the mucosa for 10 min produced a significant increase of GMBF for over 30 min (Fig. 9). In mice lacking IP receptors, however, cicaprost did not cause any increase in GMBF, and the values remained in the same range before and after the exposure to cicaprost.

**Discussion**

PGs, either endogenous or exogenous derivatives, act on multiple receptors (Coleman et al., 1994). In a previous study, we investigated the relation between EP receptor subtypes and gastric protection against HCl/ethanol in rats using various EP agonists and found that exogenous PGE$_2$ affords gastric cytoprotection mediated by EP1 receptors (Araki et al., 2000). We also reported that adaptive gastric cytoprotection is mediated by endogenous PGs, mainly PGE$_2$ through EP1 receptors (Takeuchi et al., 2001a). On the other hand, it is known that capsaicin also affords gastric protection by stimulating afferent C-fibers (Holzer and Sametz, 1986), and this action is partly dependent on endogenous PGs (Takeuchi et al., 1991a,b; Uchida et al., 1991; Brzozowski et al., 1993). However, it remains unexplored which EP receptor subtypes or other prostanoid receptors are responsible for this phenomenon. The present study showed that capsaicin-induced gastric protection is facilitated by endogenous PGs through activation of EP2 and IP receptors, although PG biosynthetic activity in the stomach remained unchanged after the challenge with capsaicin.

First, we confirmed that exogenous PGE$_2$ given i.v. potently prevented the development of HCl/ethanol-induced gastric lesions, and this action was significantly attenuated by the EP1 antagonist ONO-AE-829. These results are consistent with our previous findings using various EP agonists in rats (Araki et al., 2000). This contention was also verified in EP receptor knockout mice, with the protection disappearing in the mice lacking EP1 receptors (Araki et al., 2000; Takeuchi et al., 2001a). Thus, the present together with previous data strongly suggest that the protective action of exogenous PGE$_2$ in the stomach is mainly mediated by activation of EP1 receptors.

Second, it is known that endogenous PGs play a role in gastric cytoprotection induced by oral administration of capsaicin (Takeuchi et al., 1991b, Uchida et al., 1991; Brzozowski et al., 1993). In the present study, the gastroprotective action of capsaicin against HCl/ethanol was observed to be dose-dependent, and the effect at 10 mg/kg was totally attenuated by chemical ablation ofafferent neurons, confirming that this action is mediated by stimulation of capsaicin-sensitive sensory neurons. Interestingly, the protective effect of capsaicin was also significantly mitigated by prior administration of indomethacin. This result suggests the involvement of endogenous PGs in the protective action of capsaicin. However, in contrast to adaptive cytoprotection induced by a mild irritant (Takeuchi et al., 2001a), the capsaicin effect was not affected by the selective EP1 antagonist ONO-AE-829. Furthermore, we found that neither stimulation of sensory neurons by capsaicin nor sensory deafferentation affected the mucosal PGE$_2$ contents in the stomach. Many investigators have shown that mild irritants increased PGE$_2$ generation in the stomach (Konturek et al., 1982; Takeuchi et al., 2001a). These results suggest that although endogenous PGs are involved in the phenomenon of gastric protection induced by both mild irritants and capsaicin, the mode of action seems to be different in these two cases. It is assumed that stimulation of afferent neurons by capsaicin does not increase PG generation in the stomach but exerts protective action in the stomach, partly dependent on endogenous PGs. Certainly, the absence of a measurable increase of PG content does not exclude a possibility that there were localized increases of PG biosynthesis in insufficient magnitude to affect the measurement.

Fig. 9. Effect of cicaprost on GMBF in wild-type and IP receptor knockout mice under urethane anesthetized conditions. Cicaprost was applied to the chamber in a concentration of 5 μg/ml (100 μl) for 10 min, and the GMBF was measured before and after the application. Data are presented as percentage of basal values and represent the means ± S.E. from five to six mice. ∗, significant difference from the corresponding basal values (time 0), $P < 0.05$.
endogenous PGs of this action. Indeed, significant protection by capsaicin was also observed in knockout mice lacking EP1 and EP3 receptors, confirming that the capsaicin-induced gastric protection has nothing to do with the EP1 and EP3 receptors. We could not confirm the involvement of EP2 receptors in this action simply because EP2 knockout mice were not available in our laboratory.

In contrast, we found that capsaicin failed to exhibit cytoprotection against HCl/ethanol-induced gastric lesions in IP receptor knockout mice. We previously found that 20 mM sodium taurocholate as a mild irritant protected the stomach against HCl/ethanol, even in IP receptor knockout mice, similar to in wild-type mice, suggesting no involvement of PGJ2 in the mechanism of adaptive cytoprotection (Takeuchi et al., 2001b). We also reported that 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 (Takeuchi et al., 2001a). The present data 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 contributes to the protective action of capsaicin is unknown. Previous studies suggest that endogenous PGs may sensitize the sensory neurons to nociceptive stimulation (Ohishi et al., 1999; Ueno et al., 2000). Boku et al. (2001) recently reported a lack of release of calcitonin gene-related peptide in response to mild injury in the stomach of IP receptor knockout mice. Ohishi et al. (1999) demonstrated using IP receptor knockout mice that PGJ2 is a major nociceptive mediator in the acetic acid-induced writhing reaction. Because the capsaicin-induced gastric cytoprotection was attenuated by indomethacin and disappeared in IP receptor knockout mice, it is assumed that endogenous PGJ2 plays a supportive role in the mechanism of capsaicin-induced gastric cytoprotection, probably by sensitizing the sensory neurons. As shown in the present study, however, capsaicin did not have any effect on either PGE2 production in the rat stomach or PGJ2 production in the mouse stomach. Thus, it is possible that PGs generated constitutively might maintain the sensitivity of these neurons to capsaicin stimulation.

It is known that intragastric capsaicin increases GMBF in the rat stomach, and this effect is attenuated by sensory deafferentation after capsaicin pretreatment (Holzer et al., 1991; Matsumoto et al., 1992; Brzozowski et al., 1993). Although gastric hyperemia is not the exclusive mechanism of gastric cytoprotection as induced by PGE2 or capsaicin-sensitive afferent neurons (Stroff et al., 1996; Araki et al., 2000), GMBF is considered to be a factor in capsaicin-induced gastric protection under certain experimental conditions (Holzer et al., 1991; Takeuchi et al., 1993). We previously reported that the gastric hyperemic response to capsaicin was also significantly mitigated by indomethacin, suggesting an involvement of endogenous PGs in this action (Matsumoto et al., 1992). In the present study, we confirmed that intragastric capsaicin caused a marked increase of GMBF in wild-type mice, in an indomethacin-sensitive manner. This effect was similarly observed in EP1 or EP3 receptor knockout mice but totally disappeared in the animals lacking IP receptors, similar to the gastroprotective action of capsaicin. We also observed that IP receptor knockout mice failed to respond to cicaprost, the PGJ2 agonist, by increasing the GMBF, confirming the absence of IP receptors in these knockout mice. Because in a preliminary study we confirmed a significant increase of GMBF after i.v. infusion of isoproterenol in these knockout mice, it is assumed that the failure of cicaprost to increase the GMBF in these animals is not a nonspecific phenomenon but due to the absence of IP receptors. These data may provide functional evidence for a modulatory role of IP receptors in facilitation by endogenous PGs of gastric protection mediated by capsaicin-sensitive afferent neurons. The present results taken together suggest that capsaicin provides gastric cytoprotection as well as gastric hyperemic response, essentially through the stimulation of sensory neurons and partly depending on endogenous PGs. The latter, i.e., facilitation by endogenous PGs of this effect, is mediated by EP2 and IP receptors, probably sensitizing the sensory neurons to capsaicin, despite that capsaicin does not increase PG generation in the gastric mucosa. Because the present study was done using only one injury model and because the mechanisms for gastric protection are different depending on the model, further study is certainly required to verify these points using other different injury models. In addition, it remains unknown whether endogenous PGs modulate the capsaicin action through interaction with VR1, although capsaicin exhibits gastric protection through activation of VR1 (Yamamoto et al., 2001). Recent studies demonstrated that endogenous phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P2] inhibits VR1 and the repression can be alleviated by agents that activate phospholipase C (Premkumar and Ahern, 2000; Chuang et al., 2001). Indeed, bradykinin potentiates VR1 activation by capsaicin through hydrolysis of PtdIns(4,5)P2 in a phospholipase C-dependent manner (Chuang et al., 2001). PGs might sensitize these afferent neurons to capsaicin through EP2/IP receptors, by somehow releasing VR1 from PtdIns(4,5)P2-mediated inhibition. Certainly, further study is needed to verify this point.

Acknowledgments
We thank Professor Shu Narumiya (Kyoto University Faculty of Medicine) for kindly supplying EP1, EP3, and IP receptor knockout mice. We also thank Ono Pharmaceutical Co. Ltd. for kindly providing ONO-NT-012 and ONO-AE-829.

References
Boku K, Ohno T, Sueki T, Hayashi H, Hayashi I, Katoro M, Murata T, Narumiya S, Sagi
jeni K, and Majima M (2001) Adaptive cytoprotection mediated by prostaglan-
din IP2 is attributable to sensitization of CRGP-containing sensory nerves. Gastro-
enterology 120:134–143.
Coleman RA, Smith WL, and Narumiya S (1994) Classification of prostaglandin recep-
subtypes have distinctive effects on jejunal afferent sensitivity in the rat. Gastroenterology 119:1580–1588.


