Prostaglandin E₂ Protects Gastric Mucosal Cells from Apoptosis via EP₂ and EP₄ Receptor Activation

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Prostaglandin E₂ (PGE₂) has a strong protective effect on the gastric mucosa in vivo; however, the molecular mechanism of a direct cytoprotective effect of PGE₂ on gastric mucosal cells has yet to be elucidated. Although we reported previously that PGE₂ inhibited gastric irritant-induced apoptotic DNA fragmentation in primary cultures of guinea pig gastric mucosal cells, we show here that PGE₂ inhibits the ethanol-dependent release of cytochrome c from mitochondria. Of the four main subtypes of PGE₂ receptors, we also demonstrated, using subtype-specific agonists, that EP₂ and EP₄ receptors are involved in the PGE₂-mediated protection of gastric mucosal cells from ethanol-induced apoptosis. Activation of EP₂ and EP₄ receptors is coupled with an increase in cAMP, for which a cAMP analogue was found here to inhibit the ethanol-induced apoptosis. The increase in cAMP is known to activate both protein kinase A (PKA) and phosphatidylinositol 3-kinase pathways. An inhibitor of PKA but not of phosphatidylinositol 3-kinase blocked the PGE₂-mediated protection of cells from ethanol-induced apoptosis, suggesting that a PKA pathway is mainly responsible for the PGE₂-mediated inhibition of apoptosis. Based on these results, we considered that PGE₂ inhibited gastric irritant-induced apoptosis in gastric mucosal cells via induction of an increase in cAMP and activation of PKA, and that this effect was involved in the PGE₂-mediated protection of the gastric mucosa from gastric irritants in vivo.

Prostaglandins (PGs), one of the major groups of chemical mediators in the mammalian body, are involved in numerous physiological reactions, such as inflammation and cellular differentiation (1). PGs, especially PGE₂, also have strong cytoprotective effects on the gastric mucosa as a consequence of various indirect mechanisms that include increased epithelial mucus production and bicarbonate secretion (2, 3), inhibition of gastric motility (4), inhibition of acid secretion (5), amelioration of mucosal blood flow (6), inhibition of free radical and enzyme release from neutrophils (7), and vascular, luminal, and/or extrinsic and intrinsic neural mechanisms (8). In contrast, it is still unclear as to whether or not PGE₂ directly protects gastric mucosal cells from various gastric irritants.

Gastropathy, such as gastric ulcer and gastritis, is caused by damage to the gastric mucosa due to its exposure to various gastric irritants such as ethanol and acids. It appears that these gastric irritants damage the gastric mucosa by inducing not only necrosis but also apoptosis in gastric mucosal cells (9). For example, a stimulated rate of apoptosis of gastric mucosal cells was reported at the onset of gastric ulceration (10). Apoptosis associated with Helicobacter pylori infection was suggested to be involved in the development of atrophic gastritis caused by H. pylori infection (11). We recently reproduced such gastric irritant-induced apoptosis in vitro by using primary cultures of guinea pig gastric mucosal cells. Various gastric irritants (non-steroidal anti-inflammatory drugs (NSAIDs), ethanol, hydrogen peroxide, and hydrochloric acid) induced apoptotic DNA fragmentation, chromatin condensation, and caspase activation (9, 12–14). We also found that these gastric irritants induced apoptosis through a common pathway in which mitochondrial dysfunction plays an important role (13). In order to examine the direct cytoprotective effect of PGE₂ on gastric mucosal cells, we investigated previously the effect of PGE₂ on this gastric irritant-induced apoptosis in cultured guinea pig gastric mucosal cells and found that PGE₂ inhibited the apoptosis caused by various gastric irritants (ethanol, hydrogen peroxide, and hydrochloric acid) (15). The molecular mechanism governing this inhibitory effect of PGE₂ on apoptosis has, however, yet to be elucidated. For example, although PGE₂ receptors have been pharmacologically subdivided into four main subtypes (EP₁, EP₂, EP₃, and EP₄) (16), the EP subtype involved in the PGE₂-mediated inhibition of apoptosis has not been revealed. In this study, we examine the molecular mechanism of this PGE₂-mediated inhibition of ethanol-induced apoptosis in gastric mucosal cells, and we suggest that PGE₂ inhibits gastric irritant-induced apoptosis through EP₂- and EP₄-mediated increases in cAMP and activation of protein kinase A (PKA).

EXPERIMENTAL PROCEDURES

Chemicals, Media, and Animals—Fetal bovine serum and trypsin were purchased from Invitrogen. RPMI 1640 was obtained from Nissui Pharmaceutical Co. (Tokyo, Japan). Pronase E and type I collagenase were purchased from Pronase E and type I collagenase (Kaken Pharmaceutical Co. (Kyoto, Japan) and Nitta Gelatin Co. (Osaka, Japan), respectively. P(4-Br-cinnamylamino)-5-isoquinolinesulfonamide (H-89), PGE₂, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma. Peptides from the 3Faculties of Pharmaceutical Sciences, Okayama University and PRESTO, Japan Science and Technology Corporation, Okayama 700-8530, Japan. Tel./Fax: 81-86-251-7958; E-mail: mizushima@pharm.okayama-u.ac.jp.

The abbreviations used are: PGs, prostaglandins; pCPT-cAMP, 8-(4-chlorophenylthio)-cAMP; PKA, protein kinase A; PKB, phosphatidylinositol 3-kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NSAIDs, non-steroidal anti-inflammatory drugs; H-89, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide; AMC, aminomethylcoumarin; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid.

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for the assay of caspases were from Peptide Institute, Inc. (Osaka, Japan). ONO-DO-004 (an EP agonist), ONO-AE-219-01 (an EP agonist), ONO-NT-012 (an EP agonist), and ONO-AE-181-209 (an EP agonist) were gifts kindly provided by Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Anti-cytochrome c antibody was from Pharmingen. Antibodies against actin, caspase-3, caspase-8, and caspase-9 were from Santa Cruz Biotechnology (Santa Cruz, CA). Male guinea pigs (4 weeks of age) were purchased from Shimizu Co., Ltd. (Kyoto, Japan). All experiments and procedures described here were approved by the Animal Care Committee of Okayama University.

Preparation and Culture of Gastric Mucosal Cells—Gastric mucosal cells were isolated from guinea pig fundic glands as described previously (17). Isolated gastric mucosal cells (1 × 10^6 cells/dish) were cultured at 37 °C for 48 h in RPMI 1640 containing 0.3% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in type I collagen-coated plastic culture plates (Iwaki) under the conditions of 5% CO₂, 95% air at 37 °C. After removing non-adherent cells by washing with RPMI 1640, cells that were attached to the plate at about 50% confluence were used. Guinea pig gastric mucosal cell preparations cultured under these conditions have been characterized previously (17, 18), with the majority (about 90%) of cells being identified as pit cells. Treatment of Cells with Ethanol and PGE₂—Cells were preincubated with PGE₂ for 2 h and were then exposed to ethanol by replacement of the entire bathing medium with fresh medium containing ethanol and the same concentration of PGE₂. For monitoring cell viability, cells were incubated with MTT solution at a final concentration of 1 mg/ml for 2 h. Isopropanol alcohol and hydrochloric acid were added to the cells at final concentrations of 50% and 20 mM, respectively. The absorbance of each sample at 570 nm was determined by a spectrophotometer using a reference wavelength of 630 nm (19).

Assay for Caspase Activation—The activities of caspase-3, caspase-8, and caspase-9 were determined as described previously (20, 21). Briefly, cells were collected by centrifugation and suspended in extraction buffer (50 mM PIPES (pH 7.0), 50 mM KCl, 5 mM EGTA, 2 mM MgCl₂, and 1 mM DTT). Suspensions were sonicated and centrifuged, after which the supernatants were incubated with fluorogenic peptide substrates (Ac-DEVD-MCA (caspase-3), Ac-IETD-MCA (caspase-8), and Ac-LEHD-MCA (caspase-9)) in reaction buffer (100 mM HEPES-KOH (pH 7.5), 10% sucrose, 0.1% CHAPS, and 1 mg/ml bovine serum albumin) for 15 min at 37 °C. The release of aminomethylcoumarin (AMC) was determined using a fluorescence spectrophotometer. One unit of protease activity was defined as the amount of enzyme required for releasing 1 pmol of AMC/min.

Release of Cytochrome c from Mitochondria—After ethanol treatment, cells were washed in fractionation buffer (250 mM sucrose, 20 mM HEPES/KOH (pH 8.0), 10 mM KCl, 1.5 mM MgCl₂, 1 mM EDTA, and 1 mM EGTA) and resuspended in the same buffer supplemented with protease inhibitors. After homogenization on ice using a tight fitting Dounce homogenizer, nuclei were spun down at 3,000 rpm for 10 min. The supernatant was further spun at 14,000 rpm for 25 min for separation into cytosolic and mitochondrial fractions. The mitochondrial fraction was then resuspended in buffer D (50 mM Tris/HCl (pH 7.2), 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.05% SDS). Protein concentrations in these fractions were determined by the Bradford method. Samples (4 μg) were applied to 15% polyacrylamide gels containing SDS and subjected to electrophoresis, and then proteins were immunoblotted with the anti-cytochrome c-3, caspase-8, or caspase-9 antibody.

Statistical Analysis—All values are expressed as the means ± S.E. A Student’s t test for unpaired results was performed for the evaluation of differences between the groups. Differences were considered to be significant for values of p < 0.05.

RESULTS

Effect of PGE₂ on Ethanol-induced Apoptosis—We reported previously (15) that PGE₂ inhibited apoptosis induced by the treatment of guinea pig gastric mucosal cells in primary culture with 3% ethanol for 4 h. In this study, conditions were changed slightly for inducing apoptosis in that gastric mucosal cells were incubated with 4% ethanol for 6 h. Fig. 1 shows that PGE₂ inhibited ethanol-induced apoptosis under these conditions. In this way, PGE₂ (0.1–10 μM) attenuated an ethanol-induced decrease in cell viability (Fig. 1). In this experiment, cells were first incubated with PGE₂ (preincubation step) and then with ethanol in the presence of the same concentration of PGE₂ as in the preincubation step. Cell viability was determined by the MTT assay. Values are means ± S.D.; n = 3. **, p < 0.01.

The release of cytochrome c from mitochondria activates the intrinsic pathway of apoptosis (15). In this study, we examined the effect of PGE₂ on the activation of various caspases, including caspase-3, where caspase activities were examined by the use of fluorogenic peptide substrates (Ac-DEVD-MCA (caspase-3), Ac-IETD-MCA (caspase-8), and Ac-LEHD-MCA (caspase-9)) (22). Since these peptides can be cleaved by other caspases (for example, caspase-7 can recognize and cleave Ac-DEVD-MCA (21)), it is more appropriate to describe our findings in terms of caspase-3, caspase-8, or caspase-9 activity, respectively. Ethanol treatment clearly activated all of the caspases tested (Fig. 2A), as has been found previously (13). PGE₂ suppressed the activation of not only caspase-3-like activity but also that of caspase-9- and caspase-8-like activities (Fig. 2A). By employing immunoblotting experiments, using specific antibodies against these caspases, we observed pro-caspase-3, pro-caspase-8, and pro-caspase-9 cleavage by ethanol treatment (Fig. 2B), as has been found previously (13). PGE₂ suppressed the cleavage of each of these pro-caspases (Fig. 2B).

The release of cytochrome c from mitochondria activates caspase-9 in collaboration with Apaf-1 (23). We reported previously (13) that various gastric irritants activated caspase-3, caspase-8, and caspase-9 in gastric mucosal cells. We also reported that PGE₂ suppressed the ethanol-mediated activation of caspase-3 (15). Therefore, we examined the effect of PGE₂ on the activation of various caspases, including caspase-3, where caspase activities were examined by the use of fluorogenic peptide substrates (Ac-DEVD-MCA (caspase-3), Ac-IETD-MCA (caspase-8), and Ac-LEHD-MCA (caspase-9)). Since these peptides can be cleaved by other caspases (for example, caspase-7 can recognize and cleave Ac-DEVD-MCA (21, 22)), it is more appropriate to describe our findings in terms of caspase-3, caspase-8, or caspase-9 activity, respectively. Ethanol treatment clearly activated all of the caspases tested (Fig. 2A), as has been found previously (13). PGE₂ suppressed the activation of not only caspase-3-like activity but also that of caspase-9- and caspase-8-like activities (Fig. 2A). By employing immunoblotting experiments, using specific antibodies against these caspases, we observed pro-caspase-3, pro-caspase-8, and pro-caspase-9 cleavage by ethanol treatment (Fig. 2B), as has been found previously (13). PGE₂ suppressed the cleavage of each of these pro-caspases (Fig. 2B).

The release of cytochrome c from mitochondria activates caspase-9 in collaboration with Apaf-1 (23). We reported previously (13) that various gastric irritants, including ethanol, stimulated the release of cytochrome c from mitochondria. Therefore, PGE₂-mediated suppression of ethanol-induced activation of caspase-9-like activity (Fig. 3) suggests that PGE₂ inhibits the ethanol-stimulated release of cytochrome c from mitochondria.

We therefore examined the effect of PGE₂ on the ethanol-dependent release of cytochrome c from the mitochondria. As shown in Fig. 3, the amount of cytochrome c in the cytosolic or mitochondrial fraction was increased or decreased, respectively, by the ethanol treatment, suggesting that ethanol stimulated the release of cytochrome c from the mitochondria. In the presence of PGE₂, however, the ethanol-induced release of...
cytochrome $c$ from the mitochondria was inhibited (Fig. 3). Therefore, it would appear that the target of PGE$_2$ for inhibiting apoptosis is located upstream of mitochondrial dysfunction in the ethanol-induced apoptosis pathway. Although the inhibition of ethanol-induced caspase-9 activation by PGE$_2$ was partial (Fig. 2), that of release of cytochrome $c$ was almost complete (Fig. 3), suggesting that the ethanol-induced apoptosis partially involves cytochrome $c$-independent activation of caspase-9, which was reported recently (24, 25) in other cell types.

**Identification of EP Receptors Involved in the PGE$_2$-mediated Protection of Cells from Ethanol-induced Apoptosis**—PGE$_2$ receptors have been pharmacologically subdivided into four main subtypes, EP$_1$, EP$_2$, EP$_3$, and EP$_4$ (16). We used agonists specific for each EP receptor in order to identify EP receptors involved in the PGE$_2$-mediated protection of gastric mucosal cells from ethanol-induced apoptosis. Both ONO-AE1-259-01 (an EP$_2$ agonist) and ONO-AE1-329 (an EP$_4$ agonist) suppressed the ethanol-induced decrease in cell viability (Fig. 1). We also found that each of these agonists suppressed the ethanol-mediated activation of caspase-3, caspase-8, and caspase-9-like activities to much the same extent as did PGE$_2$ (Fig. 5A). Furthermore, each of these agonists suppressed the cleavage of pro-caspase-3, pro-caspase-8, and pro-caspase-9 (Fig. 5B). Based on findings reported previously (26) using these agonists (ONO-AE1-259-01 and ONO-AE1-329), we detected the presence of pro-caspase-3, pro-caspase-8, and pro-caspase-9 in the sample (Fig. 5B).

**Fig. 2. Effect of PGE$_2$ on caspase-activation by ethanol.** Cultured gastric mucosal cells were preincubated with the indicated concentrations of PGE$_2$ for 2 h. Cells were further incubated for 6 h with 4% ethanol (EtOH) in the presence of the same concentration of PGE$_2$ as in the preincubation step. Cell lysates were prepared, and caspase-3-, -8-, or -9-like activities were measured by a fluorometric assay using Ac-DEVD-MCA, Ac-IETD-MCA, and Ac-LEHD-MCA for each of the caspases, respectively. One unit of protease activity was defined as the amount of enzyme required for releasing 1 pmol of AMC/min. Values are means ± S.D.; n = 3; *p < 0.05 (A). Pro-caspase-3, caspase-8, and caspase-9 cleavage was monitored by immunoblotting with specific antibodies against these caspases. The relative intensity of each band to control was shown. For control experiment, the amount of actin in each sample was monitored (B).

**Fig. 3. Effect of PGE$_2$ on ethanol-induced release of cytochrome $c$ from mitochondria.** Cultured gastric mucosal cells were preincubated with PGE$_2$ (1 μM) for 2 h. Cells were further incubated for 6 h with 4% ethanol (EtOH) in the presence of the same concentration of PGE$_2$ as in the preincubation step. After subcellular fractionation, cytosolic and mitochondrial fractions were analyzed by immunoblotting with an antibody against cytochrome $c$. As control, the amount of actin in cytosolic fraction was monitored by immunoblotting with an antibody against actin (A). The relative amounts of cytochrome $c$ to actin in cytosolic fractions were determined and expressed (B).
and ONO-AE1-329), it is reasonable to postulate that, for the concentrations of each of them used in the experiments described in Figs. 4 and 5, they act as specific agonists for the EP2 or EP4 receptor. On the other hand, neither ONO-DI-004 (an EP1 agonist) nor ONO-NT-012 (an EP3 agonist) suppressed the ethanol-induced decrease in cell viability (Fig. 4, A and C). We also found that neither of these agonists suppressed the ethanol-mediated activation of caspase-3-, caspase-8-, and caspase-9-like activities (Fig. 5A) and ethanol-mediated cleavage of pro-caspase-3, pro-caspase-8, and pro-caspase-9 (Fig. 5B). On the evidence presented in previous papers (4, 26, 27), where these agonists (ONO-DI-004 and ONO-NT-012) were used, the concentrations employed in the experiments whose results are described in Figs. 4 and 5 should have been enough to activate their respective EP receptor. Consequently, the results provided in Figs. 4 and 5 suggest that EP2 and EP4 but not EP1 and EP3 receptors are involved in the PGE2-mediated protection of gastric mucosal cells from ethanol-induced apoptosis.

**Signal Transduction Pathway for PGE2-mediated Protection of Cells from Ethanol-induced Apoptosis**—The intracellular signaling pathways differ among the various EP receptor subtypes; EP1 is coupled to Ca2+ mobilization, whereas EP2 receptor activation inhibits adenylate cyclase activity, in contrast to EP3 and EP4 receptor activation in which adenylate cyclase activity is stimulated (16). Therefore, the results described above suggest that stimulation of adenylate cyclase activity (increase in cAMP) is involved in the PGE2-mediated protection of gastric mucosal cells from ethanol-induced apoptosis. To test this hypothesis, a cAMP analogue, pCPT-cAMP, was used. As shown in Fig. 6, pCPT-cAMP attenuated the ethanol-induced apoptosis in a dose-dependent manner. The concentration of pCPT-cAMP required for expressing this activity was much the same as that for other cAMP-dependent phenomena (28–30). Therefore, it seems that activation of adenylate cyclase coupled with activation of EP2 and EP4 receptors plays an important role in the signal transduction mechanism associated with the PGE2-mediated protection of gastric mucosal cells from ethanol-induced apoptosis.

The increase in cAMP then activates two types of kinase, PKA and phosphatidylinositol 3-kinase (PI3K), that are important for various signal transduction mechanisms related to apoptosis (31). On this basis, we next examined the involvement of these kinases in the PGE2-mediated protection of gastric mucosal cells from ethanol-induced apoptosis, using an inhibitor for each kinase. H-89 (an inhibitor of PKA) blocked the PGE2-mediated protection of cells from ethanol-induced apoptosis in a dose-dependent manner (Fig. 7A). It is reasonable to consider that H-89 used in the experimental results described in Fig. 7A acts as a specific inhibitor for PKA based on the results of previous papers where similar concentrations of this inhibitor were used (32). H-89, at the concentrations used in the experimental results shown in Fig. 7A, did not affect cell viability in the absence of ethanol and PGE2 or in the presence of ethanol only (data not shown). On the other hand, wortmannin (an inhibitor of PI3K) did not affect the PGE2-mediated protection of gastric mucosal cells from ethanol-induced apoptosis (Fig. 7B). Higher concentrations of wortmannin (more than 20 nM) showed toxicity in the absence of ethanol (data not shown). Based on previous papers (33) where wortmannin was used, the concentrations of this drug used in the experiments described here should have been enough to inhibit PI3K. Therefore, it seems that PKA rather than PI3K is involved in the PGE2-mediated protection of cells from ethanol-induced apoptosis in gastric mucosal cells.

**DISCUSSION**

A number of clinically used anti-ulcer drugs are related in their action to achieving increased intracellular levels of PGE2. Furthermore, NSAIDs, one of the major causes of gastric ulcers, are thought to damage the gastric mucosa by inhibiting cyclooxygenase and decreasing the levels of circulating PGE2 at the gastric mucosa. Therefore, it is clear that PGE2 is one of the most important protective factors for the gastric mucosa in vivo. PGE2 protects the gastric mucosa both directly and indirectly. Compared with indirect PGE2-protective mechanisms, the mechanism of the direct protection is relatively unclear. For example, EP receptors involved in the indirect protection of the gastric mucosa by PGE2 have been revealed as follows: inhibition of gastric motility and stimulation of bicarbonate secretion by PGE2 are mediated by EP1 receptor (34, 35); the
FIG. 6. Effect of cAMP analogue on ethanol-induced apoptosis. Cultured gastric mucosal cells were preincubated with the indicated concentrations of pCPT-cAMP or PGE₂ (1 μM) for 2 h and then further incubated for 6 h with 4% ethanol (EtOH) in the presence of the same concentration of pCPT-cAMP or PGE₂ as that used in the preincubation step. Cell viability was determined by the MTT assay. Values are means ± S.D.; n = 3. **, p < 0.01.

FIG. 7. Effects of an inhibitor of PKA or PI3K on PGE₂-mediated protection of cells from ethanol-induced apoptosis. Cultured gastric mucosal cells were preincubated with 1 μM PGE₂ in the presence of indicated concentrations of H-89 (PKA inhibitor) (A) or wortmannin (PI3K inhibitor) (B) for 2 h. Cells were further incubated for 6 h with 4% ethanol (EtOH) in the presence of the same concentration of H-89 (A) or wortmannin (B) and PGE₂ as in the preincubation step. Cell viability was determined by the MTT assay. Values are means ± S.D.; n = 3. *, p < 0.05; **, p < 0.01; ***, p < 0.001 (versus ethanol-only treated group). #, p < 0.05; ##, p < 0.01 (versus ethanol and PGE₂-treated group).

Fig. 5. Effects of EP receptor agonists on ethanol-induced caspase activation. Cultured gastric mucosal cells were preincubated with ONO-DI-004 (EP₁ agonist; 10 μM), ONO-AE1-259-01 (EP₂ agonist; 1 μM), ONO-NT-012 (EP₃ agonist; 1 μM), ONO-AE1-329 (EP₄ agonist; 1 μM), or PGE₂ (1 μM) for 2 h and then further incubated for 6 h with 4% ethanol (EtOH) in the presence of the same concentration of each agonist or PGE₂ as that used in the preincubation step. Cell lysates were prepared, and caspase-3-, -8-, or -9-like activities were measured by a fluorometric assay using Ac-DEVD-MCA, Ac-IETD-MCA, and Ac-LEHD-MCA, respectively. One unit of protease activity was the amount of enzyme required for releasing 1 pmol of AMC/min. Values are means ± S.D.; n = 3. *, p < 0.05; **, p < 0.01 (A). Pro-caspase-3, caspase-8, and caspase-9 cleavage was monitored by immunoblotting with specific antibodies against these caspases. The relative intensity of each band to control was shown. For control experiment, the amount of actin in each sample was monitored (B).
stimulation of mucin production by PGE2 is mediated by EP4 receptor (36); the increase in gastric mucosal blood flow by PGE2 is mediated by EP3 receptor (37); and inhibition of acid secretion by PGE2 is mediated by EP3/EP4 receptors (5, 38). However, at the present time we have had no concrete evidence as to the role of EP receptors in the direct protection of the gastric mucosa by PGE2.

In a continuation of our previous work (15) that showed that PGE2 inhibited gastric irritant-induced apoptosis in primary cultures of gastric mucosal cells, we examined this phenomenon here in order to understand the underlying molecular mechanism of the direct protection of the gastric mucosa by PGE2. By using agonists specific for each receptor, we have identified that both EP2 and EP4 receptors are involved in the PGE2-mediated protection of gastric mucosal cells from ethanol-induced apoptosis. Relating this to our conclusions, it was recently reported (39) that inhibition of irradiation-induced apoptosis by PGE2 in intestinal epithelium of the jejunum was mediated by the EP2 receptor, using a knockout mouse of this receptor. Since we reported previously that all gastric irritants tested (NSAIDs, ethanol, hydrogen peroxide, and hydrochloric acid) induced apoptosis through a common pathway in which mitochondrial dysfunction plays an important role (9, 13), we assumed that PGE2 inhibited apoptosis by these gastric irritants other than ethanol through EP2 and EP4 receptors. Therefore, it appears that PGE2 protects the gastric mucosa by various mechanisms (both direct and indirect) via different EP receptors.

The conclusion arrived at above is apparently not consistent with some previous reports. The expression of EP receptors in the gastrointestinal tract of mouse has been examined by in situ hybridization studies where it was shown that the expression of EP2 mRNA could be detected in gastric mucosal cells; however, expression of EP2 mRNA was not detected in any types of cells from the stomach by this method (40). We consider that this experiment cannot exclude the possibility that EP2 is weakly expressed in gastric mucosal cells, because it was recently reported (39) that EP2 is expressed in the stomach of the mouse, using RNAase protection assay. Furthermore, it was recently found that the gene encoding EP2 is inducible in macrophages; EP2 mRNA was detected only after a change of medium or following the addition of LPS to the medium (41). Therefore, it is also possible that an unknown stimulus under our culture conditions induces EP2 mRNA expression. In such a case, the anti-apoptotic activity of PGE2 in normal gastric mucosa may be mainly mediated by EP2, whereas EP2 could be involved in such activity only in the presence of pathological conditions such as gastritis. Unfortunately, genes encoding the EP receptors in guinea pig have not been cloned, meaning that these possibilities cannot be further examined at present.

It has also been reported that the EP1 receptor is mainly responsible for the gastro-protective effect of PGE2 in vivo through the inhibition of gastric motility (37, 42). In these reports, the authors examined an acute phase of gastric lesions (1 h after administration of high doses of gastric irritants), suggesting that they damaged gastric mucosal cells mainly through necrosis (not apoptosis). We reported previously (15) that gastric irritant-induced apoptosis, but not necrosis, was inhibited by PGE2 in cultured gastric mucosal cells. Therefore, we consider that PGE2 protects the gastric mucosa from necrosis via an EP1-mediated inhibition of gastric motility and from apoptosis via an EP2/EP4-mediated direct inhibition of apoptosis.

We have also revealed here a part of the intracellular signal transduction pathway for the PGE2-mediated protection of cells from ethanol-induced apoptosis in gastric mucosal cells. By using a cAMP analogue and inhibitors for PKA and PI3K, we propose that PGE2-activated EP2/EP4 receptors stimulate adenylyl cyclase activity, thus increasing cAMP, which then activates PKA and brings about an inhibition of apoptosis. Inhibition of apoptosis by cAMP has been reported for various cell types (43–46). cAMP apparently inhibits apoptosis through activation of PKA (28, 31) or PI3K-mediated (28, 47) processes depending on the difference in cell types. PKA can phosphorylate Bcl-2 and Bcl-2 is a marker for Bcl-2 and Bcl-2 can suppress release of cytochrome c from mitochondria (50). Therefore, activation of PKA by PGE2 in our system may phosphorylate Bcl-2, which could then explain the PGE2-mediated suppression of ethanol-induced release of cytochrome c from mitochondria (Fig. 3). However, since we could not detect the band of phosphorylated BAD in guinea pig gastric mucosal cells using antibodies directed against phosphorylated human BAD (data not shown), we are unable to be certain of this point at this time. Alternatively, since PKA was reported to induce Bcl-2 expression via activation of cAMP-responsive element-binding protein (51), it is also possible that the PGE2-mediated protection of cells from ethanol-induced apoptosis is explained by this mechanism.

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