Involvement of cyclooxygenase-2 in gastric mucosal hypertrophy in gastrin transgenic mice


Involvement of cyclooxygenase-2 in gastric mucosal hypertrophy in gastrin transgenic mice. Am J Physiol Gastrointest Liver Physiol 290: G519–G527, 2006. First published October 27, 2005; doi:10.1152/ajpgi.00113.2005.—Gastrin stimulates gastric acid secretion and promotes gastric mucosal growth (10). Gastrin promotes gastric mucosal growth, and hypergastrinemia induces gastric mucosal hypertrophy. Recently, it has been reported that gastrin induces cyclooxygenase-2 (COX-2) in human gastric and colorectal cancer cell lines. However, whether COX-2 is involved in gastrin-induced gastric mucosal growth in vivo is unknown. We investigated the role of COX-2 in gastrin-induced gastric mucosal hypertrophy using gastrin transgenic mice. Hypergastrinemic mice [mice with mutated gastrin under the control of the β-actin promoter (ACT-GAS mice)] received the COX-2 inhibitor celecoxib (0, 200, or 500 mg/kg of diet) from 5 wk of age and were killed at 16 or 24 wk. Some ACT-GAS mice received celecoxib from 16 wk and were killed at 24 wk. Eighty-week-old ACT-GAS mice without celecoxib treatment were also examined. The thickness of the gastric mucosa, cell populations, COX-2 expression, and PGE2 levels were evaluated. All ACT-GAS mice showed gastric mucosal hypertrophy, and four of six 80-wk-old ACT-GAS mice developed gastric cancer. COX-2 was expressed in interstitial cells of the hypertrophic gastric mucosa and gastric cancers. Moreover, PGE2 levels in the gastric mucosa of ACT-GAS mice were significantly higher than those of normal mice. With treatment with celecoxib, PGE2 levels, the gastric mucosal thickness, and the number of total gastric cells per gastric gland of ACT-GAS mice were significantly decreased. The decrease in gastric mucosal thickness was caused by a reduction of foveolar hyperplasia. The thickness of glands and the number of Ki67-positive cells were not significantly changed. In conclusion, COX-2 contributes to gastrin-induced mucosal hypertrophy of the stomach.

prostaglandin E2; gastric cancer; foveolar hyperplasia

THE GASTRIC HORMONE gastrin stimulates gastric acid secretion and promotes gastric mucosal growth (10). In humans, hypergastrinemia is believed to induce gastric mucosal hypertrophy and to be a risk factor for gastric cancer, particularly in the presence of Helicobacter pylori (1, 25). In a gastrin transgenic (Tg) mouse model (INS-GAS mouse), long-term hypergastrinemia induced gastric mucosal hypertrophy caused by foveolar hyperplasia and led to gastric cancer (39). However, COX-2 inhibition markedly reduced gastric mucosal hypertrophy and tumorous growth of the gastric mucosa in COX-2/microsomal PGE synthase 1 (mPGES-1) Tg mice in which PGE2 is abundantly synthesized in vivo (24). Moreover, Oshima et al. (22) have shown that remarkable hypertrophy and tumorous growth of the gastric mucosa is observed in COX-2/microsomal PGE synthase 1 (mPGES-1) Tg mice in which PGE2 is abundantly synthesized in the stomach (22). In both ACT-GAS and COX-2/mPGES-1 Tg mice, gastric mucosal hypertrophy mainly consists of foveolar hyperplasia (16, 22).

Therefore, we hypothesized that COX-2 is involved in gastric mucosal hypertrophy and gastric cancer development induced by hypergastrinemia. In this study, we evaluated COX-2 expression in hypertrophic gastric mucosa and gastric cancer developed in hypergastrinemic ACT-GAS mice and investigated the effects of the COX-2 selective inhibitor celecoxib on mucosal hypertrophy.

In addition, it has been recently reported that gastrin induces cyclooxygenase-2 (COX-2) in human gastric and colorectal cancer cell lines and in the gastric mucosa of rats and that COX-2 selective inhibitor reverses the trophic properties of gastrin in colorectal cancer (15, 34, 44). However, whether COX-2 is involved in gastrin-induced gastric mucosal growth in vivo is unknown.

The enzyme COX catalyzes the first step of the synthesis of prostanoids. COX-2 is one of the isoforms of COX and is not detectable or is expressed at only very low levels in normal tissues, with the exceptions of tissues such as the brain, testis (42), tracheal epithelia (38), and kidney (4). It has been reported that COX-2 is not, or is very weakly, expressed in the stomach in physiological conditions (11, 18, 37). However, COX-2 is induced by various stimuli such as growth factors and cytokines, both in inflammatory conditions and during tumorigenesis (6). COX-2 has been reported to be induced in a size-dependent manner in gastric and intestinal polyps (13, 27, 32). Therefore, there is a possibility that COX-2 expression generally accompanies tissue growth. COX-2 is also upregulated in gastritis and gastric cancer induced by H. pylori (2, 30). Interestingly, COX-2 inhibition markedly reduces gastric mucosal hypertrophy induced by H. pylori infection in C57BL/6 mice (40). COX-2 inhibition also suppressed gastric cancer induced by N-methyl-N-nitro-N-nitrosoguanidine in rats (8).

Among the COX-2 products, PGE2 is thought to be the most responsible for tissue growth. It has been demonstrated that long-term treatment of rats with 16,16-dimethyl-PGE2 induces gastric mucosal thickening, mainly consisting of foveolar hyperplasia (24). Moreover, Oshima et al. (22) have shown that remarkable hypertrophy and tumorous growth of the gastric mucosa is observed in COX-2/microsomal PGE synthase 1 (mPGES-1) Tg mice in which PGE2 is abundantly synthesized in the stomach (22). In both ACT-GAS and COX-2/mPGES-1 Tg mice, gastric mucosal hypertrophy mainly consists of foveolar hyperplasia (16, 22).

Therefore, we hypothesized that COX-2 is involved in gastric mucosal hypertrophy and gastric cancer development induced by hypergastrinemia. In this study, we evaluated COX-2 expression in hypertrophic gastric mucosa and gastric cancer developed in hypergastrinemic ACT-GAS mice and investigated the effects of the COX-2 selective inhibitor celecoxib on mucosal hypertrophy.

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MATERIALS AND METHODS

Animals. ACT-GAS mice were generated by introducing a mutated human gastrin cDNA under the control of the β-actin promoter, as we have previously reported (16). The peptide product of the mutated human gastrin cDNA contains two mutations. One mutation is a processing site at the NH2 terminus of gastrin: Asp-Pro4-Ser3-Lys2 (native), which is changed to -Asp-Arg2-Arg1-Lys1 (mutant). This tetrabasic site is efficiently cleaved by furin, which is distributed in various cell types (43). The other mutation is after glycine at the COOH terminus of gastrin, with the progastrin sequence inserted by insertion of a stop codon. With these modifications, the mutated progastrin is efficiently cleaved and amidated, even in non-neuroendocrine cells (5). The gastrin titer of ACT-GAS mice homozygously expressing gastrin cDNA was 5- to 10-fold higher than that of wild-type (WT) mice at 10 wk of age. ACT-GAS mice do not have cleaved glycine-extended gastrin or unprocessed progastrin (16).

Study design. ACT-GAS mice were allocated to four groups (Tg-A, -B, -C, and -D), and WT mice were allocated to three groups (WT-A, -B, and -C). Figure 1 summarizes the celecoxib treatment schedules. The Tg-B and WT-B groups were treated with a low dose (200 mg/kg of diet) of the COX-2 selective inhibitor celecoxib (Pfizer Pharmaceuticals) from 5 wk of age until the end of the study. The Tg-C and WT-C groups were treated with a high dose of celecoxib (500 mg/kg of diet) from 4 wk until the end of the study. The Tg-D group was treated with a high dose of celecoxib (500 mg/kg of diet) from 16 wk until the end of the study. The Tg-A and WT-A groups did not receive celecoxib. Because ACT-GAS mice clearly show gastric mucosal hypertrophy from about 12 wk of age (16), we examined the gastric mucosa of ACT-GAS mice at 16 and 24 wk of age. Five mice from each of the Tg-A, -B, and -C and WT-A, -B, and -C groups were killed at 16 wk of age. Five or seven mice from each of the Tg-A, -B, -C, and -D and WT-A, -B, -C groups were killed at 24 wk of age. In addition, six ACT-GAS and four WT mice that had not been treated with celecoxib were also killed at the age of 80 wk.

The study protocol was approved by the animal committee of Kyoto University.

Histological examination and quantification of mucosal thickness. Gastric tissue specimens were fixed in neutral buffered 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). The thickness of the entire gastric mucosa, foveolae, and glandules in the fundus was measured by microscopy. Briefly, the gastric mucosa of the fundus was scanned along the major axis, which was sectioned vertically to the surface of gastric mucosa. We measured the thickness of 10 complete longitudinal profiles of the entire gastric mucosa, foveolae, and glandules and then determined their averages. In addition, the number of total gastric cells and parietal cell per gastric unit was also counted under high magnification in 10 complete longitudinal profiles and averaged. Because the gastric mucosal hypertrophy in ACT-GAS mice is not uniform, we measured the thickness, total gastric cell counts, and parietal cell counts in the thickest parts in one animal. The five researchers choosing the sections to scan were blind to the identity of the treatment group.

RT-PCR. Total RNA was extracted from the gastric mucosa of WT and ACT-GAS mice. RNA extraction was performed using TRIzol reagent (Invitrogen; Carlsbad, CA), and first-strand cDNA was prepared from 5 μg RNA using SuperScript III Reverse Transcriptase (Invitrogen). PCRs were then performed using LA Taq (TAKARA BIO; Tokyo, Japan). The PCR primers were as follows: COX-1, forward 5′-AGTCGAGAGACTCTCGCTCTGG-3′ and reverse 5′-CAGAAATGGTGAACGAGGCTG-3′; COX-2, forward 5′-TTTGTGATGCATTTCAACAGCAGAT-3′ and reverse 5′-CAGTATTGGAGAACAGATGGGATT-3′; and β-actin, forward 5′-GTGGGCGCTCTAGGCACCAAA-3′ and reverse 5′-CTCTTGTGATCTACGGAGATTC-3′ (539 bp).

PCP products were separated on 1.2% agarose gels and analyzed. The levels of expression of COX-1 and COX-2 were measured semi-quantitatively by comparison with β-actin. PCR products (COX-1 and COX-2) were confirmed by sequence analysis.

Western blot analysis. To analyze COX-1 and COX-2 expressions in the stomach, frozen tissues from the gastric fundus of the mice were homogenized and lysed in a buffer containing 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 50 mM NaF, and Complete Mini protease inhibitor cocktail (Roche Molecular Biochemicals; Mannheim, Germany). Lysates were placed on ice for 30 min and centrifuged at 15,000 rpm for 20 min. After denaturation, 30 μg of protein extracts were fractionated by SDS-PAGE and transferred to immobilin-P membranes (Millipore; Bedford, MA). Membranes were incubated with anti-COX-1 goat antibody (Santa Cruz Biotechnology; Santa Cruz, CA) or anti-COX-2 rabbit antibody (Cayman Chemical; Ann Arbor, MI) for 1 h at 37°C and then for 12 h at 4°C. Proteins were detected using the enhanced chemiluminescence system (Amersham Pharmacia Biotech; Buckinghamshire, UK). COX-2 standard protein (Santa Cruz Biotechnology) was used as a control. The film exposure time was 5 min for COX-2, 5 min for COX-1, and 5 s for β-actin. The band density was quantified using NIH Image, and the COX-2-to-β-actin ratio was evaluated. The same experiments were performed three times, and representative data are shown.

Immunohistochemistry. Specimens were fixed in neutral buffered 10% formalin, embedded in paraffin, and sectioned to a thickness of 5 μm. Sections were deparaffinized, rehydrated, and incubated with 3% H2O2 in methanol for 20 min to quench endogenous peroxidase activity. Subsequently, specimens were blocked with normal goat serum for 30 min and incubated for 2.5 h at room temperature with rabbit polyclonal anti-COX-2 antibody (1:100, Cayman Chemical) or for 30 min at room temperature with rat anti-Ki67 antibody (1:50, DAKO; Copenhagen, Denmark). The immune complex was visualized using the Vectastain Elite Kit (Vector Laboratories; Burlingame, CA) according to the manufacturer’s protocol. Sections incubated with normal rabbit serum served as negative controls. Antibody absorption studies were performed with sections treated in the same manner except that murine COX-2 blocking peptide (Cayman Chem...
ical) was added to the anti-COX-2 antibody in a 1:1 (wt/wt) ratio and incubated at 37°C for 1 h before application. The number of Ki67-positive cells per gastric unit was counted under high magnification in 10 complete longitudinal profiles in the thickest part in one animal, and the average number was calculated.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay. Epithelial cell apoptosis was determined in situ from paraffin-embedded tissue sections of the stomach of celecoxib-treated and untreated 24-wk-old AGT-GAS mice and untreated WT mice of the same age by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. TUNEL staining was performed using the ApopTag kit (Intergen) as described by the manufacturer.

Measurement of PGE2. The gastric mucosa was homogenized at 4°C in lysis buffer. Homogenates were centrifuged at 12,000 rpm for 20 min at 4°C. PGE2 levels in the supernatant of each sample were determined by enzyme immunoassay using a Prostaglandin E2 Monoclonal Enzyme Immunoassay Kit (Cayman Chemical) according to the manufacturer’s protocol.

Measurement of serum gastrin levels. Serum gastrin levels were measured by radioimmunoassay (41) with a commercially available kit (Gastrin-RIAKIT II, Dinabot; Tokyo, Japan).

Statistical analysis. Data are presented as means ± SE. Differences in thickness of the entire gastric mucosa, foveolae, and glands and numbers of parietal cells and Ki67-positive cells were analyzed using Student’s t-test. A P value of <0.05 was considered significant for two-tailed tests.

RESULTS

ACT-GAS mice develop gastric mucosal hypertrophy and COX-2 is expressed in interstitial cells. First, we examined the stomachs of ACT-GAS and WT mice at the age of 80 wk. ACT-GAS mice showed marked gastric mucosal hypertrophy compared with WT mice (Fig. 2). Gastric mucosal hypertrophy was mainly composed of foveolar hyperplasia and was accompanied by a decrease of the thickness of the glandular compartment and the number of parietal cells. To examine the expression levels of COX mRNA and protein, total RNA and tissue lysates were extracted from the gastric mucosa of ACT-GAS and WT mice, and RT-PCR and Western blot analysis were performed. COX-2 mRNA and protein expression were clearly observed in ACT-GAS mice, whereas their expression levels were very low in WT mice (Fig. 3A). We also quantified COX-2 and β-actin band density in Western blotting using NIH Image. The COX-2-to-β-actin ratio in ACT-GAS mice was significantly higher than that in WT mice (2.35 ± 1.26 vs. 0.69 ± 0.05, P < 0.05). In contrast, COX-1 mRNA and protein levels were not different between ACT-GAS and WT mice (Fig. 3B). Next, we examined the localization of COX-2 by immunohistochemistry. In ACT-GAS mice, COX-2 protein was localized in interstitial cells in the lamina propria of the stomach (Fig. 3, C and D). On the other hand, immunoreac-

Fig. 2. Hematoxylin and eosin (H&E)-stained sections of stomachs of ACT-GAS (A) and WT mice (B) at the age of 80 wk. Bars = 200 μm.

Fig. 3. A and B: cyclooxygenase (COX)-1 and COX-2 expressions in ACT-GAS and WT mice at 80 wk of age. RT-PCR and Western blot analysis showed that COX-2 mRNA and protein expression levels in stomachs of 80-wk-old ACT-GAS mice were higher than those in WT mice at the same age (A), whereas COX-1 mRNA and protein expression levels in stomachs of ACT-GAS and WT mice were similar (B). For Western blotting, COX-2 standard protein (0.5 μg) was used as a control (right lane). Data are representative of 3 independent experiments. C and D: immunohistochemistry showed that COX-2 was expressed in interstitial cells in stomachs of 80-wk-old ACT-GAS mice. Bars = 200 (C) and 100 μm (D). E: antigen absorption studies were performed with sections treated in the same manner except that murine COX-2 blocking peptide was added to anti-COX-2 antibody. Bar = 200 μm.
tivity was not observed in the same specimens, when immuno- 
nohistochemistry was performed after murine COX-2 blocking 
peptide was added to the anti-COX-2 antibody before applica-
tion (Fig. 3E). In WT mice, COX-2 was not detected by immuno-

COX-2 is abundantly expressed in the gastric cancer that de-
velops in ACT-GAS mice. Gastric tumors were observed in 
four of six ACT-GAS mice at the age of 80 wk (Fig. 4, A and B), whereas no tumors were seen in four WT mice of the same age. Histologically, all tumors showed epithelial atypia, hyperchromia, and loss of cellular polarity, although invasion into the submucosa or vasculature was not observed. Thus the tumors that developed in the ACT-GAS mouse stomach were well-differentiated intramucosal adenocarcinoma, and this finding is compatible with the report of Wang et al. (39), in which the development of gastric cancer in hypergastrinemic INS-GAS mice was observed. Immunohistochemistry showed that COX-2 was abundantly expressed in interstitial cells in the gastric cancer (Fig. 4, C and D).

The COX-2 inhibitor celecoxib reduces gastric mucosal hypertrophy in ACT-GAS mice. Next, to investigate the role of COX-2 in gastric mucosal hypertrophy induced by gastrin, we administered the COX-2 selective inhibitor celecoxib to ACT-GAS and WT mice. Because gastric mucosal hypertrophy was clearly observed from about 12 wk of age in ACT-GAS mice, we examined the gastric mucosa at the ages of 16 and 24 wk. To confirm that COX-2 protein is truly expressed in the stomach of ACT-GAS mice at the age of 16 and 24 wk, tissue lysates were extracted from the stomachs of 16- and 24-wk-old ACT-GAS and WT mice. Western blot analysis was performed using an anti-COX-2 antibody. Although COX-2 protein was detected in the stomachs of both 16- and 24-wk-old ACT-GAS and WT mice, its level in ACT-GAS mice was much higher than in WT mice (Fig. 5, A and B). We also quantified COX-2 and β-actin band density in Western blotting using NIH Image. The COX-2-to-β-actin ratio in ACT-GAS mice was significantly higher than that in WT mice (16 wk: 0.384 ± 0.014 vs. 0.140 ± 0.024, P < 0.05; and 24 wd: 0.314 ± 0.015 vs. 0.121 ± 0.018, P < 0.05).

The mucosal thickness and total gastric cell counts per gastric gland of ACT-GAS mice were significantly greater than those of WT mice at both 16 and 24 wk of age (Tables 1 and 2). With treatment with celecoxib at 200 and 500 mg/kg of diet, the gastric mucosal hypertrophy and increased total gastric cell counts per gastric gland observed in ACT-GAS mice were significantly reduced compared with those in nontreated ACT-GAS mice (P < 0.05; Tables 1 and 2). Moreover, treatment

Fig. 4. Development of well-differentiated adenocarcinoma in stomachs of aged ACT-GAS mice. A and B: H&E-stained sections of gastric cancer that developed in 80-wk-old ACT-GAS mice. Bars = 500 (A) and 100 μm (B). C and D: immunohistochemistry showed that COX-2 protein was expressed exclusively in interstitial cells in the gastric cancer that developed in 80-wk-old ACT-GAS mice. Bars = 200 (C) and 100 μm (D). E: antigen absorption studies were performed with sections treated in the same manner except that murine COX-2 blocking peptide was added to anti-COX-2 antibody. Bar = 200 μm.
with celecoxib at 500 mg/kg of diet from 16 to 24 wk also significantly reduced gastric mucosal hypertrophy ($P < 0.05$). In contrast, celecoxib did not have a significant effect on the gastric mucosal thickness of WT mice at any dose (Table 1). Representative H&E-stained sections of stomachs of 24-wk-old ACT-GAS mice with and without celecoxib treatment (200 or 500 mg/kg of diet) from 5 to 24 wk of age are shown in Fig. 6.

**Celecoxib reduce foveolar hyperplasia in stomachs of ACT-GAS mice.** We also examined which cells in the gastric mucosa were affected by the treatment with celecoxib. In addition, to investigate the effects of celecoxib on cellular proliferation, the number of Ki67-positive cells was also counted. Table 2 and 3 summarized the effects of celecoxib on the thickness of the gastric foveolar and glandular parts and the numbers of total gastric cells, parietal cells, and Ki67-positive cells. In the gastric mucosa of 24-wk-old ACT-GAS mice, the foveolar thickness was significantly higher than that of WT mice, whereas the thickness of the glandular part was not different from that of WT mice. Moreover, the numbers of total gastric cells and Ki67-positive cells per gastric gland in 24-wk-old ACT-GAS mice were also significantly higher than those in WT mice at the same age. With the treatment with celecoxib at 200 or 500 mg/kg of diet from 5 wk of age, the gastric foveolar thickness of 24-wk-old ACT-GAS mice was significantly reduced ($P < 0.05$; Table 3). However, gastric glandular thickness and parietal cell counts per gastric gland were not altered by celecoxib treatment at any dose (Tables 2 and 3). In addition, the average number of Ki67-positive cells per gastric gland in ACT-GAS mice was not affected by celecoxib treatment (Table 2). Representative immunohistochemistry sections of the stomachs of 24-wk-old ACT-GAS mice with or without celecoxib treatment, using an anti-Ki67 antibody, are shown in Fig. 7.

**Celecoxib induced apoptosis of foveolar cells in ACT-GAS mice.** Several reports have demonstrated that one of the COX-2 products, PGE$_2$, prolongs the survival of foveolar cells with resulting foveolar hyperplasia (9, 35, 36). Therefore, we evaluated the apoptosis index of the gastric cells in celecoxib-treated and untreated ACT-GAS mice. Celecoxib (200 or 500 mg/kg of diet) induced apoptosis of foveolar cells in ACT-GAS mice. The apoptosis index of the total gastric cells in celecoxib-treated (200 or 500 mg/kg of diet) ACT-GAS mice was significantly higher in that in ACT-GAS mice not treated with celecoxib (Table 4).

**PGE$_2$ levels in ACT-GAS mice were higher than in WT mice.** Among the COX-2 products, PGE$_2$ is thought to be the most responsible for tissue growth. Therefore, we measured PGE$_2$ levels in stomachs of ACT-GAS and WT mice at the age of 24 wk. PGE$_2$ levels in stomachs of ACT-GAS mice were significantly higher those in WT mice ($P < 0.05$). Moreover, the elevated levels of PGE$_2$ in the stomachs of ACT-GAS mice were significantly reduced by celecoxib treatment (Table 5).

**Celecoxib did not affect serum gastrin levels in ACT-GAS mice.** It is possible that celecoxib affects gastrin expression in ACT-GAS mice; therefore, we measured serum gastrin levels in both celecoxib-treated and untreated ACT-GAS mice. Serum gastrin levels in celecoxib-treated (200 and 500 mg/kg of diet) ACT-GAS mice were 1,070 ± 334.6 and 858.7 ± 145.3 pg/ml, respectively. These values were not significantly different from those in untreated ACT-GAS mice (880 ± 145.6 pg/ml).

**DISCUSSION**

In this study, we clearly demonstrated that hypertrophy of the gastric mucosa of hypergastrinemic ACT-GAS mice is associated with enhanced expression of COX-2 in the gastric mucosa and that inhibition of COX-2 by the specific COX-2 antagonist celecoxib significantly reduced hypertrophy of the gastric mucosa in ACT-GAS mice. These results suggest that COX-2 is involved in gastrin-induced gastric mucosal hypertrophy in ACT-GAS mice. Moreover, gastric mucosal hypertrophy in ACT-GAS mice was also reduced by celecoxib treatment from 16 wk of age, when gastric mucosal hypertrophy had already developed. Therefore, COX-2 inhibition also seems able to reduce the gastric mucosal hypertrophy that has already developed in response to hypergastrinemia.

Although much effort has been made to elucidate how COX-2 is induced in various tumors and tissues, the precise mechanisms still remain unknown. It has been reported that gastrin induces COX-2 in some human gastric and colorectal cancer cell lines and in rat gastric mucosal epithelial cells (15, 17, 44). In the present study, however, COX-2 was expressed...
in interstitial cells of the lamina propria of stomachs of ACT-GAS mice. Although this observation is compatible with several previous reports (13, 23, 29) showing that COX-2 is expressed in interstitial cells in intestinal polyps and gastric hyperplastic polyps, it should noted that interstitial cells in the stomach do not possess the CCK-2 receptor (21). Moreover, we also confirmed that gastrin does not affect the COX-2 expression in the mouse fibroblast cell line NIH3T3 (data not shown). Therefore, it is unlikely that gastrin directly induces COX-2 expression in interstitial cells in the stomach. We and others (13, 27, 32) have reported that COX-2 is induced in a size-dependent manner in gastric and intestinal polyps. Thus it may be considered that induction of COX-2 in ACT-GAS mice is partly due to the development of hypertrophy of the gastric mucosa. Finally, the possibility of infection with Helicobacter species should be considered, because various reports (30, 33, 40) have demonstrated that H. pylori infection induces COX-2 expression in the stomach. However, Helicobacter species could not be detected in the stomachs of our ACT-GAS mice using PCR that can detect Helicobacter species (data not shown).

How is COX-2 involved in gastric mucosal hypertrophy in hypergastrinemic ACT-GAS mice? It is possible that COX-2 inhibition affects gastrin expression levels in ACT-GAS mice. Gastrin expression levels in ACT-GAS mice were not significantly affected by celecoxib in the present study.

In the present study, the mucosal hypertrophy observed in ACT-GAS mice mainly consisted of foveolar hyperplasia. Moreover, celecoxib reduced this foveolar hyperplasia in ACT-GAS mice, whereas it affected neither the thickness of the glandular part nor the numbers of parietal cells per gastric gland. In addition, celecoxib did not affect the increased number of Ki67-positive cells. Therefore, COX-2 seems to play roles in gastric foveolar hyperplasia without increasing cellular proliferation in hypergastrinemic ACT-GAS mice. We also showed in this study that PGE2 levels in the gastric mucosa of ACT-GAS mice were significantly higher than those in WT mice and that administration of celecoxib lowered the increased PGE2 levels in the gastric mucosa of ACT-GAS mice. In this respect, it is important to note that various reports (9, 35, 36) have demonstrated that PGE2 induces gastric foveolar hyperplasia by prolonging cell survival without increasing the mitotic index. We also confirmed that apoptotic gastric foveolar cells were increased by the treatment with celecoxib in ACT-GAS mice using TUNEL method. Therefore, it is suggested that PGE2 is produced by COX-2 in interstitial cells in the lamina propria of the stomach of ACT-GAS mice and is responsible at least in part for prolongation of the survival of foveolar cells, with resulting foveolar hyperplasia (35, 36).

Table 3. Effects of celecoxib on gastric foveolar thickness and glandular thickness in ACT-GAS mice at 24 wk of age

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<th>Foveolae, μm</th>
<th>Glandules, μm</th>
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<tr>
<td>WT mice</td>
<td></td>
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<tr>
<td>No celecoxib</td>
<td>107.5±22.1</td>
<td>300.5±12.8</td>
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<tr>
<td>ACT-GAS mice</td>
<td></td>
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<tr>
<td>No celecoxib</td>
<td>347.6±69.9*</td>
<td>325.2±128.8</td>
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<tr>
<td>200 mg/kg of diet celecoxib</td>
<td>250.8±47.5†</td>
<td>294.0±48.4</td>
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<td>500 mg/kg of diet celecoxib</td>
<td>249.7±63.7†</td>
<td>307.1±70.2</td>
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Values are means ± SE; n = 5 or 7 mice. Celecoxib treatment was performed between 5 and 24 wk of age. *P < 0.05 vs. WT mice without celecoxib treatment; †P < 0.05 vs. ACT-GAS mice without celecoxib treatment.
Several mechanisms may be considered for the prolongation of foveolar cell survival by PGE₂. PGE₂ has been reported to increase mucosal blood flow (20), mucus production and bicarbonate secretion (12, 14), and to inhibit acid secretion (45) and gastric motility (31). Moreover, we (19) have previously shown that the PGE₂ EP4 receptor is present in foveolar cells of the stomach, and recently it has been reported that PGE₂ directly protects pig gastric foveolar cells from apoptosis via EP2 and EP4 receptor activation (7). Therefore, PGE₂ may directly affect foveolar cells and inhibits their apoptosis.

Because celecoxib did not completely reverse the gastric mucosal hypertrophy induced by hypergastrinemia in this study, it is evident that there exist COX-2-independent mechanisms for the gastric mucosal hypertrophy observed in hypergastrinemic ACT-GAS mice. In this regard, transforming growth factor-α (TGF-α) and heparin-binding epidermal growth factor were increased in hypergastrinemic INS-GAS mice (39), and TGF-α is known to induce foveolar hyperplasia with increased DNA synthesis (28). This mechanism seems to be independent of COX-2, because COX-2 inhibitor did not affect the number of Ki67-positive proliferative cells in the stomach of hypergastrinemic ACT-GAS mice. Alternatively, we have previously demonstrated that gastrin directly stimulates the growth of foveolar cell precursors by inducing its own receptors (19) and that gastrin induces Reg protein production, which has an antiapoptotic effect on gastric mucosal cells (3, 26). These mechanisms also seem to be involved in gastrin-induced gastric mucosal hypertrophy.

We have shown in this study that intramucosal gastric cancer developed in four of six ACT-GAS mice at the age of 80 wk, which was preceded by foveolar hyperplasia. So far, only Wang et al. (39) have directly demonstrated that hypergastrinemic mice (INS-GAS mice) develop gastric cancer without other carcinogens. The results of our present study are congruent with their study and support the notion that hypergastrinemia may promote gastric cancer. In addition, we found in this study that COX-2 was overexpressed not only in cancer tissues but also in the preceding hyperplastic gastric mucosa in hypergastrinemic mice. Moreover, the COX-2 inhibitor celecoxib could inhibit the foveolar hyperplasia observed in our mouse model. Because the presence of precancerous lesions such as mucosal atrophy and intestinal metaplasia are usually associated with hypergastrinemia (25), whether the COX-2 inhibitor can prevent the development of gastric cancer from such a condition needs to be clarified in future studies.

In conclusion, this is the first report demonstrating that COX-2 contributes to gastrin-induced gastric mucosal hypertrophy in vivo.

Table 4. Effects of celecoxib on the apoptosis index of gastric cells at 24 wk of age in ACT-GAS mice

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<tr>
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<th>Apoptosis Index, %</th>
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<tr>
<td>WT mice</td>
<td>0.77±0.22</td>
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<tr>
<td>No celecoxib</td>
<td></td>
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<tr>
<td>ACT-GAS mice</td>
<td>2.10±0.47*</td>
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<tr>
<td>No celecoxib</td>
<td></td>
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<tr>
<td>200 mg/kg of diet celecoxib</td>
<td>3.24±0.68†</td>
</tr>
<tr>
<td>500 mg/kg of diet celecoxib</td>
<td>4.26±1.39†</td>
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Values are means ± SE; n = 5 or 7 mice. Celecoxib treatment was performed between 5 and 24 wk of age. *P < 0.05 vs. WT mice without celecoxib treatment. †P < 0.05 vs. ACT-GAS mice without celecoxib treatment.

Table 5. PGE₂ levels in the gastric mucosa of 24-wk-old WT and ACT-GAS mice with or without celecoxib

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<thead>
<tr>
<th></th>
<th>PGE₂, pg/mg total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT mice</td>
<td>199.7±41.3</td>
</tr>
<tr>
<td>No celecoxib</td>
<td></td>
</tr>
<tr>
<td>ACT-GAS mice</td>
<td>292.0±65.9*</td>
</tr>
<tr>
<td>No celecoxib</td>
<td></td>
</tr>
<tr>
<td>200 mg/kg of diet celecoxib</td>
<td>189.4±61.0†</td>
</tr>
<tr>
<td>500 mg/kg of diet celecoxib</td>
<td>179.0±58.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 or 7 mice. Celecoxib treatment was performed between 5 and 24 wk of age. *P < 0.05 vs. WT mice without celecoxib treatment; †P < 0.05 vs. ACT-GAS mice without celecoxib treatment.
REFERENCES


25. Wang TC, Dangler CA, Chen D, Goldenberg JR, Koh T, Raychowdhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, and Fox JG. Synergistic interaction between hypergastrinemia and Helicobacter infec-


