We investigated the cyclooxygenase (COX) isoforms as well as prostaglandin E receptor EP subtypes responsible for acid-induced gastric HCO$_3^-$ secretion in rats and EP receptor-knockout (-/-) mice. Under urethane anesthesia, a chambered stomach (in the presence of omeprazole) was perfused with saline, and HCO$_3^-$ secretion was measured at pH 7.0 using a pH-stat method and by adding 2 mM HCl. Mucosal acidification was achieved by exposing the stomach for 10 min to 50 or 100 mM HCl. Acidification of the mucosa increased the secretion of HCO$_3^-$ in the stomach of both rats and WT mice, in an indomethacin-inhibitable manner. The acid-induced gastric HCO$_3^-$ secretion was inhibited by prior administration of indomethacin and SC-560 but not rofecoxib in rats and mice. Acidification increased the PGE$_2$ content of the rat stomach, and this response was significantly attenuated by indomethacin and SC-560 but not rofecoxib. This response was also attenuated by ONO-8711 (EP1 antagonist) but not AE3-208 (EP4 antagonist) in rats and disappeared in EP1 (-/-) but not EP3 (-/-) mice. PGE$_2$ increased gastric HCO$_3^-$ secretion in both rats and WT mice, and this action was inhibited by ONO-8711 and disappeared in EP1 (-/-) but not EP3 (-/-) mice. These results support a mediator role for endogenous PGs in the gastric response induced by mucosal acidification and clearly indicate that the enzyme responsible for production of PGs in this process is COX-1. They further show that the presence of EP1 receptors is essential for the increase in the secretion of HCO$_3^-$ in response to mucosal acidification in the stomach.

**Key words:** gastric and duodenal HCO$_3^-$ secretion, cyclooxygenase, prostaglandin E$_2$, prostaglandin EP receptor subtype, rat EP receptor knockout mice
INTRODUCTION

The secretion of HCO$_3^-$ from surface epithelial cells is one of the mucosal defensive mechanisms and plays an important role in protecting the gastroduodenal mucosa against acid (1-3). The physiological regulation of this secretion involves several factors such as prostaglandins (PGs), peptides and neuronal factors (2,4-6), yet endogenous PGs are particularly important in the local control of the process. We have previously investigated the roles of cyclooxygenase (COX) isoforms and PGE receptor (EP) subtypes in acid-induced duodenal HCO$_3^-$ secretion, and found that the secretion was stimulated by endogenous PGs produced by COX-1 through activation of EP3/EP4 receptors (7-9). It is also known that the increase in duodenal HCO$_3^-$ secretion is mediated by the stimulation of adenylate cyclase (AC) activity and an elevation in intracellular adenosine 3', 5'-cyclic monophosphate (cAMP) levels (1,2,10,11) and that the activation of EP3/EP4 receptors results in an increase of intracellular cAMP via G protein (9,12).

Endogenous PGs also play an important role in acid-induced HCO$_3^-$ secretion in the stomach (1,4), yet the roles of COX isoforms and EP receptor subtypes in this process remain to be explored. Although we previously reported that PGE$_2$ stimulates gastric HCO$_3^-$ secretion mediated by activation of EP1 receptors (7), it remains unknown whether the acid-induced response in the stomach also requires the presence of EP1 receptors or whether EP1 receptors are really expressed in the surface epithelial cells responsible for this secretion.

The present study was designed to investigate the role of COX isoforms in acid-induced HCO$_3^-$ secretion in the stomach, using selective COX-1 and COX-2 inhibitors, and the EP receptor subtype responsible for the responses to acid and PGE$_2$, using selective EP agonists and antagonists, in rats and EP receptor knockout mice. We also examined the expression of EP1 receptors in RGM1 cells derived from surface epithelial cells of the rat gastric mucosa.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (200-220 g) and C57BL/6 mice (25-30 g) were used. Mice lacking EP1- or EP3-receptors were generated as described previously (13,14). In brief, the mouse genes encoding the EP1 and EP3 receptors were individually disrupted, and chimeric mice were generated. These animals were then back-crossed with C57BL/6 mice, and the resulting heterozygous litter mates were bred to produce homozygous EP1- or EP3 knockout mice. The distribution of the EP1 or EP3-receptor genes was verified by northern blot hybridization, which failed to detect messenger RNAs encoding the respective receptors in EP1 (-/-) or EP3 (-/-) mice. These rats and knockout mice were deprived of food but allowed free access to tap water for 18 hr before the experiments. Experiments were performed using 4~7 animals under anesthesia with
urethane (1.25 g/kg) given intraperitoneally (i.p.). All experimental procedures described here were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

**Determination of HCO$_3^-$ Secretion**

HCO$_3^-$ secretion was determined in a chambered stomach according to previously published methods (7). In brief, the stomach was exposed, mounted on a chamber (exposed area: 3.1 cm$^2$ for rats and 1 cm$^2$ for mice) and perfused with saline that was gassed with 100% O$_2$ and kept in a reservoir. Gastric HCO$_3^-$ secretion was measured at pH 7.0 by using a pH-stat method (Hiranuma Comtite-8, Mito, Japan) and by adding 2 mM HCl to the reservoir. To unmask HCO$_3^-$ in the stomach, acid secretion was completely inhibited by omeprazole given i.p. at a dose of 60 mg/kg. Omeprazole at this dose has been shown to have no influence on gastric HCO$_3^-$ secretion in rats (15). After basal HCO$_3^-$ secretion had well stabilized, the following prostanoids were given intravenously (i.v.) as a single bolus injection in rats or by topical application to the chamber in mice, and the secretion of HCO$_3^-$ was measured for 1 hr thereafter; PGE$_2$ (1 mg/kg), 17-phenyl PGE$_2$ (EP1 agonist: 0.3 mg/kg) and ONO-AE1-329 (EP4 selective agonist: 3 µg/kg) (7,9). In some animals, the effects of ONO-8711 (EP1 antagonist: 10 mg/kg) and ONO-AE3-208 (EP4 antagonist: 1 mg/kg) on gastric HCO$_3^-$ secretion induced by PGE$_2$ were examined (9). ONO-8711 was given subcutaneously (s.c.) 1 hr before PGE$_2$, while ONO-AE3-208 was given i.v. 10 min before. The secretion of HCO$_3^-$ was also stimulated by perfusing the chamber with 50 mM or 100 mM HCl for 10 min (mucosal acidification), respectively, in the case of mice or rats. In addition, the effects of various COX inhibitors such as indomethacin (5 mg/kg), SC-560 (10 mg/kg) and rofecoxib (10 mg/kg) on the acid-induced HCO$_3^-$ secretion were examined by administering these agents intraduodenally (i.d.) 1 hr before the acidification. These selective COX inhibitors at the dose used have been shown to suppress COX-1 and COX-2 activity in the gastric mucosa (16).

**Measurement of Mucosal PGE$_2$ Content**

The mucosal PGE$_2$ content in the rat stomach was measured after exposure to acid (100 mM HCl) for 10 min, in the absence or presence of indomethacin, SC-560 and rofecoxib. The stomach was perfused with saline or 100 mM HCl for 10 min. Thirty minutes later, the entire gastric mucosa was removed, weighed, and put in a tube containing 100% ethanol plus 0.1 M indomethacin (17). Then, the samples were minced with scissors, homogenized, and centrifuged at 12000 r.p.m. for 10 min at 4°C. The supernatant of each sample was used to measure the level of PGE$_2$ by EIA using a PGE$_2$-kit (Cayman Chemical Co., Ann Arbor, MI). Indomethacin (5 mg/kg), SC-560 (10 mg/kg) or rofecoxib (10 mg/kg) was administered i.d. 1 hr before the acid treatment.

**Analyses for Gene Expression of COX-1/COX-2 and EP Receptors by RT-PCR**

COX-1/COX-2:

The rats were killed under deep ether anesthesia 30 min after exposure of the stomach to saline or 100 mM HCl for 10 min. The mucosa was removed, frozen in liquid nitrogen, and stored at −80°C prior to use. Gastric tissue samples were pooled from 2–3 rats for extraction of total RNA, which was prepared by a single-step acid phenol-chloroform extraction procedure using TRIZOLE (GIBCO BRL, Gaithersburg, MO). Total RNA primed by random hexadeoxy ribonucleotide was reverse-transcribed with a SUPERSCRIPT preamplification system (GIBCO BRL). The sequences of sense and antisense primers for rat COX-1 and COX-2 are listed in Table 1 (18,19). For the rat glyceraldehyde-3-phosphate dehydrogenase (G3PDH), a constitutively expressed gene, the sequences were 5’-GAACGGGAAGCTCACTGGCATGGC-3’ for the sense primer and 5’-TGAGGTCCACCACCCCTGTTGCTG-3’ for the antisense primer, giving rise to a 310-bp PCR
An aliquot of the RT reaction product served as a template in 35 cycles of PCR with 1 min of denaturation at 94°C, 0.5 min of annealing at 58°C and 1 min of extension at 72°C on a thermal cycler. A portion of the PCR mixture was electrophoresed in 1.8% agarose gel in TAE buffer (Tris buffer 40 mM, EDTA 2 mM and acetic acid 20 mM; pH 8.1), and the gel was stained with ethidium bromide and photographed.

**EP1~EP4 receptors:** Expression of EP1~EP4 receptor mRNAs was examined in whole mucosa of the stomach and duodenum as well as RGM1 cells. As described in the case of COX mRNA expression, the mucosa was removed, frozen in liquid nitrogen, and stored at –80°C prior to use. Using these samples and RGM1 cells, total RNA was extracted, primed by random hexadeoxy ribonucleotide and reverse-transcribed with the SUPERSCRIPT preamplification system. The sequences of sense and antisense primers for rat EP1~EP4 are also listed in Table 1 (20).

**Table 1. Sequences of Sense and Antisense Primers for Rat COX-1, COX-1, and EP1~4 Subtypes**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sequences</th>
<th>PCR Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-1</td>
<td>5’-AACCGTGTGTGTGACTTGGCTGAA-3’ 5’-AGAAAGAGCCCATCAGCGTCTAG-3’</td>
<td>887 bp</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX-2</td>
<td>5’-TGATGACTCACCACCTCCATG-3’ 5’-AATGGTGAAAGGTGCTCCGCCGC-3’</td>
<td>702 bp</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP1 receptor</td>
<td>5’-CCCAGGGTGCCCATAATCATC-3’ 5’-GGGCTAGGTGGTTGAAG-3’</td>
<td>778 bp</td>
</tr>
<tr>
<td>Sense</td>
<td></td>
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<tr>
<td>Antisense</td>
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</tr>
<tr>
<td>EP2 receptor</td>
<td>5’-CGCCCTCCACACGACTGGAAT-3’ 5’-AAGCAGCGCATGCTCAACAC-3’</td>
<td>1178 bp</td>
</tr>
<tr>
<td>Sense</td>
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</tr>
<tr>
<td>Antisense</td>
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<tr>
<td>EP3 receptor</td>
<td>5’-TGCTGGCGCTCCAGCTGGAGTT-3’ 5’-GCATTGCTCTCAGCCTCTG-3’</td>
<td>666 bp</td>
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<tr>
<td>Sense</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td></td>
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</tr>
<tr>
<td>EP4 receptor</td>
<td>5’-CCCTGCAGCGCTCAGTGACTTT-3’ 5’-CTTGCTCCCGAGGTGTTTCAGT-3’</td>
<td>488 bp</td>
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<tr>
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<tr>
<td>Antisense</td>
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</tr>
</tbody>
</table>

Preparation of Drugs

Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), prostaglandin E₂ (PGE₂: Funakoshi, Tokyo, Japan), ONO-AE1-329, ONO-AE3-208, ONO-8711 (Ono, Osaka, Japan), indomethacin, (Sigma Chemicals, St. Louis, MO.), SC-560 (Cayman Chemical, Ann Arbor, MI), rofecoxib (synthesized in our laboratory) and omeprazole (Astra Zeneca, Mõndal, Sweden). Indomethacin, SC-560 and rofecoxib were suspended in saline with a drop of Tween 80 (Wako, Osaka, Japan). Omeprazole was suspended in a 0.5% carboxymethyl cellulose solution. 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 per 100 g body weight (rat) for i.d., s.c. or i.p. administration, given i.v. in a volume of 0.1 ml per 100 g body weight (rat), or applied topically to the chamber in a volume of 0.1 ml (mouse). Control animals received saline in place of the active agent.
Statistics

Data are presented as the mean±SE from 4–7 rats 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

Effects of Various COX Inhibitors on Acid-Induced Gastric HCO$_3^-$ Secretion

Under the present experimental conditions, the stomach spontaneously secreted HCO$_3^-$ at a steady rate of 0.2–0.4 µEq/10 min during a 90 min test period, in the rats given omeprazole (60 mg/kg, i.p.) to inhibit acid secretion. The secretion of HCO$_3^-$ was markedly increased by acidification of the mucosa following exposure to 100 mM HCl for 10 min, reaching roughly 2 times the pre-exposure values, and remained elevated for 1 hr thereafter; the ΔHCO$_3^-$ output after acid treatment was 1.0±0.3 µEq/hr (Fig. 1A and 1B). Intraduodenal administration of indomethacin (5 mg/kg) had no effect on basal HCO$_3^-$ secretion but significantly inhibited the increased response to the acidification, the ΔHCO$_3^-$ output being 0.17±0.22 µEq/hr, about 1/6 of that obtained in vehicle-treated animals. Likewise, a significant suppression of the acid-induced HCO$_3^-$ response was observed on pretreatment with SC-560 (10 mg/kg), the selective COX-1 inhibitor, and the ΔHCO$_3^-$ output was 0.14±0.15 µEq/hr, the inhibition being about 85%. By contrast, rofecoxib (10 mg/kg), the selective COX-2 inhibitor, did not affect either basal HCO$_3^-$ secretion or the increased response to acid; ΔHCO$_3^-$ output induced by acid was 1.0±0.15 µEq/hr, which was not significantly different from that observed in vehicle treated rats.

Effects of COX Inhibitors on Mucosal PGE$_2$ Content After Acidification

Mucosal PGE$_2$ content in the normal rat stomach was 9.2±2.3 ng/g tissue. Acidification of the mucosa significantly increased the PGE$_2$ content 30 min later, to about 2.2 fold the control level, the value being 22.5±4.3 ng/g tissue (Fig. 2). The biosynthesis of PGE$_2$ in response to the acidification was totally blocked by prior administration of indomethacin (5 mg/kg, i.d.), and the PGE$_2$ content decreased significantly to a level even lower than that in the normal mucosa without acidification. Likewise, SC-560 (10 mg/kg), given i.d. 1 hr before the acid treatment, also inhibited the increase in PGE$_2$ as effectively as indomethacin. By contrast, pretreatment of the animals with rofecoxib (10 mg/kg, i.d.) did not significantly affect the increase in PGE$_2$ production following acidification; the PGE$_2$ level was 17.8±3.2 ng/g tissue, which was equivalent to that observed in vehicle-treated animals.

Gene Expression of COX-1/COX-2 in The Stomach after Acidification

Both G3PDH and COX-1 mRNAs were observed in the gastric mucosa of control rats, with exposure to saline for 10 min (Fig. 3). The expression of
G3PDH was not changed in the mucosa following acidification with 100 mM HCl for 10 min. In contrast, the gene expression of COX-2 was not detected in the gastric mucosa 30 min after exposure to either saline or 100 mM HCl.

**Effects of EP Antagonists on Acid-Induced Gastric HCO$_3^-$ Secretion**

It was previously found that PGE$_2$ stimulates gastric HCO$_3^-$ secretion through the activation of EP1 receptors while it stimulates duodenal HCO$_3^-$ secretion mediated by EP3/EP4 receptors (5,7,9). To confirm the involvement of EP1 receptors in the acid-induced gastric response, we examined the effects of ONO-8711 (the EP1 antagonist) and ONO-AE3-208 (the EP4 antagonist) on the
secretion of $\text{HCO}_3^-$ induced in the rat stomach by mucosal acidification and compared them with those on the PGE$_2$-induced secretion.

Acidification of the mucosa by 100 mM HCl for 10 min caused an increase of $\text{HCO}_3^-$ secretion, the $\Delta\text{HCO}_3^-$ output being $1.0\pm0.3$ µEq/hr. The response induced by mucosal acidification was significantly inhibited by prior administration of ONO-8711 (10 mg/kg), the EP1 antagonist, the $\Delta\text{HCO}_3^-$ output in the presence of ONO-8711 being $0.24\pm0.12$ µEq/hr, which is only 23.7% of that observed in
control rats given saline (Fig. 4). By contrast, the EP4 antagonist ONO-AE3-208 (1 mg/kg) had no effect on the response induced by acidification of the mucosa. Likewise, gastric HCO$_3^-$ secretion was significantly increased by i.v. administration of PGE$_2$ (1 mg/kg), the ΔHCO$_3^-$ output being 1.6±0.4 µEq/hr. As evident in Fig. 5, this response was significantly inhibited by ONO-8711 (10 mg/kg) but not ONO-AE3-208 (1 mg/kg). The secretion of HCO$_3^-$ in the stomach was also markedly stimulated by the EP1 agonist 17-phenyl PGE$_2$ (1 mg/kg, i.v.) but not the EP4 agonist ONO-AE1-329 (3 µg/kg, i.v.).

_Gastric HCO$_3^-$ Secretion in EP1 or EP3 Knockout Mice_

Under urethane anesthesia, the mouse stomach spontaneously secreted HCO$_3^-$ at a steady rate of 0.3–0.5 µEq/hr during a 90 min test period. Gastric HCO$_3^-$
secretion increased in response to luminal exposure to PGE\(_2\) (0.3 mg/ml) in wild-type mice; the ΔHCO\(_3^-\) output (1.02±0.06 μEq/hr) was about 2.5 fold greater than basal values (0.38±0.02 μEq/hr). As shown in Fig. 6A, this response was almost absent in EP1 receptor-knockout mice, and the ΔHCO\(_3^-\) output remained in the same range (0.42±0.07 μEq/hr) before and after PGE\(_2\) treatment.

Gastric HCO\(_3^-\) secretion was increased in response to mucosal acidification (50 mM HCl for 10 min) in wild-type mice; the ΔHCO\(_3^-\) output (0.90±0.10) was about two fold greater than basal values (Fig. 6B). This process was almost totally attenuated by indomethacin (5 mg/kg) or SC-560 (10 mg/kg) but not rofecoxib (10 mg/kg) given i.d. 1 hr before the acid treatment, suggesting a major mediator role for endogenous PGs derived from COX-1 in the acid-induced HCO\(_3^-\) secretion. A marked increase in the secretion of HCO\(_3^-\) in response to acid was also observed in

![Figure 5](image_url)

**Figure 5.** Effects of PGE\(_2\), 17-phenyl PGE\(_2\) and AE1-329 on gastric HCO\(_3^-\) secretion in rats. PGE\(_2\) (1 mg/kg), 17-phenyl PGE\(_2\) (0.3 mg/kg) or AE1-329 (3 μg/kg) was given intravenously. In some animals, AE3-208 (1 mg/kg) or ONO-8711 (10 mg/kg) was given i.v. 10 min or s.c. 30 min before the administration of PGE\(_2\), respectively. In Fig. A, the data are presented as % of basal values and represent the mean±SE of values determined every 15 minutes from 5~6 rats. Fig. B shows total net HCO\(_3^-\) output for 1 hr after the acidification, and the data are presented as the mean±SE from 5~6 rats. Significant difference at P<0.05; *from control; # from vehicle.
Figure 6A. Gastric HCO$_3^-$ secretion induced by PGE$_2$ in wild-type and EP receptor-knockout mice. PGE$_2$ (0.3 mg/ml) was applied luminally to the stomach for 10 min. Total HCO$_3^-$ output was obtained for 1 hr before and after PGE$_2$ treatment. Values are the mean±SE from 4–6 mice. Significantly different at P<0.05; *from basal secretion.

Figure 6B. Gastric HCO$_3^-$ secretion induced by mucosal acidification in wild-type and EP receptor-knockout mice. The mucosa was exposed to 50 mM HCl for 10 min. In control animals, the mucosa was exposed to saline for 10 min. In wild-type mice, indomethacin (5 mg/kg), SC-560 (10 mg/kg) or rofecoxib (10 mg/kg) was given intraduodenally 1 hr before the acidification. Values show total HCO$_3^-$ output obtained for 1 hr before and after mucosal acidification, and are presented as the mean±SE from 4–6 mice. Significant difference at P<0.05; *from basal secretion in the corresponding group; # from values in wild-type mice.
EP3 receptor-knockout mice, similar to wild-type mice, the ΔHCO$_3^-$ output being 0.92±0.06 µEq/hr, which was significantly greater than basal values (0.55±0.06 µEq/hr). However, in the mice lacking EP1 receptors, the gastric mucosa did not respond to acidification by secreting more HCO$_3^-$, and the ΔHCO$_3^-$ output remained in the same range (0.6–0.7 µEq/hr) before and after the acid treatment.

**Expression of EP Receptor Subtypes in RGM1 Cells**

Since it was found that both PGE$_2$ and mucosal acidification stimulated gastric HCO$_3^-$ secretion mediated by activation of EP1 receptors, we examined whether or not EP1 receptors are expressed in rat surface epithelial cells, using RGM1 cells, which are known to be derived from normal rat surface epithelial cells. The gene expression of all EP receptor subtypes (EP1–EP4) was observed in the entire gastric or duodenal mucosa (Fig. 7). Likewise, the expression of mRNAs of all EP receptor subtypes, including EP1 receptors, was detected in RGM1 cells.

**DISCUSSION**

The importance of PGE$_2$ in the local regulation of gastroduodenal HCO$_3^-$ secretion has been well documented (1,2,4,21). Endogenous PGE$_2$ mediates the
acid-induced secretion of HCO$_3^-$, the response being very important in the mucosal defense against injury (3,8). The present study supports a mediator role for endogenous PGs in the gastric response induced by mucosal acidification in rats, and clearly indicates that the enzyme responsible for production of PGs in this process is COX-1. We have previously reported that duodenal responses to PGE$_2$ and mucosal acidification are mediated through the activation of EP3/EP4 receptors (7,9,22) while the stimulatory effect of PGE$_2$ on HCO$_3^-$ secretion in the stomach is mediated by EP1 but not EP3/EP4 receptors (9,23). However, it remains undetermined which EP receptor subtype is involved in acid-induced HCO$_3^-$ secretion in the stomach. The present study showed for the first time that the presence of EP1 receptors is also essential for the increase in the secretion of HCO$_3^-$ in response to mucosal acidification in the stomach.

The factors that govern mucosal HCO$_3^-$ secretion include neurohumoral factors and luminal acid (1,4-6). Notably, the ability of the mucosa to respond to acid is critical to the maintenance of the surface pH gradient and protection of the mucosa, and this process seems to be mediated mainly by endogenous PGs (3,24). There are two forms of COX; COX-1, a constitutive enzyme expressed in many tissues, including the gastroduodenal mucosa (19,25-27), and COX-2, whose message and protein are normally undetectable in most tissues but whose expression can be induced by proinflammatory or mitogenic agents (28). Even the exposure to acid might up-regulate COX-2 expression in the stomach. However, the present study showed that acid-induced HCO$_3^-$ secretion in the stomach of both rats and mice was significantly abrogated by the selective COX-1 inhibitor SC-560 but not the selective COX-2 inhibitor rofecoxib, clearly suggesting the involvement of COX-1/PGs in this response. This contention was supported by the finding that the acidification did not up-regulate the expression of COX-2 mRNA in the rat gastric mucosa and that SC-560 but not rofecoxib significantly attenuated the increase in mucosal PGE$_2$ content in response to acidification. Certainly, the message of COX-1 was observed in the normal mucosa and its expression remained unchanged following the mucosal acidification. Sawaoka et al (29) reported the up-regulation of COX-2 expression in the stomach as early as 40 min after acid treatment. This discrepancy can be explained by different experimental procedures; they administered 0.6 M HCl in the stomach, while we acidified the stomach with 100 mM HCl for 10 min. Indeed, we observed no damage in the gastric mucosa after acidification with 100 mM HCl, either macroscopically or histologically. This is also supported by our previous data showing that topical application of 100 mM HCl did not cause any decrease in transmucosal potential difference in rat stomachs, suggesting that the gastric mucosal integrity is kept intact after acidification with 100 mM HCl (30). Thus, it is assumed that COX-1 is the enzyme responsible for production of PGs that play a crucial role in the secretion of HCO$_3^-$ induced by acidification of the
gastric mucosa. Similar results have been obtained in the acid-induced HCO$_3^-$ response in the duodenum (8).

Consistent with previous observations (7,23), the present study showed that both PGE$_2$ and 17-phenyl PGE$_2$ but not ONO-AE1-329 increased gastric HCO$_3^-$ secretion and that the stimulatory action of PGE$_2$ in the stomach was significantly abrogated by ONO-8711, the EP1 antagonist, but not ONO-AE3-208, the EP4 antagonist. These results are in contrast to those in the duodenum (9) and strongly suggest the involvement of EP1 receptors in the HCO$_3^-$ response to PGE$_2$ in the stomach. Furthermore, the present study showed for the first time that the response to the mucosal acidification was also attenuated by ONO-8711 and totally disappeared in EP1 but not EP3 receptor-knockout mice. Certainly, acidification of the mucosa stimulated production of PGs to increase the levels of endogenous PGE$_2$ in the stomach. Because the HCO$_3^-$ response to acid is mediated by endogenous PGs and because PGE$_2$ stimulates the secretion of HCO$_3^-$ in the stomach through the activation of EP1 receptors, it is reasonable that the acid-induced response in the stomach is mediated by EP1 receptors through enhanced production of endogenous PGs.

Narumiya and his group examined using in-situ hybridization the localization of mRNAs of the EP receptors in the stomach (31,32). They showed that strong signals for EP1 transcripts occurred in the smooth muscle cells in the muscularis mucosa while moderate EP3 mRNA expression was detected in the epithelial cells and also in the neurons of the myenteric ganglia. The same group of investigators also demonstrated by northern blot analysis significant expression of EP3 and EP4 receptors in the gastroduodenal mucosal layer containing epithelial cells (32). These results are compatible with our previous observation that HCO$_3^-$ secretion, one of the epithelial functions, is mediated by both EP3 and EP4 receptors in the duodenum, yet do not support the present finding that the response in the stomach is totally mediated by EP1 receptors. Then, we examined the gene expression of EP receptor subtypes, including EP1, in RGM1 cells derived from rat gastric epithelial cells. As evidenced in Figure 6, all the subtypes of EP receptors, including EP1, were detected in RGM1 cells as well as the whole stomach and duodenum. So, it is not unreasonable that the acid-induced HCO$_3^-$ response in the stomach is mediated by endogenous PGs via stimulation of EP1 receptors expressed on the surface epithelial cells.

It is believed that the acid-induced secretion of HCO$_3^-$ is mediated via an axonal reflex pathway, in addition to endogenous PGs (6). Since this response is almost totally inhibited by indomethacin, it is speculated that the afferent side of this reflex pathway is also influenced by PGs, probably by facilitating the neuronal excitation in response to H$^+$. Indeed, we previously reported that acid-induced HCO$_3^-$ secretion in the gastroduodenal mucosa was significantly attenuated by chemical ablation of capsaicin-sensitive afferent neurons and that the stimulatory action of capsaicin was also suppressed by indomethacin (5,33).
The EP1 receptors, which are a prerequisite for the acid-induced gastric $\text{HCO}_3^-$ secretion, might be on cells on the afferent side of the reflex pathway. However, we recently found that mucosal acidification stimulates gastric $\text{HCO}_3^-$ secretion in different mechanisms, depending on the concentration of acid; the response caused by 100 mM HCl is mediated by only PGs while that caused by 200 mM HCl is mediated by both capsaicin-sensitive afferent neurons and PGs (34). These results suggest that topical application of 100 mM HCl by itself increases PG biosynthesis in the stomach but does not acidify the mucosa enough to activate these afferent neurons. We further investigated this point and found that gastric $\text{HCO}_3^-$ secretion in response to acid is regulated by two independent mechanisms, one mediated by PGs and the other by sensory neurons and nitric oxide (NO), and that the acid-induced $\text{HCO}_3^-$ secretion in the normal stomach is totally mediated by endogenous PGs, but when the stomach is made slightly permeable to acid, the response is markedly facilitated by sensory neurons and NO (35). Under the present experimental conditions (acidification with 100 mM HCl), it is thus assumed that PGE$_2$ released locally directly stimulates the epithelial cells to result in an increase of $\text{HCO}_3^-$ secretion and does not stimulate the reflex pathway on the afferent side, even if EP1 receptors are expressed on the sensory neurons.

The EP receptor subtypes are coupled with different signal transduction systems; the activation of EP1 receptors causes an elevation of intracellular Ca$^{2+}$ levels via Ca$^{2+}$ channels, independent of phosphoinositol turnover, while that of EP2 and EP4 receptors results in an elevation of intracellular cAMP levels (12). We previously reported that forskolin, a stimulator of adnylate cyclase, did not increase $\text{HCO}_3^-$ secretion in the stomach, suggesting no involvement of cAMP in this process (36). On the other hand, the gastric $\text{HCO}_3^-$ response to PGE$_2$ was inhibited by a Ca$^{2+}$ antagonist verapamil, but not affected by an inhibitor of phosphodiesterase isobutylmethylxanthine (7). These results are compatible with the idea that the secretion of $\text{HCO}_3^-$ in the stomach is totally mediated by activation of EP1 receptors, coupled with an elevation of intracellular Ca$^{2+}$ levels (12).

Given the above findings, the present study supports a mediator role for endogenous PGs in gastric $\text{HCO}_3^-$ secretion induced by mucosal acidification in rats, and clearly indicates that the enzyme responsible for production of PGs in this process is COX-1. Furthermore, the present study shows for the first time that the presence of EP1 receptors is essential for the increase of $\text{HCO}_3^-$ secretion in response to mucosal acidification as well as PGE$_2$ in the stomach, suggesting a distinctive mechanism of regulation from that in the duodenum where EP3/EP4 receptors are involved in the response.

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