Distinct mechanisms of acid-induced HCO₃⁻ secretion in normal and slightly permeable stomachs

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Aihara, Eitaro, Yoko Sasaki, Fumitaka Ise, Kazutomo Kita, Yoko Nomura, and Koji Takeuchi. Distinct mechanisms of acid-induced HCO₃⁻ secretion in normal and slightly permeable stomachs. Am J Physiol Gastrointest Liver Physiol 291: G464–G471, 2006.—We investigated the regulatory mechanism of acid-induced HCO₃⁻ secretion in the slightly permeable rat stomach after an exposure to hyperosmolar NaCl. Under urethane anesthesia, a rat stomach was mounted on a chamber and perfused with saline, and the secretion of HCO₃⁻ was measured at pH 7.0 using a pH-stat method and by adding 2 mM HCl. Acidification of the normal stomach with 100 mM HCl increased HCO₃⁻ secretion, and this response was totally inhibited by pretreatment with indomethacin but not Nω-nitro-L-arginine methyl ester (L-NAME) or chemical ablation of capsaicin-sensitive afferent neurons. Exposure of the stomach to 0.5 M NaCl deranged the unstirred mucus layer without damaging the surface epithelial cells. The stomach responded to 0.5 M NaCl by secreting slightly more HCO₃⁻, in an indomethacin-inhibitable manner, and responded to even 10 mM HCl with a marked rise in HCO₃⁻ secretion, although 10 mM HCl did not have an effect in the normal stomach. The acid-induced HCO₃⁻ response in the NaCl-treated stomach was significantly but partially attenuated by indomethacin, L-NAME, or sensory deafferentation and was totally abolished when these treatments were combined. These results suggest that gastric HCO₃⁻ secretion in response to acid is regulated by two independent mechanisms, one mediated by prostaglandins (PGs) and the other by sensory neurons and nitric oxide (NO). The acid-induced HCO₃⁻ 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.

gastric HCO₃⁻ secretion; mucosal acidification; capsaicin-sensitive afferent neurons; prostaglandin; nitric oxide; rat

THE SECRETION OF HCO₃⁻ PLAYS A CRUCIAL ROLE IN THE PROTECTION OF THE GASTRODUODENAL MUCOSA FROM ACID (5, 10). THE SECRETION INCREASES IN RESPONSE TO MUCOSAL ACIDIFICATION, AND THE PROCESS IS MEDIATED BY MULTIPLE FACTORS, INCLUDING ENDOGENOUS PROSTAGLANDINS (PGs) AND NITRIC OXIDE (NO) AS WELL AS NEURONAL PATHWAYS (12, 22, 29, 30). RECENTLY, WE FOUND THAT THE MECHANISM UNDERLYING ACID-INDUCED HCO₃⁻ SECRETION IN THE STOMACH DIFFERED DEPENDING ON THE CONCENTRATION OF ACID; THE RESPONSE CAUSED BY 100 mM HCl WAS MITIGATED ONLY BY PGs, WHEREAS THAT CAUSED BY 200 mM HCl WAS MITIGATED BY BOTH CAPSAICIN-SENSITIVE AFFERENT NEURONS AND NO IN ADDITION TO PGs (1). IT IS ASSUMED THAT 100 mM HCl APPLIED TOPICALLY TO THE STOMACH INCREASES PG BIOSYNTHESIS BY ITSELF BUT DOES NOT ACIDIFY THE MUCOSA ENOUGH TO ACTIVATE AFFERENT NEURONS, RESULTING IN AN INCREASE OF HCO₃⁻ SECRETION THAT IS TOTALY DEPENDENT ON ENDOGENOUS PGs BUT NOT AFFERENT NEURONS OR NO.

MUCUS ADHERENT TO THE LUMINAL SURFACE OF THE MUCOSA PROVIDES A ZONE OF LOW TURBULENCE (UNSTIRRED LAYER), ALLOWING THE DEVELOPMENT OF A GRADIENT FOR HCO₃⁻ FROM THE LUMINAL SIDE (5, 10, 24). SMALL AMOUNTS OF HCO₃⁻ PROTECT THE MUCOSA AGAINST LARGE AMOUNTS OF ACID BY NEUTRALIZING HYDROGEN IONS THAT DIFFUSE BACK INTO THE MUCUS LAYER. THIS, IT IS POSSIBLE THAT THE MUCUS LAYER HAMPERS THE PENETRATION OF LUMINAL ACID AT 100 mM HCl, SO THAT THE ACID TREATMENT AT THIS CONCENTRATION DOES NOT ACIDIFY THE MUCOSA ENOUGH TO ACTIVATE THESE AFFERENT NEURONS. A HYPERTONIC NaCl SOLUTION HAS BEEN USED TO REMOVE THE MUCUS LAYER FROM THE SURFACE EPITHELIAL CELLS. THIS TECHNIQUE PROVIDES CONDITIONS WHERE THE EFFECT OF LUMINAL ACID ON THE SECRETION OF HCO₃⁻ CAN BE EXAMINED WITHOUT DISRUPTION OF THE MUCUS LAYER.

IN THE PRESENT STUDY, WE EXAMINED THE EFFECT OF MUCOSAL ACIDIFICATION ON HCO₃⁻ SECRETION IN THE STOMACH AFTER A BRIEF EXPOSURE TO 0.5 M NaCl AND INVESTIGATED THE REGULATORY MECHANISM OF THIS RESPONSE IN THE SLIGHTLY PERMEABLE STOMACH COMPARED WITH THE INTACT STOMACH.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (220–260 g, Nippon Charles River, Shizuoka, Japan) were used. The animals were deprived of food but allowed free access to tap water for 18 h before the experiments. All studies were performed under urethane anesthesia (1.25 g/kg ip) using ~4–8 animals/group. All experimental procedures described here were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Determination of gastric HCO₃⁻ secretion. Gastric HCO₃⁻ secretion was measured in the chambered stomach as described previously (22, 28). In brief, the abdomen was incised, and the stomach was exposed, mounted on a chamber (the exposed area: 3.1 cm²), and superfused with isotonic saline (154 mM NaCl) that was gassed with 100% O2 and kept in a reservoir. The secretion of HCO₃⁻ was measured at pH 7.0 by using a pH-stat method (Hiranuma Comitee, Nippon Medical Products, Japan) and by adding 2 mM HCl to the reservoir. To unmask HCO₃⁻ in the stomach, acid secretion was completely inhibited by omeprazole given intraperitoneally at a dose of 60 mg/kg. Omeprazole at this dose has been shown to have no influence on gastric HCO₃⁻ secretion in rats (6). After the basal HCO₃⁻ secretion had well stabilized, the mucosa was exposed to 10–200 mM HCl for 10 min, and the secretion of HCO₃⁻ was measured before and after the exposure. After the application of HCl, the mucosa was rinsed several times with saline, another 2 ml of saline was instilled, and the perfusion was resumed. The rate of HCO₃⁻ secretion was low for a little while after the acidification but gradually increased, reaching plateau levels within 5–10 min thereafter. In half the number of animals subjected to the acid treatment, the stomach was exposed to 0.5 M NaCl for 10
min immediately before the acidification (5–100 mM HCl). In some cases, the effect of (±)-1-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamine (NOR-3; a NO donor) on HCO₃⁻ secretion was examined. This agent (3 mg/ml) was applied topically to the chamber for 10 min. In addition, the effects of several treatments on the acid-induced change in HCO₃⁻ secretion were examined in the stomach with or without prior exposure to 0.5 M NaCl. Indomethacin (5 mg/kg) was given subcutaneously 30 min before the acidification while Nω-nitro-l-arginine methyl ester (l-NAME; 20 mg/kg) was given subcutaneously 3 h before the acidification, because this agent acutely increased HCO₃⁻ secretion through a neural reflex due to an increase of blood pressure (2, 26). Chemical ablation of capsaicin-sensitive afferent neurons was achieved with repeated subcutaneous injections of capsaicin (total dose: 100 mg/kg) once daily for 3 days, 2 wk before the experiment (15, 22, 30). The injections were performed under ether anesthesia, and the rats were pretreated with terbutaline (0.1 mg/kg im) and aminophylline (10 mg/kg im) to counteract the respiratory impairment associated with capsaicin. To check for the effectiveness of the treatment, a drop of capsaicin solution (0.1 mg/ml) was instilled onto one eye of each rat, and wiping movements were counted as previously reported.

In other experiments, we measured the amount of luminal acid loss when the mucosa was exposed to 50, 100, and 200 mM HCl without or with 50 mM HCl with prior exposure to 0.5 M NaCl in animals pretreated with omeprazole to inhibit acid secretion. The stomach was exposed to 2 ml of 50–200 mM HCl solution for 10 min, and the solution was then recovered. Luminal acid loss was determined from analysis of the collected acid solution. Each sample was analyzed for volume and acid concentration, which was determined by automatic titration of an aliquot with 50 mM NaOH to pH 7.0 (Autoburette, Comitite-7, Hiranuma, Tokyo, Japan). The amount of luminal acid loss was calculated as the difference between the product of the final volume and concentration and the product of the initial volume and concentration. In half the number of animals, the stomach was exposed for 10 min to 2 ml of 0.5 M NaCl immediately before the acid treatment.

**Determination of the gastric potential difference.** The methods used for determining transmucosal potential difference (PD) and gastric perfusion were as described in our previous study (32). Animals were pretreated with omeprazole (60 mg/kg ip) to inhibit acid secretion. The abdomen was opened through a midline incision, and the stomach was exposed, mounted on an ex vivo chamber, and superfused at a flow rate of 1 ml/min with isotonic saline kept in a reservoir. The PD was determined using two agar bridges, one positioned in the chamber and the other in the abdominal cavity. Changes in PD were monitored continuously on a two-pen recorder (U-228, Tokai-irika, Tokyo, Japan). After the basal PD had stabilized, the perfusion system was interrupted, and the solution in the chamber was withdrawn. The mucosa was then exposed for 10 min to 2 ml of 0.5 M NaCl. After the application of NaCl, the mucosa was rinsed with saline, another 2 ml of saline was instilled, and the perfusion was resumed.

**Histological evaluation of gastric mucosa.** The gastric mucosa was examined with a light microscope after an exposure to 0.5 M NaCl for 10 min. The mucosa was excised immediately after the NaCl treatment, and the tissue sample was immersed in 10% formalin, sectioned at 5 μm, and stained with hematoxylin and eosin as well as periodic acid-Schiff (PAS).

**Determination of mucosal PGE₂ content.** Mucosal PGE₂ levels were determined in the stomach under various conditions. The stomach mounted on a chamber was exposed for 10 min to 0.5 M NaCl, 10 mM HCl, or 100 mM HCl. In some cases, the mucosa was exposed to 10 mM HCl immediately after the NaCl treatment. Indomethacin (5 mg/kg) was given subcutaneously 1 h before NaCl or HCl treatment. Immediately after the exposure, the corpus mucosa was excised, weighed, and put in a tube containing 100% methanol plus 0.1 M indomethacin (9). The samples were minced with scissors, homogenized, and then centrifuged for 10 min at 12,000 rpm at 4°C. The supernatant of each sample was used for the determination of PGE₂ by enzyme immunoassay using a PGE₂ kit (Cayman Chemical, Ann Arbor, MI).

**Measurement of luminal NO content.** The release of NO from the stomach was determined indirectly as an amount of NO metabolites, NO₂⁻ and NO₃⁻. The stomach mounted on a chamber was exposed for 10 min to 0.5 M NaCl or 10–200 mM HCl and perfused with saline before and after the exposure. In some cases, the mucosa was exposed to 10 mM HCl immediately after the NaCl treatment. The perfusate collected for 30 min before and after the exposure was centrifuged for 15 min at 3,000 rpm and stored at −80°C until the assay. NO was measured in aliquots of the samples by the Griess method (11) after the reduction of nitrate to nitrite with nitrate reductase. Nitrates were incubated with Griess reagent (0.1% naphthylene diamine dihydrochloride and 1% sulfanilamide in 2.5% H₃PO₄) for 10 min at room temperature, and the absorbance was measured at 550 nm. l-NAME (20 mg/kg) was given subcutaneously 3 h before the exposure to 0.5 M NaCl plus 10 mM HCl.

**Preparation of drugs.** The drugs used were urethane (Tokyo kasei, Tokyo, Japan), HCl and NaCl (Nacalai tesque, Kyoto, Japan), l-NAME and indomethacin (Sigma Chemicals, St. Louis, MO), and NOR-3 (Dojindo, Kumamoto, Japan). Capsaicin was dissolved in a Tween 80-ethanol solution [10% ethanol, 10% Tween 80, and 80% saline (wt/wt/wt)]; Wako, Osaka, Japan]. NOR-3 was first dissolved in DMSO and then diluted with saline to the desired concentration. Other agents were dissolved in saline. Each agent was prepared immediately before use and given in a volume of 0.5 ml/100 g body wt in the case of intraperitoneal or subcutaneous administration, in a volume of 0.1 ml/100 g body wt in the case of intramuscular administration, or applied topically to the chamber in a volume of 2 ml/rat. Control animals received saline in place of active agents.

**Statistics.** Data are presented as means ± SE from approximately 4 to 8 animals/group. Statistical analyses were performed using a two-tailed Dunnett’s multiple-comparison test, and P values of <0.05 were regarded as significant.

**RESULTS**

**Effect of mucosal acidification on HCO₃⁻ secretion in normal stomachs.** Under urethane anesthesia, the rat stomach spontaneously secreted HCO₃⁻ at a rate of 0.2–0.4 μeq/min during the test period. Mucosal exposure to acid (10–100 mM HCl) for 10 min increased the secretion of HCO₃⁻ in a concentration-dependent manner, with the net HCO₃⁻ output at 200 mM HCl being 1.8 ± 0.2 μeq/h (Fig. 1). The HCO₃⁻ response to 100 mM HCl was all but totally inhibited by a prior administration of indomethacin (5 mg/kg sc) but was not significantly affected by either l-NAME (20 mg/kg sc) or chemical ablation of capsaicin-sensitive afferent neurons (Fig. 2A). By contrast, the response induced by 200 mM HCl was significantly mitigated by both indomethacin and l-NAME as well as by sensory deafferentation (Fig. 2B). When the inhibitory effect of indomethacin was compared for the responses to 100 and 200 mM HCl, it was evident that the effect was much greater in the case of the response to 100 mM HCl.

**Effect of hypertonic NaCl on HCO₃⁻ secretion in stomachs.** Under chambered conditions in the presence of omeprazole (inhibition of acid secretion), the rat stomach generated a PD of approximately −50 to −55 mV (mucosa negative) and maintained relatively constant values during the test period. Mucosal exposure to 0.5 M NaCl for 10 min caused a slight but significant reduction in the transmucosal PD from 52.6 ± 3.1 to 44.3 ± 2.6 mV immediately after the exposure, and the reduced PD quickly reverted after the removal of NaCl from
the chamber, with the values reaching ~80% of the preexposure level within 60 min. Mucosal exposure to 0.5 M NaCl for 10 min caused no damage in the mucosa visible macroscopically 1 h after the exposure. Histologically, surface epithelial cells maintained continuity even after an exposure to 0.5 M NaCl, and the amount of the PAS-positive substance adherent to the surface cells was slightly reduced (Fig. 3). The mucosal permeability to hydrogen ion was also examined in normal and 0.5 M NaCl-treated stomachs in the absence of endogenous acid secretion. When the mucosa was exposed to 50, 100, and 200 mM HCl for 10 min in normal stomachs, the amount of luminal acid loss observed during an exposure to 50 mM HCl was markedly increased in the stomach preexposed to 0.5 M NaCl, reaching values of 23.6 ± 1.9 μeq/10 min, ~3 or 1.5 times greater than those observed at 50 or 100 mM HCl in the normal stomach, respectively. Although the acid loss observed in the 0.5 M NaCl-treated stomach during an exposure to 50 mM HCl was a little lower than that observed in the normal stomach exposed to 200 mM HCl, there was no statistically significant difference between these two groups.

After an exposure to 0.5 M NaCl for 10 min, the rate of HCO₃⁻ secretion was slightly but significantly increased to 170% of basal levels, with the net HCO₃⁻ output being 0.6 ± 0.1 μeq/h (Fig. 4A). This response was almost totally prevented by a prior administration of indomethacin (5 mg/kg sc), with the inhibition being over 90% (Fig. 4B). By contrast, the response to 0.5 M NaCl was not significantly affected by either a prior administration of L-NAME or chemical ablation of capsaicin-sensitive afferent neurons.

**Mucosal acidification on HCO₃⁻ secretion in stomachs pretreated with hypertonic NaCl.** The secretion of HCO₃⁻ in the stomach exposed for 10 min to 0.5 M NaCl was further enhanced by subsequent acidification of the mucosa (5–50 mM HCl) in a concentration-dependent manner; the net HCO₃⁻ output induced by 50 mM HCl was equivalent to that caused by 200 mM HCl in normal stomachs (Fig. 5). The secretion of HCO₃⁻ was not further enhanced by acidification of the mucosa with 100 mM HCl, with the net HCO₃⁻ output being equivalent to that obtained with 50 mM HCl (data not shown). When the net HCO₃⁻ output caused by acidification in normal and 0.5 M NaCl-treated stomachs was plotted against various concentrations of HCl, it was found that the curve obtained in the latter stomach shifted to the left, i.e., lower concentrations of HCl (Fig. 6).

The increased HCO₃⁻ secretion caused by 0.5 M NaCl was further enhanced by a subsequent exposure to 10 mM HCl for 10 min, with the net HCO₃⁻ output being 1.4 ± 0.2 μeq/h, and this response was also totally inhibited by a pretreatment with...
indomethacin (Fig. 7). Likewise, the response to 10 mM HCl was significantly but partially attenuated by both L-NAME (20 mg/kg sc) and sensory deafferentation, with the degree of inhibition by these two treatments being equivalent, about 60%.

Mucosal PGE2 content in the stomach after acidification with or without hypertonic NaCl treatment. The level of PGE2 in the gastric mucosa of normal rats was 7.2 ± 0.6 ng/g tissue. Mucosal exposure to 0.5 M NaCl for 10 min stimulated the generation of PGs to significantly increase the PGE2 content of the stomach (Fig. 8). In contrast, an exposure to 10 mM HCl for 10 min did not affect PG production in the stomach, and the mucosal PGE2 content remained at the basal level in control stomachs. However, the PGE2 content was significantly increased after an exposure of the mucosa to 100 mM HCl, with the value being about two times the control level, and the effect was equivalent to that induced by 0.5 M NaCl alone. A prior administration of indomethacin significantly inhibited the increase in mucosal PGE2 content after an exposure to 0.5 M NaCl or 100 mM HCl. The combined exposure to 0.5 M NaCl and 10 mM HCl also significantly increased the mucosal PGE2 content, yet the degree of the increase was almost equivalent to that observed after an exposure to 200 mM HCl alone. This response was totally attenuated by a prior administration of L-NAME (20 mg/kg sc).

Effect of NOR-3 on gastric HCO3− secretion and PGE2 levels. Because the HCO3− response induced by 10 mM HCl in the 0.5 M NaCl-preexposed stomach was significantly inhibited by L-NAME, it is possible that NO also stimulates the secretion of HCO3− in the stomach, as in the duodenum (22). To confirm the stimulatory role of NO in gastric HCO3− secretion, the effect of the NO donor NOR-3 on HCO3− secretion was examined. NOR-3 applied to the chamber for 10 min increased the secretion of HCO3− in the stomach from 0.2 ± 0.1 to 0.5 ± 0.1 eq/10 min, with the net HCO3− output being 1.0 ± 0.2 eq/h. This response was significantly inhibited by a prior administration of indomethacin, with the net HCO3− output being 0.3 ± 0.1 eq/h. This agent also increased PGE2 production in the gastric mucosa, and the PGE2 content was 7.4 ± 0.5 ng/g tissue, which was significantly greater than that (4.9 ± 0.4 ng/g tissue) in the control stomach. The stimulatory effect of NOR-3 on PGE2 production was almost totally attenuated by a prior administration of indomethacin.

DISCUSSION

The secretion of HCO3− from the gastroduodenal mucosa increases in response to luminal acid, and this response plays a particularly important role in protecting the mucosa from acid (25). Previous studies have demonstrated that this process in the duodenum is regulated by multiple factors, including PGs, NO, and sensory neurons (12, 22, 29, 30), yet there is limited information on the response in the stomach.

HCO3− secretion in the duodenum was increased by acidification of the mucosa even with 10 mM HCl, and this response was blocked by both indomethacin, L-NAME, and the ablation...
of capsaicin-sensitive afferent neurons (22, 26). In the stomach, however, the response occurred on acidification with 50 mM or more of HCl, suggesting a different threshold for the response compared with the duodenum (1, 26). Furthermore, we have recently found that the mechanism underlying the response in the stomach differed depending on the degree of acidification, i.e., the concentration of the acid solution to which the mucosa was exposed (1). The response caused by 100 mM HCl is mediated only by PGs, whereas that caused by 200 mM HCl is mediated by both capsaicin-sensitive afferent neurons and NO in addition to endogenous PGs. We previously examined the effect of HCl at various concentrations (0.1–0.35 M) on transmucosal PD of rat stomachs and found that the PD was not decreased by HCl even at 0.25 M (27). Thus, it is likely that 200 mM HCl, unlike 0.5 M NaCl, does not damage surface epithelial cells. Indeed, we reported that gastric HCO₃⁻ secretion in response to 200 mM HCl was completely attenuated by the death of the animal by saturated KCl (1), suggesting a totally active process of this secretion.

On the other hand, capsaicin-sensitive afferent neurons are activated not only by capsaicin but also by hydrogen ions as well, resulting in the liberation of calcitonin gene-related peptide (CGRP) from the nerve ending, which then stimulates...
These results led us to speculate that 100 mM HCl applied topically to the stomach increases PG biosynthesis but does not acidify the mucosa enough to activate the afferent neurons, resulting in an increase of \( \text{HCO}_3^- / H_2CO_3 \) secretion totally mediated by endogenous PGs but not NO or afferent neurons. By the way, the secretion of mucus is also stimulated by both PGs, NO and sensory neurons, similar to the secretion of \( \text{HCO}_3^- / H_2CO_3 \) (3, 16, 23). Therefore, it is possible that below a concentration of 100 mM, the acid is captured, in large part, by the mucus-\( \text{HCO}_3^- \) barrier and does not acidify the mucosa enough to stimulate these afferent neurons.

In the present study, we found that a brief exposure (10 min) of the mucosa to 0.5 M NaCl increased the basal rate of \( \text{HCO}_3^- / H_2CO_3 \) secretion in the stomach. Although the application of the NaCl solution, which is slightly hypertonic (3.2 times), caused a small reduction in gastric PD, this treatment did not damage the surface epithelium histologically and only deranged the mucus gel layer. In the present study, gastric PD was measured in animals pretreated with omeprazole to inhibit acid secretion. Thus, the changes in PD observed after 0.5 M NaCl treatment would reflect mostly those in tissue electrical resistance as well as those in electrode/solution junction potentials. Because mucosal exposure to 0.5 M NaCl apparently sloughed off the mucus layer, it may be assumed that a small decrease in PD after 0.5 M NaCl treatment is caused by changes in liquid junctional potentials due to derangement of mucus layer. On the other hand, it is known that hypertonic saline acts as a mild irritant to the stomach and induces adaptive cytoprotection mediated by endogenous PGs (20, 21). As expected, we found that the mucosal exposure to 0.5 M NaCl significantly increased the PGE\(_2\) content of the stomach. In addition, the
secretion of HCO$_3^-$ in response to 0.5 M NaCl was totally prevented by a prior administration of indomethacin, suggesting that HCO$_3^-$ appeared in the lumen via an active process mediated by endogenous PGs and not through passive diffusion. We have previously reported that the gastric alkaline response that occurred in the stomach after an exposure to 1 M NaCl or 20 mM taurocholate was not affected by indomethacin, suggesting a passive diffusion of HCO$_3^-$ (20, 32, 33). Certainly, after the exposure of the stomach to these irritants, the PD was markedly reduced and surface epithelial cells were extensively sloughed. Because the response to 0.5 M NaCl was not affected by either l-NAME or the ablation of capsaicin-sensitive afferent neurons, it is assumed that neither NO nor sensory neurons are involved in the regulatory mechanism of this process.

One of the most important findings of the present study is that the threshold concentration of acid required for stimulating HCO$_3^-$ secretion was significantly decreased in the permeable stomach after an exposure to 0.5 M NaCl. The minimum concentration of acid required for stimulating HCO$_3^-$ secretion in the stomach preexposed to 0.5 M NaCl was 5 mM, roughly 10 times less than that required in the normal stomach. Furthermore, the HCO$_3^-$ response induced by 10 mM HCl was significantly abrogated by a prior administration of l-NAME and sensory deafferentation, suggesting the involvement of NO and sensory neurons in this process. The mucosal PGE$_2$ content significantly increased after treatment with 0.5 M NaCl and was not further enhanced by an additional exposure to 10 mM HCl. It is possible that 0.5 M NaCl treatment enhances the epithelial permeability to increase acid back diffusion, resulting in acidification of the mucosa. As expected, acid loss from the lumen during exposure to 50 mM HCl was markedly increased in the stomach preexposed to 0.5 M NaCl, reaching a value of 23.6 ± 1.9 μeq/10 min, ~3 or 1.5 times greater than that observed in the normal stomach exposed to 50 or 100 mM HCl, respectively, and a little lower than that observed during an exposure to 200 mM HCl. Thus, the threshold concentration of HCl for acidification of the mucosa efficiently to stimulate the HCO$_3^-$ secretion mediated by both PG and sensory neurons is between 100 and 200 mM HCl in the normal stomach. In addition, because the 0.5 M NaCl treatment sloughed off the mucus layer without damaging surface cells, it is assumed that luminal acid can acidify the mucosa efficiently without being trapped with the mucus gel. Because of these two factors, even a low concentration of acid can acidify the mucosa sufficiently to activate afferent neurons. It is known that endogenous PGs play a crucial role in the gastric functional responses induced by these afferent neurons, probably sensitizing the neurons through EP1 receptors (17, 34). In addition, activation of these afferent neurons causes CGRP, which, in turn, stimulates the production and release of NO (26). Indeed, we observed that the luminal release of NO remained unchanged after the exposure of the stomach to 0.5 M NaCl or 10–100 mM HCl alone yet significantly increased after exposure to 10 mM HCl in the 0.5 M NaCl-treated stomach. Thus, it is understandable that indomethacin totally inhibited the secretion of HCO$_3^-$ in the permeable stomach, whereas both l-NAME and sensory deafferentation only partially attenuated the response.

At present, how acidification stimulates NO release in the stomach or which cell type is responsible for NO release remain unknown. Various types of cells, including surface epithelial cells, enteric neurons, and endothelial cells, are capable of producing NO. Brown et al. (4) reported that cells in the elutriator fraction rich in mucous epithelial cells exhibit the most activity of the constitutive type of NO synthase in the stomach, localizing in parallel with NADPH-dependent diaphorase activity. On the other hand, acid-induced HCO$_3^-$ secretion is mediated via an axonal reflex pathway in addition to endogenous PGs and NO (13, 22, 29, 30). In the present study, this process was indeed attenuated by the functional ablation of capsaicin-sensitive sensory neurons. Other studies have also shown that the gastric hyperemic response induced by acid back diffusion is mediated by NO released by the stimulation of sensory neurons (14, 18). Thus, it is also possible that mucosal acidification increases NO release through the stimulation of capsaicin-sensitive sensory neurons. On the other hand, several studies have reported that NO or NO donors stimulate PG production in various organs and cells (8, 35, 36). Uno et al. (35) reported that the NO donor S-nitroso-N-acetyl penicillamine stimulates PGE$_2$ production in rat gastric epithelial cells. Furukawa et al. (8) reported that both NOR-3 and dibutyryl guanosine-3’,5’-cyclic monophosphate increased PGE$_2$ production in isolated bullfrog duodenum, suggesting a NO/cGMP-dependent increase in PG production. In the present study, the increased PGE$_2$ generation after the mucosal acidification was blocked not only by indomethacin but also by l-NAME. In addition, we also observed that NOR-3, at a dose that stimulated HCO$_3^-$ secretion, increased PGE$_2$ production in the gastric mucosa, and these effects were both suppressed by indomethacin. These results strongly suggest an interactive role for NO and PGs in the acid-induced secretion of HCO$_3^-$ in the stomach.

Given the above findings, the present study suggests that gastric HCO$_3^-$ secretion in response to acid is regulated by two independent mechanisms, one mediated by PGs and the other by sensory neurons and NO. Normally, acid-induced HCO$_3^-$ secretion is mediated entirely by endogenous PGs, but, when the stomach is made slightly permeable to acid, as induced by an exposure to irritating substances, the response is markedly facilitated by the activation of sensory neurons and NO, suggesting the cooperative action of these three factors.

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HCO₃⁻ SECRETION IN SLIGHTLY PERMEABLE STOMACHS