Helicobacter pylori Vacuolating Cytotoxin Inhibits Duodenal Bicarbonate Secretion by a Histamine-Dependent Mechanism in Mice

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Background. The pathogenic mechanisms involved in Helicobacter pylori–induced duodenal mucosal injury are incompletely understood. In the present study, we sought to investigate the effect of H. pylori vacuolating cytotoxin (VacA) on duodenal mucosal bicarbonate (HCO₃⁻) secretion.

Methods. Concentrated bacterial culture supernatants from an H. pylori wild-type strain producing VacA with s1/m1 genotypes (P12) and from an isogenic mutant lacking VacA (P12ΔvacA) were used. HCO₃⁻ secretion by murine duodenal mucosa was examined in vitro in Ussing chambers. Duodenal mucosal histamine release was measured using enzyme-linked immunosorbent assay. The expression of histamine H₂ receptor was examined by immunohistochemical analysis.

Results. In a dose-dependent manner, the VacA-positive supernatant P12 reduced prostaglandin E₂ (PGE₂)–stimulated duodenal mucosal HCO₃⁻ secretion to a maximum of 49% (P < .0001), whereas P12ΔvacA did not result in significant inhibition (P > .05). Purified VacA had a similar effect. Histamine H₂ receptor antagonists attenuated the effect of P12 on PGE₂-induced HCO₃⁻ secretion. P12 stimulated duodenal histamine release in a dose-dependent manner, and exogenous histamine inhibited PGE₂-stimulated duodenal HCO₃⁻ secretion. H₂ receptor expression was found in duodenal epithelial cells, the enteric nerve plexus, and lymphocytes in Peyer’s patch.

Conclusions. H. pylori VacA inhibits PGE₂-stimulated duodenal epithelial HCO₃⁻ secretion by a histamine-dependent mechanism. This effect likely contributes to the damaging effect of H. pylori in the duodenal mucosa.

Helicobacter pylori infection occurs worldwide, and it is now widely accepted as a major cause of duodenal ulcer formation [1, 2]. However, the pathogenesis of H. pylori–induced duodenal ulcers is not fully understood.

H. pylori infects a majority of the human population, with a very high prevalence noted in countries with poor hygienic conditions. However, only a minority of persons infected with H. pylori developed gastroduodenal diseases. Pathogenesis depends on strain virulence, host genetic susceptibility, and environmental cofactors [3]. The vacuolating cytotoxin (VacA) gene is present in most H. pylori strains, and ~50% of H. pylori strains secrete VacA [4]. The studies have shown that VacA, especially VacA with s1/m1 genotypes, is closely associated with peptic ulceration [3–5].

Duodenal mucosal bicarbonate (HCO₃⁻) secretion plays an important role in duodenal mucosal protection against acid-peptic injury [6–9]. Patients with duodenal ulcer have significantly diminished proximal duodenal mucosal HCO₃⁻ secretion, compared with healthy volunteers [10], suggesting that diminished mucosal HCO₃⁻ secretion contributes to the pathogenesis of duodenal ulcer. In patients with duodenal ulcer associated
with *H. pylori*, duodenal HCO$_3^-$ secretion was diminished, and, after eradication of *H. pylori*, it returned to normal levels [11]. This finding indicated that *H. pylori* infection impaired the capacity of duodenal mucosal HCO$_3^-$ secretion. In a previous study, Debellis et al. [12] found that *H. pylori* cytotoxin VacA stimulated HCO$_3^-$ efflux in an isolated frog stomach model. On the other hand, intraluminal perfusion of *H. pylori* water extract was shown to inhibit acid-induced duodenal mucosal HCO$_3^-$ secretion in rats in vivo [13]. *H. pylori* water extract strongly decreased murine duodenal HCO$_3^-$ secretion in vitro within 1 h of incubation [14]. These results indicate that products secreted by *H. pylori* likely influence duodenal mucosal HCO$_3^-$ secretion. However, *H. pylori* water extract is a crude mixture that contains many unidentified components, and the nature of these products and the underlying mechanism(s) involved remain unclear.

In the present study, therefore, we investigated both the effect of *H. pylori* VacA on duodenal mucosal HCO$_3^-$ secretion and the underlying mechanisms for the VacA-induced reduction in HCO$_3^-$ secretion, by use of concentrated culture supernatants from isogenic *H. pylori* strains producing VacA with S1/m1 genotypes and from isogenic mutation strains lacking VacA. In selected experiments, we also studied the effect of highly purified VacA toxin. We sought to illustrate the pathogenesis of *H. pylori*-induced duodenal ulcer. The results demonstrated that VacA inhibits prostanoid E$_2$ (PGE$_2$)-stimulated duodenal mucosal HCO$_3^-$ secretion in a histamine-dependent manner.

**MATERIAL AND METHODS**

**Materials.** The reagents, PGE$_2$, cimetidine, ranitidine, diphenhydramine, thioperamide, and histamine, were purchased from Sigma. These reagents were dissolved in dimethyl sulfoxide (DMSO) or water for stock. Rabbit anti-histamine H$_3$ receptor affinity-purified polyclonal antibody and nonimmune mouse IgG used as negative control were obtained from Chemicon International. All other chemicals in solutions were obtained from Sigma and Calbiochem.

**Preparation and purification of *H. pylori* VacA.** The concentrated culture supernatants from an *H. pylori* wild-type strain producing VacA with S1/m1 genotypes (P12) and its isogenic mutant with a deletion of the VacA gene (P12ΔvacA) were prepared as described elsewhere [15]. In brief, *H. pylori* strain 60190 was cultured in Brucella broth supplemented with 10% fetal calf serum. The broth cultures were incubated for 2 days with mild agitation. The bacteria were pelleted by centrifugation, and the supernatant was passed through a 0.2-µm pore sterile filter. Proteins of the culture supernatant were precipitated with a 50% saturated solution of ammonium sulfate. The precipitate was resolved in PBS and underwent dialysis against PBS overnight. The protein concentration was determined using Bradford reagent. The *H. pylori* P12 vacA deletion mutant strain was constructed by polymerase chain reaction (PCR) amplification and cloning into pBluescript II SK of the upstream and downstream regions of vacA and by insertion of a cat resistance gene cassette. The correctly verified plasmid (pWS59) was used for transformation of *H. pylori* P12 to generate a nonpolar mutant. VacA was purified from culture supernatant by precipitation with a 44% saturated solution of ammonium sulfate and by gel filtration chromatography performed with the use of a Sephacryl S300 16/60 column (Pharmacia Biotech). Fractions were tested for VacA by Western blot analysis and were concentrated using Amicon Ultra 100K centrifugal filter devices. The purified toxin was assayed for functional activity in a vacuolization assay.

**Animal preparation.** All studies were approved by the committees on investigations involving animals at Hannover Medical School (Hannover, Germany) and Zunyi Medical College (Zunyi, China). Experiments were performed in NMRI mice (age, 6–12 weeks). The mice were housed in a standard animal care room with a light-dark cycle of 12:12 h, and they were allowed free access to food and water. After narcosis was briefly induced with 100% CO$_2$, the mice were killed by cervical dislocation. The abdomen of each mouse was opened by making a midline incision, and the proximal duodenum (the portion stretching approximately from 2 mm distal to the pylorus to the common bile duct ampulla) was removed and immediately placed in ice-cold iso-osmolar mannitol and indomethacin (1 µmol/L) solution (to suppress trauma-induced prostanoid release). The duodenum was opened along the mesenteric border and was stripped of external serosal and muscle layers by sharp dissection in the aforementioned ice-cold iso-osmolar mannitol and indomethacin solution.

**Ussing chamber experiments for the measurement of HCO$_3^-$ secretion.** Ussing chamber experiments were performed as described elsewhere [16, 17]. In brief, the duodenal mucosae were mounted between the 2 chambers with an exposed area of 0.196 cm$^2$ and then were placed in an Ussing chamber. The mucosal side was bathed with unbuffered HCO$_3^-$-free modified Ringer’s solution circulated by a gas lift with 100% O$_2$. The serosal side was bathed with modified buffered Ringer’s solution (pH 7.4) containing 25 mmol/L HCO$_3^-$ and gassed with 95% O$_2$ and 5% CO$_2$. Each bath contained 10.0 mL of the respective solution maintained at 37°C by use of a heated water jacket. Experiments were performed under continuous short-circuited conditions. The luminal pH was maintained at 7.40 by means of continuous infusion of 0.5 mmol/L HCl under the automatic control of a pH-stat system (a model PHM290 pH-Stat Controller; Radiometer). The volume of the titrant infused per unit of time was used to quantitate HCO$_3^-$ secretion. The measurement was recorded at 5-min intervals. The rate of luminal HCO$_3^-$ secretion is expressed as the number of micromoles per centimeters squared per hour. After a 20-min measurement of basal values, *H. pylori* P12, P12ΔvacA, or control vehicle was added to the mucosal side of
the chambers for 1 h of incubation. PGE₂ was then added to the serosal side of tissue in Ussing chambers. Changes in duodenal HCO₃⁻ secretion during the 60-min period after the addition of PGE₂ were determined to observe the effects of VacA on PGE₂-stimulated duodenal mucosal HCO₃⁻ secretion, or the incubation was continued in the absence of PGE₂ to observe the effect of VacA on basal values.

**Measurement of histamine release.** The mice were prepared as described above. Segments of proximal duodenal tissue (∼50 mg), opened along the mesenteric border and stripped of seromuscular layers, were placed in serosal Ringer’s solution gassed with 95% O₂ and 5% CO₂ at 37°C to incubate for different time points in the presence of 40 μg/mL P12 supernatant or to incubate for 45 min in the presence of various P12 supernatant concentrations. At the end of the incubation period, the incubation media were centrifuged, and the supernatants were stored at −70°C for later histamine assay. The tissues were homogenized at 4°C in perchloric acid (66%; 10 μL/mg tissue) for the extraction of histamine. The resulting suspension was centrifuged (at 425 g at 4°C for 10 min) to remove solid matter and precipitated protein, and the fluid phase was transferred to a clean tube and neutralized with the addition of an equal volume of potassium borate (1 mol/L; pH 9.25; −300 μL). The samples were then recentrifuged, and the supernatants were stored at −70°C for histamine assay. Measurement of histamine was performed using ELISA, with the use of a histamine ELISA kit (SPI-BIO) according to the manufacturer’s guidelines. Release of histamine in the incubation medium was expressed as a percentage of the total histamine content in tissue.

**Immunohistochemical analysis.** For immunohistochemical determination of histamine H₂ receptor expression, proximal duodenal tissue specimen was fixed in 10% buffered formalin solution, dehydrated, and then embedded in paraffin. The preparation was cut in 5-μm sections, deparaffinized, and treated for 10 min with citrate buffer (10 mmol/L citric acid; pH 6.0) in a microwave oven (750 W) before immunostaining was done. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide for 7 min. After incubation with rabbit anti–histamine H₂ receptor affinity-purified polyclonal antibody (Chemicon) overnight at 4°C, the sections were washed with PBS and exposed to Dako EnVision Detection Systems Peroxidase/DAB, Rabbit/Mouse (Dako) for 30 min at room temperature. Slides were rinsed in PBS, incubated with the Dako Liquid DAB Large-Volume Substrate-Chromogen System (Dako), rinsed gently with distilled water, and counterstained with hematoxylin. Coverslips were mounted using mounting media and were examined using a Leica microscope. The specificity of immunoreactivity was confirmed by negative controls in which nonimmune mouse IgG was used instead of the primary antibodies.

**Statistics.** All results are expressed as the mean ± SE. “ΔHCO₃⁻” denotes the stimulated peak responses minus the basal values (i.e., the net stimulated murine HCO₃⁻ secretion). Basal values for HCO₃⁻ secretion denote the average value determined for the 20-min period before the administration of PGE₂. Data were analyzed by 1-way analysis of variance (ANOVA), followed by Newman-Keuls post hoc test or, when appropriate, by Student’s t tests. P < .05 was considered to denote statistical significance.

**RESULTS**

**Effect of VacA on basal duodenal mucosal HCO₃⁻ secretion.** The addition of either 40 μg/mL P12 supernatant (P12) or 40 μg/mL P12ΔvacA supernatant (P12ΔvacA) to the mucosal side of the Ussing chambers caused a transient increase in the measurement of HCO₃⁻ secretion; this increase was attributable to the buffering effect of the proteinaceous components. After resolution of this response, similar steady-state HCO₃⁻ secretion was observed in the 2 groups (time

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course figure not shown) during incubation for 2 h. The basal HCO$_3^-$ secretions determined before the addition of P12 and P12ΔvacA were, respectively, 1.14 ± 0.08 and 1.21 ± 0.11 μmol/cm$^2$/h (n = 8). After incubation for 1 h, HCO$_3^-$ secretions in the presence of P12 or P12ΔvacA were, respectively, 1.23 ± 0.12 and 1.27 ± 0.14 μmol/cm$^2$/h (P > .05, compared with values obtained before the addition of P12 and P12ΔvacA). Thus, neither P12 nor P12ΔvacA altered basal HCO$_3^-$ secretion.

To assess tissue viability, 25 mmol/L luminal glucose was added at the end of the experiment. Glucose increased short-circuited current to a similar degree in P12-exposed tissue and control tissue, indicating that the viability of tissue was not influenced by P12 and P12ΔvacA (data not shown).

**VacA inhibits PGE$_2$-stimulated duodenal mucosal HCO$_3^-$ secretion.** PGE$_2$ is believed to play an important role in the regulation of duodenal mucosal HCO$_3^-$ secretion. Therefore, we investigated the effect of VacA on PGE$_2$-stimulated duodenal HCO$_3^-$ secretion. After mucosae were incubated for 1 h with 40 μg/mL P12 or 40 μg/mL P12ΔvacA, P12 markedly reduced PGE$_2$-stimulated duodenal mucosal HCO$_3^-$ secretion by 49% (P < .0001), whereas P12ΔvacA only slightly, but not significantly, reduced PGE$_2$-stimulated HCO$_3^-$ secretion by 17% (P > .05) (figure 1A and 1B). Furthermore, the inhibitory action of P12 on PGE$_2$-stimulated HCO$_3^-$ secretion was dose dependent over a range of concentrations from 5 to 100 μg/mL (figure 2). P12ΔvacA did not significantly reduce PGE$_2$-stimulated HCO$_3^-$ secretion, even at a concentration of 100 μg/mL (data not shown). We then performed experiments with purified VacA. Purified VacA, 1 μg/mL, which had exerted its functional activity at this concentration [15], produced comparable inhibition of PGE$_2$-stimulated HCO$_3^-$ secretion, reducing it by 51% (P < .001) (figure 3). The results demonstrated that VacA inhibits PGE$_2$-stimulated duodenal HCO$_3^-$ secretion.

**Histamine H$_2$ receptor antagonist attenuates inhibition of VacA on PGE$_2$-stimulated HCO$_3^-$ secretion.** A previous study showed that exogenous histamine inhibits PGE$_2$-stimulated duodenal HCO$_3^-$ secretion in rabbits [18]. To determine whether histamine released from duodenal mucosa mediates the inhibitory effect of VacA on PGE$_2$-stimulated duodenal HCO$_3^-$ secretion, histamine H$_1$ receptor antagonist diphenhydramine, H$_2$ receptor antagonist cimetidine, or H$_3$ receptor antagonist thioperamide was added into the serosal side of the chambers at the same time that P12 was added. As shown in figure 4, the histamine H$_2$ receptor antagonist cimetidine attenuated the effect of P12 on PGE$_2$-induced HCO$_3^-$ secretion (P > .05), but both H$_1$ receptor antagonist diphenhydramine and H$_3$ receptor antagonist thioperamide failed to alter the effect of P12 on PGE$_2$-induced HCO$_3^-$ secretion. Cimetidine, ranitidine, diphenhydramine, and thioperamide had no effects on basal duodenal HCO$_3^-$ secretion (data not shown). These results indicated that the inhibitory action of VacA on PGE$_2$-stimulated duodenal HCO$_3^-$ secretion may be mediated in part by the release of histamine from mucosal mast cells and/or other sources. This histamine, in turn, mediates its inhibitory effect via the H$_2$ receptor.

**VacA stimulates duodenal histamine release.** Next, we examined the effect of VacA on histamine release from the duodenal mucosa. Histamine released from tissues after incubation with P12 was expressed as a percentage of the total tissue con-

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**Figure 2.** Effects of graded doses of *Helicobacter pylori* vacuolating cytotoxin (VacA) on prostaglandin E$_2$ (PGE$_2$)-stimulated ΔHCO$_3^-$ secretion. Experiments were conducted as described in the figure 1 legend, except that various concentrations of purified VacA were added to mucosal side of tissues. Results are expressed as the mean ± SE, and, for each series, n ≥ 8. P12 inhibited PGE$_2$-stimulated HCO$_3^-$ secretion in a dose-dependent manner. (*P > .05, **P < .01, and P < .0001, compared with control, by 1-way analysis of variance with Student’s t test and Newman-Keuls post hoc test).
tent. As shown in figure 5, after incubation with 40 μg/mL P12 for 15 min, duodenal mucosal histamine release was markedly increased (*P < .05), and it reached maximal levels at 45 min (**P < .001, compared with control, by 1-way analysis of variance with Student’s t test or Newman-Keuls post hoc test).

Effect of histamine on PGE2-stimulated HCO3− secretion. To confirm the role of histamine in inhibition of VacA-induced duodenal HCO3− secretion, we further examined the effect of exogenous histamine on duodenal mucosal HCO3− secretion in mice. The results showed that histamine failed to alter murine basal duodenal HCO3− secretion but inhibited PGE2-stimulated duodenal HCO3− secretion in a dose-dependent manner (P < .0001) (figure 6). Histamine started to exert its inhibitory effect at a concentration of 10 μmol/L, and 100 μmol/L histamine inhibited PGE2-stimulated duodenal HCO3− secretion by 72%.

Expression of histamine H2 receptor on duodenal mucosa. The aforementioned results indicate that histamine H2 receptor mediates the inhibitory effect of VacA on PGE2-stimulated duodenal HCO3−

Figure 4. Effects of histamine receptor antagonists on the inhibitory action of Helicobacter pylori vaculating cytotoxin (VacA) on prostaglandin E2 (PGE2)-stimulated bicarbonate (HCO3−) secretion. Experiments were conducted as described in the figure 1 legend. Histamine H1 receptor antagonist diphenhydramine (10−4 mol/L), H2 receptor antagonists cimetidine (10−4 mol/L) and ranitidine (10−4 mol/L), or H3 receptor antagonist thioperamide (10−4 mol/L) was added to the serosal side of the chambers at the same time that P12 was added. Results are expressed as the mean ± SE, and, for each series, n ≥ 8. The histamine H2 receptor antagonists cimetidine and ranitidine, not the H1 receptor antagonist diphenhydramine and the H3 receptor antagonist thioperamide, partially reversed the inhibitory effect of VacA on PGE2-stimulated HCO3− secretion. (*P < .05 and **P < .0001, compared with control; †P > .05 and ‡P < .001, compared with P12, by 1-way analysis of variance with Student’s t test or Newman-Keuls post hoc test).

Figure 5. Effect of Helicobacter pylori vaculating cytotoxin (VacA) on duodenal mucosal histamine release. Murine duodenal mucosa was treated for various periods of time with 40 μg/mL P12 (A), for 45 min with 40 μg/mL P12 or 40 μg/mL P12ΔvacA (B), or for 45 min with various concentrations of P12 (C). Histamine release was measured as described in Materials and Methods and is expressed as a percentage of the total tissue content. Values are the mean ± SE, and, for each series, n = 8. The histamine H2 receptor antagonists cimetidine and ranitidine, not the H1 receptor antagonist diphenhydramine and the H3 receptor antagonist thioperamide, partially reversed the inhibitory effect of VacA on PGE2-stimulated HCO3− secretion. (*P < .05 and **P < .0001, compared with control; †P > .05 and ‡P < .001, compared with P12, by 1-way analysis of variance with Student’s t test or Newman-Keuls post hoc test).
secretion. We further examined the location of histamine H2 receptor within the duodenal mucosa by using an immunohistochemical method. Figure 7 shows that histamine H2 receptor was expressed on duodenal epithelial cells, the enteric nerve plexus, and lymphocytes in Peyer’s patch, indicating that histamine H2 receptors are widely expressed on the duodenal mucosa of the mouse.

**DISCUSSION**

Clinical studies have shown that patients with duodenal ulcer disease have markedly diminished basal and stimulated duodenal mucosal HCO3\(^{-}\) secretion [10, 19], suggesting that a relative lack of duodenal HCO3\(^{-}\) secretion may be a factor in the pathogenesis of duodenal ulcer. In the present study, we found that a VacA-positive *H. pylori* supernatant and purified VacA markedly reduced PGE2-stimulated duodenal HCO3\(^{-}\) secretion, whereas the supernatant of an isogenic VacA-negative mutant did not. Because the 2 supernatants from the isogenic strains differed only in the presence or absence of the VacA gene product, these results indicated that *H. pylori* VacA inhibits PGE\(_2\)-stimulated duodenal mucosal HCO3\(^{-}\) secretion. The effect of VacA on PGE\(_2\)-stimulated duodenal HCO3\(^{-}\) secretion was dose dependent. In an attempt to understand the mechanism for VacA-mediated inhibition of duodenal HCO3\(^{-}\) secretion, we measured the mucosal release of histamine by incubating the duodenal mucosa with the *H. pylori* supernatant, and we found that, in a dose-dependent manner, the VacA-positive supernatant caused a mucosal release of histamine. The concomitant application of H\(_2\) receptor antagonists, but not H\(_1\) or H\(_3\)-receptor antagonists, attenuated the inhibitory effect of VacA-positive supernatant, suggesting an inhibition of duodenal HCO3\(^{-}\) secretion via a local release of histamine acting on H\(_2\) receptors, the presence of which we could demonstrate on murine duodenal epithelial cells as well as on neural and lymphatic structures.

The precise mechanism for *H. pylori*-induced duodenal ulcer is not fully understood. The present conceivable reasons for the development of duodenal ulcer in *H. pylori*-infected subjects include the possibility that *H. pylori* infection of the antrum induces hypersecretion of gastric acid, leading to an increased duodenal acid load and giving rise to gastric metaplasia in the duodenal bulb. The gastric metaplasia leads to *H. pylori* colonization in the bulb, giving rise to duodenitis and impairing duodenal epithelial function [20 –22]. In this series of events, the major determinant is the prevalence of the virulent *H. pylori* strains and their density in the bulb [21–26]. A number of virulence factors have been identified. VacA, a highly immunogenic 95-kDa protein that induces massive vacuolization in epithelial cells in vitro, is believed to play an important role in peptic ulceration [3, 4]. In the present study, our results demonstrated that *H. pylori* VacA markedly reduced PGE\(_2\)-stimulated duodenal HCO3\(^{-}\) secretion in a dose-dependent manner; this finding may have important pathophysiologic relevance. Under physio-

![Figure 6. The effect of histamine on prostaglandin E\(_2\) (PGE\(_2\))-stimulated murine duodenal bicarbonate (HCO3\(^{-}\)) secretion.](image)

- **A.** Time course. **B.** The dose-dependent effect on \(\Delta\)HCO3\(^{-}\) (the net stimulated murine HCO3\(^{-}\) secretion). Control vehicle or histamine was added to the serosal side of tissues 20 min before stimulation with PGE\(_2\) (10\(^{-6}\) mol/L). Results are expressed as the mean ± SE, and, for each series, \(n = 7\). Histamine reduced PGE\(_2\)-stimulated HCO3\(^{-}\) secretion in a dose-dependent manner (*\(P < .05\), **\(P < .005\), †\(P < .0001\), compared with control, by 1-way analysis of variance with Student’s t test or Newman-Keuls post hoc test).
logic conditions, luminal acid is a critical stimulus of proximal duodenal mucosal HCO$_3^-$ secretion [8, 27]. Patients with duodenal ulcer have markedly diminished basal and acid-stimulated proximal duodenal mucosal HCO$_3^-$ secretion and an impaired mucosal HCO$_3^-$ response to endogenous PGE$_2$ [10, 19]. Many studies have also shown that PGE$_2$ is an important intramucosal mediator of stimulation of duodenal HCO$_3^-$ secretion by luminal acid [28–31]. Therefore, the inhibitory effect of H. pylori VacA on PGE$_2$-stimulated HCO$_3^-$ secretion may impair duodenal mucosal defense against acid injury. In addition, the stimulation of H. pylori VacA on mucosal histamine release, which is likely to occur in the stomach as well, may increase gastric acid secretion. These factors could contribute to the development of duodenal ulcer.

How does H. pylori VacA influence PGE$_2$-stimulated duodenal mucosal HCO$_3^-$ secretion? It has been shown that VacA may have proinflammatory activities and that it activates mast cells to produce proinflammatory cytokines [32–34]. Proinflammatory cytokines inhibited bile duct epithelial secretion in rats [35]. In the present study, after duodenal tissue was incubated for 1 h, VacA inhibited PGE$_2$-stimulated duodenal mucosal HCO$_3^-$ secretion. However, it does not seem likely that VacA inhibited PGE$_2$-induced duodenal HCO$_3^-$ secretion via inflammatory cytokine production in the present study, considering that the inhibitory effect of VacA occurred rapidly. This time course is probably too fast for cytokine synthesis and secretion in response to H. pylori VacA to take place [33, 36]. We speculated that VacA stimulated duodenal tissue to release some substances that mediated the action of VacA on PGE$_2$-stimulated HCO$_3^-$ secretion. Previous studies showed that histamine inhibits duodenal HCO$_3^-$ secretion stimulated by PGE$_2$ without altering basal duodenal HCO$_3^-$ secretion in rabbits [18], an effect that is inhibited by histamine H$_2$ receptor antagonist, and that some ulcerogenic agents inhibited duodenal HCO$_3^-$ secretion via a histamine-dependent mechanism [37]. Histamine is a ubiquitous chemical mediator. In the gastroduodenal mucosa, histamine is stored in mast cells and enterochromaffin-like (ECL) cells. Clinical studies found that mast cell and ECL cell densities were markedly increased in the gastric mucosa in patients with an H. pylori–infected duodenal ulcer [38, 39]. Patients with an H. pylori–infected duodenal ulcer have a lower histamine content in the oxyntic mucosa than do patients without an H. pylori–infected duodenal ulcer [40], suggesting that H. pylori infection stimulates histamine release from gastric mucosa. In experiments performed in isolated cells in vitro, it was also shown that H. pylori can stimulate histamine release from mast cells [41] and ECL cells [42]. These results suggest the involvement of histamine pathways in H. pylori infection and duodenal ulcer. Therefore, we examined the effect of histamine H$_2$, H$_3$, and H$_3$ receptor antagonists on the inhibitory action of VacA on duodenal HCO$_3^-$ secretion. We found that histamine H$_2$ receptor antagonists, both cimetidine and ranitidine, not H$_1$ and H$_2$ receptor antagonists, partly reversed the inhibitory effect of VacA on PGE$_2$-stimulated duodenal mucosal HCO$_3^-$ secretion. Further study found that VacA stimulated duodenal mucosal histamine release in a dose-dependent manner. After incubation for 15 min with VacA, duodenal mucosal histamine release was markedly increased; it reached maximal levels at 45 min, and this corresponded to development of the reduction in PGE$_2$-stimulated HCO$_3^-$ secretion. Exogenous histamine inhibited PGE$_2$-stimulated duodenal HCO$_3^-$ secretion in mice in a dose-dependent manner. Histamine H$_2$ receptor was widely expressed in duodenal mucosal epithelial cells, the enteric nerve plexus, and lymphocytes in Peyer’s patch. These results demonstrate that the inhibitory action of VacA on PGE$_2$-stimulated duodenal HCO$_3^-$ secretion may be mediated via histamine release from mucosal mast cells and/or other sources. This histamine, in turn, mediates its inhibitory effect via H$_2$ receptors. The mechanisms by which histamine inhibits PGE$_2$-stimulated duodenal HCO$_3^-$ secretion are unclear. It is possible that histamine, acting through H$_2$ receptors, either directly or indirectly activates within the duodenal mucosa a subpopulation of nerves that contain an inhibitory messenger. In addition, it is also possible that H$_2$ receptors are located on nerve plexus in the submucosa, which could release an inhibitory messenger that, in turn, activates a neural response. In either case, once the neurotransmitter is released, it is proposed to act directly on the epithelium to inhibit PGE$_2$-stimulated duodenal HCO$_3^-$ secretion. However, the precise mechanism of histamine’s action on PGE$_2$-stimulated duodenal HCO$_3^-$ secretion may be complex and will require further study.

In conclusion, H. pylori VacA inhibits PGE$_2$-stimulated duodenal epithelial HCO$_3^-$ secretion via stimulating the release of mucosal histamine, which in turn inhibits HCO$_3^-$ secretion in an H$_2$ receptor–dependent mechanism. H. pylori VacA-mediated duodenal histamine release and subsequent inhibition of duodenal HCO$_3^-$ secretion might therefore be important pathogenic factors contributing to H. pylori–associated duodenal mucosal injury.

References


