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Helicobacter Pylori and Hormones

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Helicobacter pylori affects gastric acid secretion via several mechanisms. One of these is by changing gastric regulatory physiology. The infection elevates plasma gastrin levels and decreases gastric mucosal expression of the inhibitory peptide somatostatin. These changes may be due to products of *H. pylori* itself or inflammatory cytokines released in *H. pylori* infection: acid secretion is inhibited less by a low intra-gastric pH, infusions of cholecystokinin and gastric distention in infected persons. Eradication of *H. pylori* rapidly decreases basal acid secretion and gastrin-releasing, peptide-stimulated acid secretion. There are now reports that maximally-stimulated acid secretion, a measure of the parietal cell mass, falls significantly six and 12 months after eradication of *H. pylori* from duodenal ulcer patients. This might be due to withdrawal of the trophic effect of gastrin. However *H. pylori* can also decrease gastric acid secretion, both through the mechanisms described in Dr. Cave's paper and by causing gastric mucosal atrophy with loss of parietal cells. The net effect on acid presumably depends on which mechanism predominates. The processes involved may be crucial determinants of clinical outcome. For example, infection with little atrophy and high acid secretion is associated with duodenal ulcers, while infection with atrophy and low acid secretion increases the risk of gastric cancer of the intestinal-type.

INTRODUCTION

Before *Helicobacter pylori* was discovered, the aetiology and treatment of duodenal ulcer [DU][b] disease was viewed in terms of the changes in gastric physiology found in DU patients. Since it became clear that *H. pylori* also plays a major role, we and others have examined the respective roles of, and interactions between, physiology and infection in a variety of conditions. What has emerged is that *H. pylori* is actually responsible for many of the physiological changes previously described in DU patients. Furthermore, it is becoming clear that how infection affects gastric physiology in a particular host is an important determinant of the different outcomes of *H. pylori* infection.

REGULATORY PEPTIDES AND THEIR RECEPTORS

Three regulatory peptides seem especially interesting in the pathophysiology of peptic ulcer disease and *H. pylori* infection:

Gastrins

Gastrins [1] are released from G-cells. These are located in the epithelium of the gastric antrum and duodenum, but are more abundant in the former. The main gastrin peptides are gastrin-34 (G34) and its C-terminal fragment gastrin-17 (G17). About 95 percent of antral gastrin is G17, while duodenum gastrin is about 60 percent G34 [2]. G34 and

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[b]*Abbreviations:* DU, duodenal ulcer; GRP, gastrin-releasing peptide.

G17 have similar agonist activity at the gastrin (alias CCK-B) receptor [3]. However, G34 is cleared more slowly from the circulation, so that if the same molar amount of G34 or G17 are administered *in vivo,* G34 has the greater effect [3]. G-cells are of the "open type" with microvilli extending into the gastric lumen. G-cells are stimulated to release gastrin by luminal stimuli, notably the products of protein digestion and intra-mucosal stimuli, notably gastrin-releasing peptide [1]. Gastrin acts via the blood stream to increase acid secretion. It does this both directly by stimulating parietal cells [4] and indirectly by stimulating ECL-cells to release histamine [5]. Gastrin also has a trophic effect on the gastric epithelium and particularly on the ECL-cells that it contains [6]. Prolonged hypergastrinaemia can cause ECL-cell tumors.

Somatostatins

Somatostatin peptides [7] are released from D-cells located throughout the gastrointestinal tract and many other organs. The two main forms of somatostatin S28 and S14 have different affinities for the different somatostatin receptors SSTR1-5. Recent evidence suggests that SSTR2 is most involved in physiological control of acid secretion [8]. This receptor appears to be affected similarly by S28 and S14. D-cells in the gastric antrum are "open," with microvilli extending into the gastric lumen, while D-cells of the gastric corpus are "closed" and do not contact the lumen [7]. Somatostatin is released from the antrum by luminal factors, notably acid [9-11], but also by food [12]. Curiously, although antral D-cells are in contact with the gastric lumen, there is evidence that acid affects antral D-cells indirectly through a neural reflex that may involve CGRP [13]. Somatostatin is also released by neurotransmitters including epinephrine and a wide variety of peptides including gastrin releasing peptide (GRP)/bombesin and cholecystokinin. Stimulation of somatostatin-release by small intestinal hormones such as cholecystokinin may be important in the inhibition of gastric secretion that occurs in the late postprandial period. Somatostatin has widespread inhibitory effects on endocrine and exocrine cells, including G-cells, ECL-cells and parietal cells [7].

Gastrin-releasing peptide

GRP [14] contains 27 amino acid residues. Its C-terminal decapeptide GRP10 also occurs in humans and has been called neuromedin-C [15]. GRP closely resembles the amphibian 14 amino acid-peptide bombesin. GRP is present in nerve fibers and cell bodies throughout the gastrointestinal tract, in other organs and within the central nervous system. GRP nerves are present in the oxyntic and antral regions of the stomach, but are more abundant in the former [16]. GRP is an exceedingly potent stimulant of gastrin release, and there is good evidence that it mediates gastrin release in response to vagal stimulation [17]. GRP also stimulates somatostatin release from gastric mucosal pieces *in vitro.* These appear to be indirect effects, mediated via gastrin-release in the antrum and via cholinergic and non-cholinergic mucosal nerves in the corpus [18]. Infusion of GRP into intact animals both stimulates acid secretion through gastrin release and also inhibits it [19], probably by releasing somatostatin in the gastric corpus [14]. Thus, GRP not only stimulates acid secretion via gastrin but can also inhibit acid secretion via somatostatin, which inhibits gastrin release in the antrum and acid secretion in the corpus through separate mechanisms.

KNOWLEDGE BEFORE THE DISCOVERY OF *H. PYLORI*

Much of the work on gastric pathophysiology was undertaken before the discovery of *H. pylori.* The subsequent discovery of this bacterium has taught us much, most notably we have learned the importance of integrating physiology with other disciplines such as bacteriology, immunology and histopathology.

Acid in DU disease

Before the discovery of *H. pylori*, our whole approach to DU disease was based on the finding that sufferers secrete more acid than controls. This abnormality could be divided into two elements: First, duodenal ulcer patients have been estimated to have approximately two billion parietal cells compared with about one billion in controls, leading to a maximal acid-secretory capacity that is about twice normal [20, 21]. In addition, studies showed a series of abnormalities of physiological control that might be summarized as failures of inhibitory reflexes. Basal acid secretion was increased even more than would be expected from the increased parietal cell mass [20]. A low intra-gastric pH inhibited peptone-stimulated acid secretion less than usual [22], and perhaps because of this, acid secretion persisted for longer than usual after meals [23]. Additionally, acid-secretion in response to GRP/bombesin was found to be exaggerated in DU patients. More precisely an inhibitory effect of high doses appeared lacking in DU patients [24,25].

Abnormalities of gastrin physiology in duodenal ulcer disease

Since gastrin is the most potent known stimulant of acid secretion, it was logical to ask whether its release was increased in DU patients, but studies gave mixed results. Some centers reported higher postprandial gastrin concentrations in DU patients [26], but others did not. In retrospect, this probably depended on whether the control group was infected with *H. pylori* or not. Studies using intra-gastric titration showed that a low intra-gastric pH inhibited peptone-stimulated gastrin levels less in DU patients than in controls [22]. One stimulus that consistently produced higher plasma gastrin levels in DU patients than controls was bombesin [24, 25].

Abnormalities of somatostatin in duodenal ulcer disease

Decreased mucosal somatostatin was a more consistent finding in DU disease. Studies showed less immuno-reactive somatostatin and fewer immuno-reactive D-cells in DU patients [27]. This was particularly interesting because it offered an explanation for the failure of acid-inhibitory reflexes in DU patients. However, the cause of decreased mucosal expression of somatostatin in DU disease remained mysterious until the discovery of *H. pylori*.

EFFECTS OF *H. PYLORI* ON HORMONES

When it became clear that *H. pylori* is a major etiologic factor in DU disease, we and others asked whether this infection causes some or all of the alterations in gastric physiology that had been reported in DU disease.

Gastrin

Odera et al. [28] reported that elevated gastrin levels in children with *H. pylori* fall after treatment. However they did not measure acid secretion, so it was unclear whether elevated gastrin levels were due to the achlorhydria of initial infection, which resolved on treatment. We reported elevated postprandial gastrin levels and elevated maximal acid output in infected vs. uninfected DU patients and proposed the "gastrin link" between infection of the antrum and ulcers in the duodenum in 1989 [29]. We and others confirmed that gastrin levels fall on eradication of the infection. The elevation in plasma gastrin levels occurs basally and during infusion of bombesin or GRP [26, 30, 31]. Interestingly, the excessive rise in gastrin after GRP [30] and after eating [32] is predominantly due to a rise in G17. This might be because the excessive gastrin emanates from the antrum where G17 predominates. Alternatively *H. pylori* might accelerate the cleavage of gastrin to release G17. Eradication restores the inhibitory effect of cholecystokinin on gastrin release [33] This reflex is believed to be mediated through release of somatostatin from antral D-cells.

Somatostatin

Several studies have shown that the decrease in mucosal expression of somatostatin noted in DU patients is actually due to *H. pylori* infection. Originally Kaneko et al. [34] showed diminished mucosal somatostatin peptide in association with this infection, and Moss et al. [35] showed that *H. pylori* infection also decreases mucosal somatostatin mRNA and the number of immunoreactive D-cells. Other groups have since confirmed the inhibitory effect of *H. pylori* on mucosal levels of somatostatin peptide [36-38], mRNA [37] and D-cell numbers [39]. On the other hand, Graham et al. [40] were unable to detect a difference in the number of D-cells. Such discrepancies might emanate from technical aspects such as the ability to detect cells containing small amounts of peptide. Little is known of D-cell function in *H. pylori* infection. We compared the response of mucosal somatostatin mRNA to three-hour infusions of GRP between infected and uninfected persons [41]. The results showed a significant rise in somatostatin mRNA in the infected, but not in the uninfected, group. The reason for this difference awaits elucidation, but baseline somatostatin mRNA levels were considerably diminished in the infected group, so the difference might be due to diminished autoinhibition of D-cells by somatostatin itself [42]. Whatever the explanation, the results do show that D-cells are capable of responding to GRP in *H. pylori* infection.

HORMONE-RELATED EFFECTS ON ACID SECRETION

Acid secretion in *H. pylori* infection has several quite clearly defined facets that need to be considered separately. The result is that *H. pylori* can both increase and decrease acid secretion.

Epidemic achlorhydria on first infection

Acid secretion is drastically diminished or completely absent for several weeks after first infection with *H. pylori*. This was first noted in volunteers undergoing repeated intubation. It was later realized that this was due to transmission of *H. pylori* between the subjects [43]. Achlorhydria might be due to acid-suppressing factors released by *H. pylori*. These were identified by Cave and his colleagues [44, 45] but have not been not fully characterized. They are discussed by Dr. Cave (page 91). Alternatively the immunological response to *H. pylori* might be responsible for the suppression of acid secretion. Patients with other infections such as pneumonia also show achlorhydria [46], and certain cytokines including interleukin-1 [47, 48] and tumor necrosis factor-α [49] inhibit acid secretion.

Diminished acid secretion through gastric atrophy

Epidemiological studies show that *H. pylori* gastritis can progress to atrophic gastritis and intestinal metaplasia [50, 51]. Atrophy developed in 28 percent of infected patients compared with four percent of uninfected patients over an 11-year period in Amsterdam [52]. Atrophy leads to a loss of parietal cells and acid secreting capacity. It is not currently clear whether atrophy is reversible on eradicating *H. pylori*, but if this is true, as Borody suggests [53], acid secretion might rise after eradication of *H. pylori* from patients with infection and atrophy.

Defective inhibition of acid secretion in H. pylori infection

Studies have shown that *H. pylori* infection is associated with defective inhibition of acid secretion. Elevations of acid secretion under conditions that would normally inhibit this may be due to a paucity of the inhibitory peptide somatostatin in the gastric mucosa. We found that eradication of *H. pylori* decreases basal acid secretion to about one third of

pre-eradication values in DU patients [54]. The sensitivity of parietal cells to gastrin-17 did not change [54], and the fall in acid secretion was appropriate for the accompanying fall in circulating gastrin. We also found that *H. pylori* impairs inhibition of peptone-stimulated acid secretion by a low intragastric pH. The low pH suppressed acid secretion by greater than 80 percent in uninfected persons but by less than 50 percent in infected volunteers. Thus, the infection elevated acid secretion in response to the acidic meal by about three times [55]. Acid secretion stimulated by neutral peptone was similar in the infected and uninfected groups, indicating that the abnormality lies in the inhibitory pathway. Antral distension normally inhibits pentagastrin-stimulated acid secretion, and this reflex is also attenuated by *H. pylori* infection [56]. Infusions of GRP normally exert a mixture of stimulatory and inhibitory effects on acid secretion. Stimulation is through gastrin release, while inhibition may be through somatostatin [18], which exogenous GRP probably releases indirectly via several routes including locally released gastrin, mucosal nerves, small intestinal hormones and a low intragastric pH [see above]. Acid secretion stimulated by GRP is elevated about three times in *H. pylori* infection and about six times in DU persons when compared with uninfected controls [57]. The more marked elevation of acid secretion in DU patients might be due to their greater parietal cell mass (see above). Elevated GRP-stimulated acid secretion disappears slowly during the first year after successful eradication. *H. pylori*-related elevations in acid secretion are interesting because they might be responsible for the association between this infection and DU disease. Therefore, it is important to ask whether the changes are sufficient to affect the pH in the duodenum? Hamlet et al. [58] found that infected persons did indeed have a lower intra-duodenal pH during the second hour after neutral and acidic meals than healthy controls. However, this was at least partly due to rapid gastric emptying, in addition to changes acid secretion. The findings described so far in this paragraph can largely be explained by *H. pylori* decreasing expression of somatostatin in the gastric mucosa, but Accord's interesting finding also needs to be considered. He found that basal acid secretion is higher in patients with active DUs, than in patients with inactive DUs [59], without eradication of *H. pylori*. This raises the possibility that the ulcer itself stimulates acid secretion, perhaps through a neural reflex. Alternatively the physiological abnormality in DU disease might be cyclical, with ulcers occurring when acid secretion is greatest. However, this cannot be the whole story because *H. pylori* infection is associated with elevated acid secretion during stimulation with acid-peptone and GRP, even in patients without ulcers [55, 57].

REASONS FOR DIFFERENT RESPONSES IN DIFFERENT PATIENTS

The amount of acid secreted by a particular patient presumably depends on the relative contribution of the various inhibitory and stimulatory processes described above. This merits study because the amount of acid secreted may be an important determinant of disease outcome in *H. pylori* infection. For example, we do not know why acid secretion is lost initially but then returns. Does acid secretion return because of a change in the state of the bacterium or the host's response to it? Similarly, why is acid secretion diminished by gastric mucosal atrophy in some patients but not in others? This depends partly on the time since infection. But atrophy also seems more likely if the infecting strain is CagA+ [60] and if the host is HLA-DR5 [61]. Other atrophy-producing factors, such as a high-salt diet [62] may also be involved. One important finding from Finland is that DU patients have a relative lack of corpus atrophy [51], the so-called "juvenile mucosa." Thus, DU patients might have a greater parietal cell mass because their parietal cells have not been diminished by atrophy [20]. Alternatively, the greater parietal cell mass might be in inherited trait or due to the trophic effect of gastrin [6]. In that case, the relative lack of corpus gastritis could be due to high acid secretion inhibiting the onset of atrophy, rather

than the reverse. This notion is supported by the increase in atrophy that is seen after suppression of acid secretion with potent agents such as omeprazole [63]. In view of these possibilities, it has been interesting to ask whether maximally stimulated acid output changes following eradication of *H. pylori* from different groups of patients. DU patients may be most likely to show a fall because their gastric corpus is initially relatively healthy and secreting plenty of acid. At present, the data are conflicting. Groups in Glasgow [64] and South Africa [65] found no significant change in maximal acid output seven months and 12 months, respectively, after eradication of *H. pylori* from DU patients. However, groups in London [66] and Canada [67] saw significant falls at six and 12 months. Such discrepancies are presumably due to methodological differences between the studies.

MECHANISMS OF ALTERED HORMONE PHYSIOLOGY

Products of urease

We originally proposed that alkali generated locally by *H. pylori*'s urease increases gastrin release [29]. As acid is a major D-cell stimulant, this would be expected to decrease expression of somatostatin [9, 68] and could thus produce the observed increases in gastrin and acid. Measurements of the pH in the gastric mucus layer in *H. pylori* infection have shown it to be more alkaline, although the difference is only 0.3-0.8 of a pH point [26]. Increasing intra-gastric urea did not elevate gastrin in infected persons [31], but might not be expected to do so because urease's Km of 0.2 [69] is well below the normal intra-gastric concentration of urea which is 1-2.5 mmol/l, so the enzyme is already virtually saturated with substrate. Inhibition of urease either using acetohydroxamic acid [70] or bismuth plus antibiotics did not decrease gastrin release in short-term experiments. Taken together, these results do argue quite strongly against the role of ammonia. However the possibility cannot be dismissed altogether because some ammonia production persisted during the study with acetohydroxamic acid [70], and it may be that exposure to small amounts is sufficient. Also, if alkalinization is involved the duration of the studies may have been insufficient. Plasma gastrin levels remain considerably elevated three hours after acidification of the stomach of achlorhydric patients [71].

Ammonium ions may release gastrin independent of any effect on pH. Lichtenberger et al. found elevated plasma gastrin levels in rats whose diets had been supplemented with ammonium acetate for two weeks. A study from Japan suggests that monochloramine may be a more potent stimulant of gastrin release than ammonia itself [72]. Ammonia released by *H. pylori* activates neutrophils to create oxidative bursts *in vitro* [73]. Oxidation of hydrochloric acid by neutrophils would be expected to produce hypochlorous acid, which then reacts with ammonia to produce monochloramine NH_2Cl. In short, it is currently difficult to dissect the respective roles of ammonium ions, alkalinization and monochloramine, largely due to a lack of adequate methods to selectively eliminate these factors in humans.

The possible role of inflammatory mediators

Another possibility is that cytokines released in *H. pylori* gastritis are responsible for the altered endocrine cell function. *H. pylori* infection increases mucosal expression of many cytokines including; interleukins 1β, 6 and 8, tumor necrosis factor-α, interferon-γ and platelet activating factor [74]. Several cytokines release gastrin from antral preparations *in vitro*, including interleukins 1 and 2, tumor necrosis factor-α interferon-γ and leukotrienes C_4 and D_4. We found that tumor necrosis factor-α and interferon-γ release gastrin from canine antral endocrine cells in primary culture [75]. Diffusible products of human peripheral blood mononuclear cells (lymphocytes plus monocytes) had a stronger effect [75]. This might be because the above cytokines have additive or synergistic effects when given together or because some other product of mononuclear cells is actually

involved. This is distinctly possible because the number of known cytokines is rapidly expanding. Those mentioned above were studied largely because assays are available, and these are known to affect a wide variety of other types of cells. One study demonstrates the importance of synergism between stimuli: *H. pylori* sonicates failed to release gastrin from canine antral cells but markedly stimulated G-cells primed with interleukin-8. This chemokine given alone was a relatively weak stimulant of gastrin release [76]. Interestingly the response to interleukin-8 plus *H. pylori* extract varied between strains of *H. pylori*. Another study showed the importance of the duration of cytokine-exposure. Short term treatment of canine corpus D-cells to TNF-α weakly stimulated somatostatin release, but exposure for 6 to 24 hr decreased both cell content and percent release of somatostatin in response to specific stimuli [77].

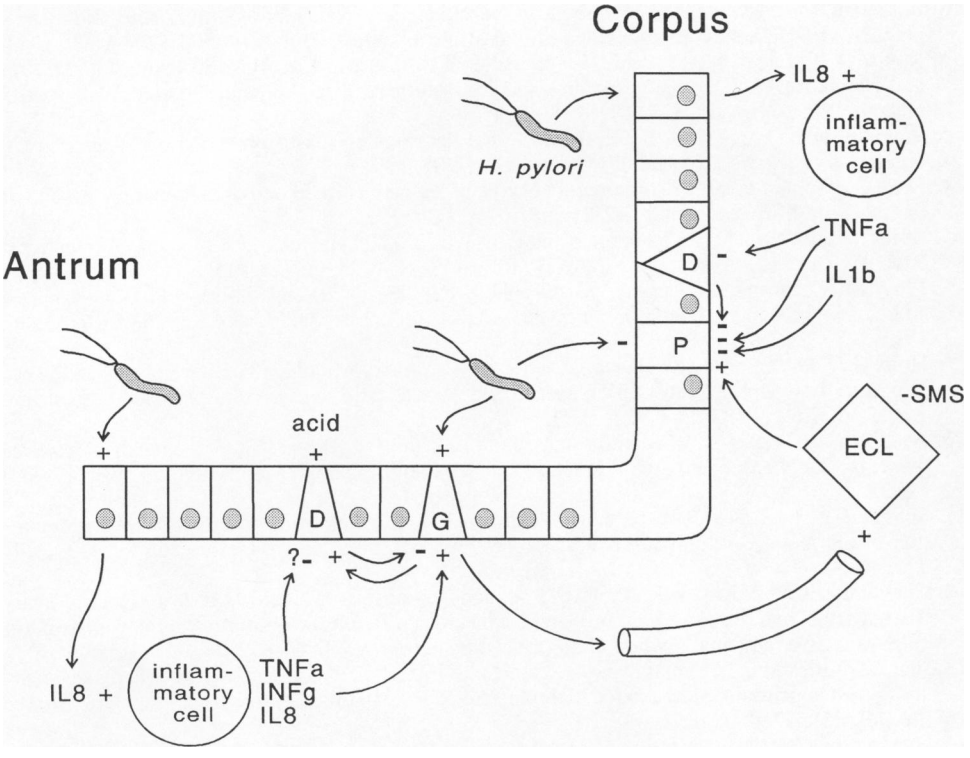

Figure 1. Some mechanisms affecting acid secretion in *H. pylori* infection. Gastrin from G-cells (G) stimulate parietal cells (P) to secrete acid, partly by stimulating enterchromaffin cells (ECL) to release histamine. D-cells (D) in the gastric corpus and antrum release somatostatin, which inhibits G-, ECL- and parietal cells. Cytokines including tumor necrosis factor-α (TNFα), interferon-γ (INFγ) and interleukin-8 (IL-8) may cause these changes in G- and D-cell function, which tend to increase acid secretion. However, *H. pylori* can also decrease acid secretion, by direct effects on parietal cells by causing atrophy and through cytokines such as interleukin-1β (IL1β).

CLINICAL PERSPECTIVE AND FUTURE STUDIES

It is now clear that *H. pylori* infection can both increase and decrease acid secretion. These different responses seem important clinically because elevation of acid secretion is found in duodenal ulcer patients, and the excess of acid may actually cause the ulcers. On the other hand, diminished acid secretion is found in patients with gastric cancer and may promote carcinogenesis by allowing the stomach to become chronically colonized with other bacteria that produce mitogens. Exploration of the mechanism of these changes in acid secretion has shown that *H. pylori* infection alters the function of gastric endocrine and exocrine cells. Future studies should aim to elucidate the molecular and cellular basis of these changes and the reasons why these effects differ between individuals.

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