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Luminal Nα-methyl histamine stimulates gastric acid secretion in duodenal ulcer patients via releasing gastrin

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Abstract

Nα-methyl histamine is an unusual histamine metabolite which is produced in the stomach infected by *Helicobacter pylori* and which was shown in animals to stimulate gastric acid secretion and to release gastrin in vitro isolated G-cells, but no information is available regarding its influence on gastric secretion and gastrin release in duodenal ulcer patients before and after *H. pylori* eradication. In this study, we compared the effects of intragastric administration of single or graded doses of Nα-methyl histamine on gastric acid secretion and plasma gastrin levels in 16 male duodenal ulcer patients (aging from 35 to 48 years and weighing 65–82 kg) before and after the eradication of *H. pylori*. Furthermore, the gastric luminal histamine and gastrin contents were determined before and after *H. pylori* eradication. In *H. pylori*-infected duodenal ulcer patients, the intragastric application of Nα-methyl histamine failed to affect gastric acid secretion or plasma gastrin levels. Following eradication of *H. pylori*, gastric luminal histamine and gastrin, and both basal gastric acid secretion and plasma gastrin levels, were significantly reduced. Nα-methyl histamine given intragastrically in graded doses to such *H. pylori*-eradicated duodenal ulcer patients was found to increase dose-dependently gastric acid output reaching at a dose of 5 mg, about 80% of histamine maximum induced by i.v. infusion of 25 μg/kg h of histamine dihydrochloride. We conclude that Nα-methyl histamine is a potent luminally active stimulant of gastrin release and gastric acid secretion in *H. pylori*-eradicated patients when luminal histamine is low but is not effective in *H. pylori* infected patients when luminal histamine is enhanced, possibly due to desensitization of gastrin (G-cells) and acid-producing (parietal) cells by Nα-methyl histamine produced excessively in *H. pylori*-infected stomach. © 2001 Published by Elsevier Science B.V.

Keywords: Histamine; Nα-Methyl histamine; Gastrin; Gastric acid secretion; *Helicobacter pylori*

1. Introduction

The major source of histamine in human stomach are numerous mast cells and the enterochromaffin-like cells located mostly in oxyntic gland area in close vicinity of the parietal cells and possessing numerous vesicular granules and cytoplasmic processes consistent with their paracrine function (Hakanson and Sundler, 1987; Hakanson et al., 1994). Recently, Nα-methyl histamine was found in the majority of human stomachs and attributed to *Helicobacter pylori* infection. Courillon Mallet et al. (1995) reported that *H. pylori* produces Nα-methyl histamine that can be detected in measurable amounts in the *H. pylori*-infected stomach. The methyl histamine compounds were reported long time ago by Code et al. (1971) to induce gastric acid stimulation in dogs comparable to that obtained by histamine, but the source of these compounds has not been revealed. Recently, Nα-methyl histamine has been found to stimulate acid production by cultured rabbit parietal cells (Beales and Calam, 1997a) and acid secretion from isolated mouse stomach (Vuyyuru and Schubert, 1997). It has been then shown to exhibit an agonist activity for histamine H_1, H_2, and H_3 receptors (Oishi et al., 1993; West et al., 1990) and to stimulate the G-cells in vitro to release gastrin (Bliss et al., 1999).

Our previous studies disclosed, unexpectedly, that regular histamine dihydrochloride is capable of releasing small but significant amounts of gastrin in humans (Konturek et al., 1999), but so far, no study was reported to confirm this observation and to determine the effect of Nα-methyl

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histamine on gastric acid secretion and gastrin release in humans with or without *H. pylori* infection. This study was designed, therefore, to compare the effects of Nα-methyl histamine with those of regular histamine on gastrin release and gastric acid secretion in *H. pylori*-infected humans with peptic ulcer before and after eradication of *H. pylori*.

The aim of our study was the following: (1) to determine the effects of luminal Nα-methyl histamine in humans on gastrin release and gastric acid secretion; (2) to compare the effects of luminal (gastric) application of Nα-methyl histamine and parenteral administration of histamine on gastrin release and gastric acid secretion; (3) to determine the influence of *H. pylori* eradication in humans on gastrin and histamine release, and (4) to propose the model implicating Nα-methyl histamine in gastrin release and gastric secretion in humans.

2. Materials and methods

Sixteen consecutive male patients, aging from 35 to 48 years (mean age 41 years) and weighing 65–82 kg (mean weight 75 kg) with active duodenal ulcer diagnosed by initial gastro-duodenoscopy, entered the study. *H. pylori* infection was verified by positive rapid urease test (*Campylobacter*-like organism test—CLO-test) performed during endoscopy using antral mucosal biopsy samples and ^{13}C-urea breath test described earlier (Bielanski, 1999). All tested patients were initially infected with *H. pylori*. They were divided into group A ($N = 10$) for studies of luminal contents of histamine and gastrin and basal gastric acid secretion, and group B ($N = 6$) for studies on the effect of Nα-methyl histamine applied intragastrically in a single dose or in graded doses on gastric secretion and gastrin release before and after the eradication of *H. pylori*. All secretory studies were performed before and after the application of an eradication anti-Hp therapy. Ethics approval was obtained from the University Research Ethics Committee wherein each patient gave informed consent.

In both groups of patients, the orogastric polyethylene tube was installed with the tip in the most dependent part of the stomach and residual gastric content was evacuated. In group A, gastric secretion was tested only under basal conditions for 30 min using continuous gastric aspiration and collecting gastric contents at 10-min intervals. In group B patients, in addition to basal gastric secretion, the intragastric (topical) Nα-methyl histamine was applied either in a single bolus dose of 5 mg or in gradually increasing doses (0.1–5.0 mg/dose), each dose given separately in 20 ml of saline and introduced into the stomach through the orogastric tube. The selection of the doses of Nα-methyl histamine was arbitrary and we started with the dose of 0.1 mg that did not cause any secretory change. Then the dose of this methyl-derivative of histamine was raised to achieve the acid secretory response close to that induced by maximal response to this amine. The collection of gastric content was stopped for 15 min after each dose used, the residual gastric content was then evacuated within 1–2 min and then the regular aspiration was restarted with gastric content collected in 10 ml aliquots for 30 min. Throughout the collection of gastric content, an i.v. infusion of saline was maintained at the rate of 100 ml/h. During the final 30 min, histamine dihydrochloride (25 μg/kg h) was added to saline infusion to assess maximal gastric acid secretion in each of the tested patients of group B as reference secretory *maximum*. Each aliquot collected from the stomach was examined by measuring its volume, HCl concentration by titration with 100 mM NaOH up to pH 7.0 using automatic titrator with autoburette (Radiometer, Copenhagen, Denmark), and acid output was calculated and expressed in millimole per 10- or 30-min period.

From each collection aliquot of gastric aspiration, the samples of about 5 ml was separated and immediately neutralized upon the aspiration to pH 7.0 by adding 300 mM NaOH and saved at −80°C for the determination of gastric luminal concentrations of histamine and gastrin by specific radioimmunoassays described previously. The blood samples were also taken at 10-min intervals, the plasma separated and frozen until radioimmunoassay of gastrin, as described before (Konturek et al., 1990). Gastrin antibody No. 4562 (final dilution, 1:100.000) was a gift of Professor J. Rehfeld from Copenhagen, Denmark. ^{125}I-gastrin was purchased from Amersham, UK). The limit of detection was 0.5 pM of sample and the IC_{50} was 18 ± 3 pM. Interassay and intra-assay coefficients of variation were 5% and 10%, respectively. Histamine concentration was also measured by commercially available radioimmunoassay kit (IBL, Hamburg, Germany), including the tubes coated with monoclonal antibodies against acetylated histamine, acetylating agent, and ^{125}I-histamine as tracer. The limit of detection was 0.1 nM histamine and the IC_{50} was 10 ± 3 nM. The anti-*N*-acylhistamine antiserum in this kit showed only negligible cross-reactivity with *N*-methylhistamine. Interassay and intra-assay coefficients of variation were 4% and 8%, respectively. The endoscopy and secretory studies with withdrawal of the gastric content and plasma samples were repeated after 4 weeks upon successful 1 week triple therapy including clarithromycin 500 mg bd, amoxycillin 500 mg bd, and omeprazole 20 mg bd. In all subjects, the CLO-test and urea breath test became negative following this therapy and patients were considered as *H. pylori*-eradicated or *H. pylori*-negative. No side-effects during the anti-*H. pylori* therapy and examination before or after the treatment were recorded.

2.1. Data analysis

All values of gastrin, histamine and gastric acid outputs are expressed as the means ± S.E.M. obtained under basal

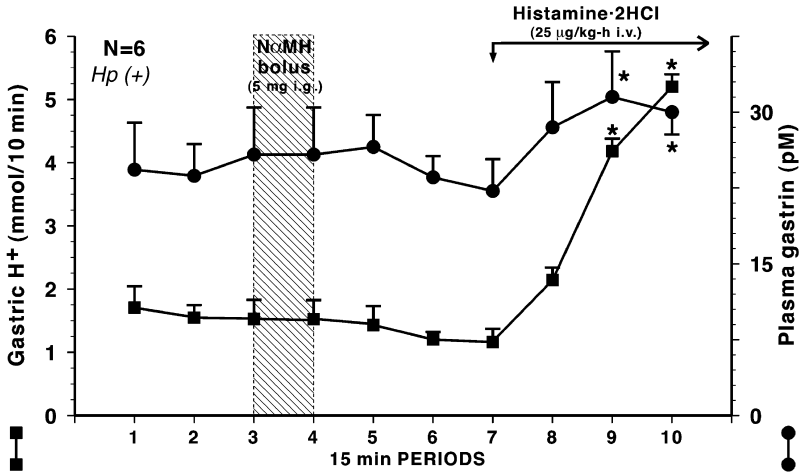

Fig. 1. Gastric acid outputs and plasma gastrin levels under basal conditions and following intragastric application of Nα-methyl histamine (5 mg/dose) and after i.v. infusion of histamine at a dose of 25 μg/kg h in six subjects of group B infected with *Helicobacter pylori*. Means ± S.E.M. of six determinations on six duodenal ulcer patients. Asterisk indicates significant ($P < 0.05$) change as compared to the values recorded before the administration of histamine.

conditions and following i.g. administration of Nα-methyl histamine or i.v. infusion of histamine. In general, rank sum test, Sperman's rank test order correlation was used for relation between independent values. A P value of less than 0.05 was accepted as significant.

3. Results

In *H. pylori*-infected duodenal ulcer patients of group A, basal gastric acid secretion averaged 2.15 ± 0.32 mmol/10 min and plasma gastrin level was about 23.4 ± 3.5 pM. Following the *H. pylori* eradication, the basal acid output and plasma gastrin concentration were significantly decreased in these patients by about 70% and 40%, respectively.

In *H. pylori*-infected patients gastric luminal histamine concentration was elevated to about 103 ± 10 pM, and accompanied by a marked release of gastrin into gastric lumen reaching about 110 ± 11 pM. Following *H. pylori* eradication, total histamine as well as gastrin levels in gastric contents were remarkably reduced by about 85% and 58%, respectively.

In *H. pylori*-infected duodenal ulcer patients of group B, the i.g. application of Nα-methyl histamine in a single bolus dose of 5 mg during basal gastric acid secretion did not cause any significant alteration of this secretion. No change in basal plasma gastrin was also recorded (Fig. 1). Infusion of exogenous histamine dihydrochloride (25 μg/kg h) resulted in about five-fold increase in acid output in these patients and small but significant rise in plasma gastrin level. In contrast, in the same patients after *H. pylori* eradication, Nα-methyl histamine applied intragastrically in a single bolus dose (5 mg/kg) for 15 min (Fig. 2) doubled the gastric acid secretion and plasma gastrin level. In separate tests, Nα-methyl histamine applied i.g. in graded doses in these *H. pylori*-eradicated

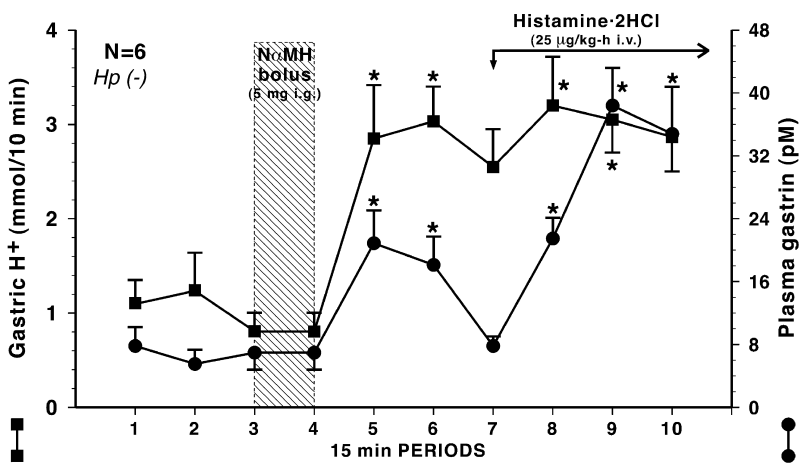

Fig. 2. Gastric acid outputs and plasma gastrin levels under basal conditions and following intragastric application of Nα-methyl histamine (5 mg/dose) and after i.v. infusion of histamine (25 μg/kg h) in six subjects of group B with eradicated *Helicobacter pylori*. Means ± S.E.M. of 10 determinations on six duodenal ulcer patients. Asterisk indicates significant ($P < 0.05$) change as compared to the values recorded before the administration of histamine.

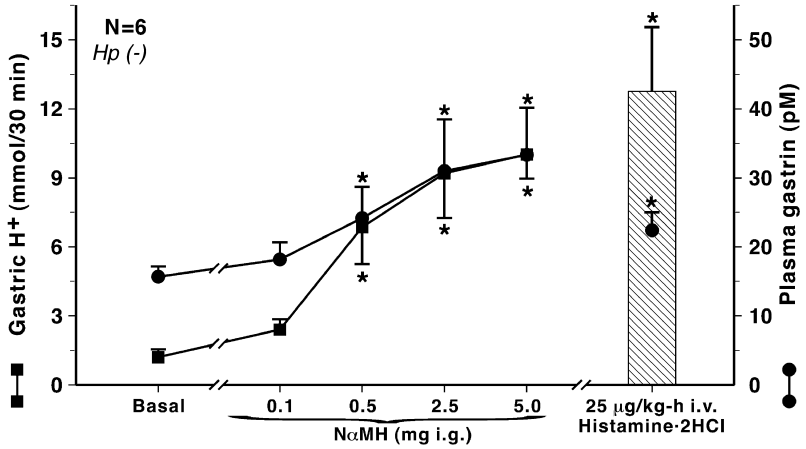

Fig. 3. Gastric acid outputs and plasma gastrin levels in six duodenal ulcer patients after eradication of *Helicobacter pylori* in response to intragastric application of gradually increasing doses of Nα-methyl histamine (0.1–5 mg/dose). For the comparison, histamine-induced maximal acid output and plasma gastrin levels in these patients are included. Asterisk indicates significant ($P < 0.05$) increase above the basal value.

subjects resulted in a dose-dependent stimulation of gastric acid secretion reaching about 80% of histamine maximum and this was accompanied by gradual increments in plasma gastrin from basal from about 16.0 ± 0.8 to about 33.2 ± 2.3 pM at the highest dose of this histamine metabolite (Fig. 3). With i.v. infusion of histamine, plasma gastrin also showed a small but significant rise over basal value but this was much less than that attained with intragastric Nα-methyl histamine.

With intragastric administration of Nα-methyl histamine at single or graded doses, no side effects were recorded and the blood pressure was normal. In contrast, the i.v. infusion of histamine resulted in the increase in heart rate by about 20 beats/min and the fall in mean arterial pressure by about 15 mm Hg. One of the histamine-infused patients fainted and the infusion had to be stopped but within about 10 min, the normal arterial pressure was restored.

4. Discussion

This study provides evidence that Nα-methyl histamine applied topically to *H. pylori*-eradicated patients results in a dose-dependent increase in gastric acid secretion reaching about 80% of histamine maximum in these subjects. In contrast, Nα-methyl histamine applied to *H. pylori*-infected stomach did not cause any significant alteration in acid secretion or plasma gastrin level while intravenous infusion of histamine dihydrochloride caused a marked increase in gastric acid secretion and a small but significant elevation of plasma gastrin both in *H. pylori*-infected and eradicated patients. This suggests that parenteral histamine directly activates the G cells to release gastrin. It is of interest, however, that *H. pylori*-infected patients showed a marked increase in luminal content of immunoreactive histamine that dramatically declined upon the eradication of *H. pylori*. The increase in histamine content in gastric mucosa of duodenal ulcer patients infected by *H. pylori* was reported previously by Bechi et al. (1996) and a "gastrin link" was proposed to explain the elevation of mucosal histamine in these patients. A threefold increase in the density of enterochromaffin-like cells was found and this has been attributed to elevated gastrin release by *H. pylori* in these patients, but no evidence was provided to support this notion. In our study, we also observed highly elevated histamine content in gastric juice probably representing the leakage of this amine from numerous mast cells and the enterochromaffin-like cells in the *H. pylori*-infected gastric mucosa as proposed before (Kurbel and Kurbel, 1995). The facts that eradication of *H. pylori* significantly reduced mucosal content of histamine, and that this decrease correlated with the reduction in gastrin release as observed in patients with successful eradication therapy (Queiroz et al., 1993), favour the concept of gastrin as a major stimulus of gastric histamine production and minimal release in duodenal ulcer patients infected with *H. pylori* (Kidd et al., 1998).

These results do not exclude the possibility that luminal histamine in duodenal ulcer patients infected with *H. pylori* originates, at least in part, from the production of this amine by bacteria themselves in the form of Nα-methyl histamine, but this requires experimental evidence. Recently, Murray et al. (2000) reported that Nα-methyl histamine measured by gas chromatography mass spectrometry was detected in gastric juice obtained only from *H. pylori*-infected patients, but not in that from uninfected subjects. However, cultured *H. pylori* in this report failed to produce detectable amounts of this amine. Although the origin of Nα-methyl histamine requires further study, it may be a stimulus of gastrin release in the *H. pylori* infected patients as suggested by the in vitro observation showing excessive release of gastrin from the G-cells in response to Nα-methyl histamine (Beales and Calam, 1997a).

As shown by this study, a strong acid stimulatory action of Nα-methyl histamine observed in *H. pylori*-eradicated patients was accompanied by a marked rise in plasma gastrin but the basal luminal histamine was remarkably reduced in these patients, suggesting that this luminal histamine could originate, at least in part, from the infected gastric mucosa and that Nα-methyl histamine could contribute to hypergastrinemia observed in *H. pylori*-positive patients. Although we did not determine directly Nα-methyl histamine in this study, it is tempting to assume that this histamine metabolite originating from the *H. pylori*-infected gastric mucosa is a potent luminally active stimulant of gastric acid secretion acting via release of antral gastrin. We propose that in the *H. pylori*-infected stomach, the feedback control of gastrin release by luminal acid is deficient and enhanced gastrin release may be due, at least in part, to excessive production of Nα-methyl histamine acting directly on G-cells, but some other *H. pylori*-related substances such as NH_3, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) could also contribute to increased gastrin release. The question remains why exogenous Nα-methyl histamine failed to affect gastric secretion and gastrin release in *H. pylori*-positive patients, but the fact that histamine content in these patients was exceedingly high suggests that this endogenous histamine could desensitize the parietal cells and G-cells to histamine, thus, preventing the action of exogenous Nα-methyl histamine on these cells.

In summary, the presence of *H. pylori* in gastric lumen appears to enhance gastrin release, at least in part, by the production of Nα-methyl histamine and possibly, also some cytokines such as IL-1β and TNF-α, that have been shown to augment the release of this hormone by other stimulants (Weghert et al., 1996; Beales and Calam, 1997b).

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