Effect of N^α-methyl-histamine on acid secretion in isolated cultured rabbit parietal cells: implications for *Helicobacter pylori* associated gastritis and gastric physiology

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Abstract

Background—*Helicobacter pylori* has been shown to produce the unusual metabolite N^α-methyl-histamine. This compound is known to be a potent agonist at inhibitory histamine H₂ receptors. There is increasing evidence implicating this receptor in the control of gastric acid secretion but the mechanism for this remains to be clarified.

Aims—To investigate the effect of N^α-methyl-histamine on the acid secretory activity of parietal cells and to determine the mechanism for such effects, thus helping to determine the role of this compound in the pathophysiology of *H. pylori* infection.

Methods—Rabbit parietal cells were isolated and enriched by collagenase-EDTA digestion and centrifugal elutriation. Following culture on Matrigel coated plates, acid secretion was assessed by ¹⁴C aminopyrine accumulation.

Results—N^α-methyl-histamine (100 μM) was as potent as histamine (100 μM) in stimulating acid secretion. This effect was reversed by ranitidine indicating it was mediated via the H₂ receptor. N^α-methyl-histamine potentiated the effects of both carbachol (increased by 280%) and gastrin (by 350%) (p<0.01). N^α-methyl-histamine had no inhibitory actions on forskolin or carbachol stimulated acid secretion suggesting that there is not an inhibitory H₂ receptor located directly on the parietal cell.

Conclusions—Bacterially produced N^α-methyl-histamine directly stimulates acid secretion by parietal cells and this may contribute to the increased acid secretion that contributes to duodenal ulceration.

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Keywords: *Helicobacter pylori*, parietal cell, N^α-methyl-histamine, H₂ histamine receptor, H₃ histamine receptor, acid secretion.

The recent discovery that *Helicobacter pylori* produces N^α-methyl-histamine¹ might explain several aspects of the pathophysiology of this infection, but the overall effect of N^α-methyl-histamine on gastric function is unclear. N^α-methyl-histamine is a potent histamine H₂ receptor agonist.² Stimulation of H₂ receptors on enterochromaffin-like (ECL) cells has an

inhibitory effect, which could explain the decreased expression of histidine decarboxylase and low mucosal histamine levels found in *H. pylori* infected gastric mucosa.³⁻⁵ The effect of histamine released from ECL, or mast cells, on the H₂ receptors on parietal cells plays a crucial part in the stimulation of acid secretion, and in the response to gastrin.^{6,7} Therefore suppression of histamine release by N^α-methyl-histamine might contribute to pathophysiological phenomena such as the temporary loss of acid secretion on first infection,^{8,9} and variations in the sensitivity of the acid secreting mucosa to gastrin in established infection.¹⁰⁻¹³ The important inhibitory effect of the highly selective H₂ receptor agonist R^α methyl-histamine on acid secretion has been demonstrated in intact animals and in a preparation of rabbit gastric glands.¹⁴⁻¹⁸ Interestingly in the gastric gland preparation⁴ R^α methyl-histamine actually inhibited acid secretion induced by both histamine and carbachol in a ranitidine insensitive manner, which led Bado *et al* to postulate the presence of inhibitory H₂ receptors on the parietal cells themselves.⁴ Therefore the first aim of this study was to test this hypothesis in a preparation of isolated parietal cells.

It seems that stimulation of H₂ receptors inhibits acid secretion, but infusion of N^α-methyl-histamine itself into intact animals has the opposite effect and increased acid secretion.¹⁹⁻²¹ The basis for this stimulation has not been fully established. It may be because N^α-methyl-histamine also has H₂ receptor agonist activity.²² Clearly if N^α-methyl-histamine acts predominantly as an H₂ receptor agonist in *H. pylori* infection its potential role in pathophysiology is different and N^α-methyl-histamine might contribute to the increased basal and stimulated acid secretion and increased sensitivity to gastrin seen in duodenal ulcer patients. Therefore the second aim of this study was to examine the H₂ agonist activity of N^α-methyl-histamine on parietal cells.

Methods

Materials

Histamine, carbachol (carbamyl chloride), gastrin-17, Earle's balanced salt solution (EBSS), bovine serum albumin (BSA), collagenase type I, collagenase type H, dithiothreitol, penicillin G, crystalline bovine insulin, hydrocortisone,

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streptomycin, gentamicin, dinitrophenol and EDTA were purchased from Sigma (Poole, UK). Triton X-100 and N-2-hydroxyethyl-piperazine-N'-ethansulphonic acid (HEPES) were from BDH-Merck (Poole, UK), N⁶-methyl-histamine was from Calbiochem (Nottingham, UK), and ¹⁴C-dimethylaminopyrine (103 mCi/mmol) was obtained from Amersham International (Amersham, UK). Ham's F12/Dulbecco's modified Eagle's culture media (F12/DMEM) (50:50 vol/vol), glutamine, Hanks's balanced salt solution (HBSS), basal medium Eagle's (BME), and fetal calf serum were obtained from Gibco (Paisley, UK). Basement membrane Matrigel was from Universal Biologicals, London, UK. Ranitidine was from Glaxo (Greenford, UK).

Parietal cell preparation

Rabbit parietal cells were isolated using a modification of previously described methods.²³⁻²⁶ New Zealand white rabbits (2–2.5 kg) were killed by overdose of sodium pentobarbitone and the stomach immediately excised, opened, and washed thoroughly in ice chilled HBSS containing 10 mM HEPES pH 7.4, 100 mg/l streptomycin, 100 mg/l penicillin G, and 0.1% BSA. The antrum and body segments were separated and then the oxyntic mucosa was removed from the submucosa by blunt dissection. After mincing the mucosa into small fragments the mucosa was then subjected to sequential digestion with collagenase and EDTA. Initially fragments were digested for 15 minutes at 37°C in a shaking water bath with type I collagenase (0.175 g/l) and type H collagenase (0.175 g/l) in BME followed by washing for one minute with Ca⁺⁺ and Mg⁺⁺ free EBSS containing 2 mM EDTA and a further 10 minute incubation with Ca⁺⁺ and Mg⁺⁺ free EBSS containing 1 mM EDTA. The cells liberated from these steps were discarded and fragments were then subjected to three further collagenase digestion steps for 15, 60, and 30 minutes respectively. The cells liberated from each of these three steps were filtered through 240 µm mesh (Lochertex, Warrington, UK) to remove undigested fragments and then washed three times with HBSS to remove remaining collagenase and then resuspended in HBSS buffer containing 0.4 mM dithiothreitol and saved at room temperature for elutriation. Cells were filtered through 64 µm mesh (Lochertex) immediately before elutriation.

Enrichment for parietal cells was carried out using a Beckman JE 5.0 elutriator rotor using the standard elutriation chamber. The system was sterilised before use by circulating 70% ethanol for 15 minutes, followed by 500 ml sterile water and 500 ml HBSS buffer. Crude cells were diluted to 3–4 million/ml and 75–100 million cells were loaded on each elutriator run. Cells were loaded at 2600 rpm at a flow rate of 21.5 ml/min. Two fractions (150 ml each), those from 2500 rpm and 21.5 ml/min and 2000 rpm and 40 ml/min respectively were discarded, these contain

bacteria, debris, and contaminating small cells. The parietal cell enriched fractions were then collected at 2000 rpm and a flow rate of 60 ml/min and 2000 rpm and 100 ml/min. HBSS containing 0.1% BSA, 10 mM HEPES, and antibiotics as above was used as the carrier and elutriation buffer. After elutriation cell viability as assessed by trypan blue exclusion always exceeded 95% and enriched preparations contained 60–70% parietal cells.

Cell culture

Cells from the parietal cell enriched fractions were collected by brief centrifugation and pooled and resuspended in culture medium (Ham's F12/DMEM 50/50 nutrient mix, containing 10% heat inactivated fetal calf serum, 10 mM HEPES pH 7.4 100 mg/l gentamicin, 2 mM glutamine, 1 µg/ml hydrocortisone, and 8 µg/ml insulin) and then cells were pre-attached by incubating for 30 minutes at 37°C in a 75 cm² Corning tissue culture flask that had been precoated with culture medium for six hours. This preincubation step removes the majority of contaminating fibroblasts, parietal cells take several hours to attach to this surface and are therefore recovered after this short preattachment step.²⁶ Parietal cells were then plated onto Matrigel coated 12 well tissue culture plates (Corning) at 0.5 million cells/well. Matrigel coated plates had been prepared by diluting Matrigel 1:9 with sterile water and then uniformly coating the wells. After setting with overnight incubation at 37°C, the remaining water was aspirated and wells were allowed to dry in a culture hood for 60 minutes. The plates were then equilibrated with culture medium before plating the cells.

The parietal cell enriched fraction was then cultured at 37°C in an atmosphere of 5% CO₂/95% air for 40 hours.

Intracellular accumulation of ¹⁴C-dimethylaminopyrine was used as a measure of functional acid secretory activity.²⁵⁻²⁷ Cultured cells were washed once with 2 ml EBSS containing 0.1% BSA, 10 mM HEPES pH 7.4, 2 mM glutamine, 0.22% NaHCO₃ to remove dead and non-adherent cells and then 1 ml of the above medium was added and 0.04 µCi aminopyrine and the various test substances were added simultaneously to each well. Each condition was tested in triplicate. Cells were then incubated for 30 minutes at 37°C in an atmosphere of 5% CO₂/95% air. Incubations were terminated by removing the medium from each well using a vacuum pump and then washing twice with 1 ml EBSS solution. Cells were then lysed with 1 ml 1% triton X-100. Aliquots of cell lysates and incubation media were then counted in Optiphase Safe (Wallac, Milton Keynes, UK) using a Beckman LS 1801 liquid scintillation counter with DPM correction. Aminopyrine accumulation ratios were calculated as previously described.^{25, 26} Dinitrophenol (0.1 mM) was added to separate wells to assess non-specific incorporation and values were subtracted from test values.²³

Statistical analysis

All data represent the sum of five to eight animal preparations. Data are expressed as mean (SEM) and normalised to the maximal histamine or forskolin output for that preparation as appropriate to correct for differences in separate animal preparations. Student's paired *t* test was used for analysis. A *p* value of <0.05 was regarded as significant.

Results

N^α-methyl-histamine stimulated aminopyrine accumulation in cultured parietal cells in a dose dependent manner (Fig 1). Basal, unstimulated AP accumulation was an AP ratio of 454 (117) and this rose to 1770 (263) (mean (SEM)) (*p*<0.001) after stimulation with N^α-methyl-histamine (10⁻⁴ M, which was 116 (14.9)% of the maximal stimulation obtained with histamine (10⁻⁴ M: 100% or an AP ratio of 1589 (50)). N^α-methyl-histamine concentrations of 10⁻⁴ to 10⁻⁶ M (*p*<0.001) and 10⁻⁷ M (*p*<0.05) all produced a significant stimulation of AP accumulation, the EC₅₀ was 3.9×10⁻⁷ M. The stimulatory effects of N^α-methyl-histamine were antagonised by the H₂ receptor antagonist ranitidine (Fig 1) in a dose dependent manner. Ranitidine at a concentration of 10⁻⁷ M shifted the dose response curve to the right (*p*<0.01) and 10⁻⁵ M completely abolished the stimulatory effects of N^α-methyl-histamine (*p*<0.01), consistent with the stimulatory action being mediated via the H₂ receptor. In keeping with this there was no antagonism of the effects of N^α-methyl-histamine with the muscarinic antagonist atropine and similar displacement of the dose response curve occurred with the alternative H₂ antagonist famotidine (data not shown).

N^α-methyl-histamine also produced a dose dependent enhancement of the stimulation of acid secretion seen with both carbachol (10⁻⁴ M) and gastrin (10⁻⁷ M). The stimulatory

effect of carbachol alone (AP ratio 1212 (269) or 62% of the maximal response of corresponding histamine maximal stimulation) was augmented in a dose dependent manner by N^α-methyl-histamine: addition of N^α-methyl-histamine 10⁻⁷ to 10⁻⁴ M led to an increase in acid secretion, which was greater than an additive response to the two agents separately (Fig 2) (*p*<0.01). Similarly the relatively weak acid stimulatory response of gastrin alone was potentiated by N^α-methyl-histamine (Fig 2) (*p*<0.01). This potentiating effect was also abolished by ranitidine (10⁻⁵ M) indicating it was mediated via the H₂ receptor (Fig 2). As would be expected from this action at the H₂ receptor the addition of N^α-methyl-histamine to maximal doses of either histamine or the adenylate cyclase activator forskolin did not lead to any further increase in acid secretion (not shown). Ranitidine alone had no effect on either the basal AP accumulation or the stimulatory effects of carbachol and gastrin, suggesting that these secretagogues were acting directly on the parietal cell and that in this enriched preparation there was no stimulation via the intermediary of histamine secreting cells.

To investigate the potential role for an inhibitory H₃ histamine receptor subtype in regulation of parietal cell function acid secretion was measured in cells simultaneously exposed to concentrations of N^α-methyl-histamine of 10⁻⁹ to 10⁻⁷ M and stimulated with either carbachol (10⁻⁴ M) or forskolin (10⁻⁵ M) and in the presence or absence of ranitidine (10⁻⁷ M). The H₃ receptor is known to be of much higher affinity than either the H₁ or H₂ types^{2,22} and that N^α-methyl-histamine is an extremely potent agonist,² thus the doses used in this part of the study would be selective for the H₃ receptor.² Additionally ranitidine at this concentration has been shown to have no effect on binding of N^α-methyl-histamine to H₃ receptors in the brain and would also prevent any interaction with the H₂ receptor. As shown in Figure 3 there was a degree of potentiation of carbachol stimulated acid secretion with N^α-methyl-histamine 10⁻⁷ M but this was

Figure 1: Aminopyrine accumulation over 30 minutes in isolated, cultured parietal cells after stimulation with N^α-methyl-histamine and the inhibitory actions of ranitidine (10⁻⁷ and 10⁻⁵ M) on N^α-methyl-histamine induced AP accumulation. ****p*<0.001 v basal, **p*<0.05 v basal, ***p*<0.01 v N^α-methyl-histamine stimulated, (mean (SEM), *n*=6-8).

Figure 2: Effect of increasing concentrations of N^α-methyl-histamine on the aminopyrine uptake over 30 minutes induced by carbachol (10⁻⁴ M) and gastrin (10⁻⁷ M) and the effect of adding ranitidine (10⁻⁵ M) ***p*<0.01 v carbachol or gastrin alone (mean (SEM), *n*=5).

Figure 3: Effect on aminopyrine uptake of adding N^α-methyl-histamine (10^{-5} – 10^{-7} M) to forskolin (FSK: 10^{-5} M) or carbachol (CBH: 10^{-4} M) and the effect of ranitidine (RAN: 10^{-7} M) on these combinations. ** $p < 0.01$ v carbachol alone, (mean (SEM), $n=5$).

blocked by ranitidine and there was no evidence of any inhibition of the acid secretion using these doses of N^α-methyl-histamine when cells were stimulated by activation of the cholinergic receptor (carbachol) or directly activating adenylate cyclase (forskolin).

Discussion

These studies in isolated parietal cells confirm that N^α-methyl-histamine is a potent directly acting acid secretagogue. It was almost as potent as histamine itself. This stimulatory action was antagonised by H₂ blockers suggesting it was mediated via this receptor. Previous studies have shown that N^α-methyl-histamine is a potent secretagogue when infused in vivo in animals, at least as potent as histamine,^{19–21} although the effect on isolated parietal cells has not been previously studied. Although recognised as a potent H₃ agonist it is clear that N^α-methyl-histamine is relatively non-selective in its actions and it has been shown previously to activate H₂ receptors in preparations of cardiac atria.^{28–29} Thus in this parietal cell preparation the dominant effect seems to be stimulation via the H₂ receptor and suggests any N^α-methyl-histamine formed in vitro in the *H. pylori* infected gastric body is likely to lead to increased gastric acid secretion.

N^α-methyl-histamine also showed potentiation of the actions of carbachol and gastrin, which were also dependent on the H₂ receptor. Such potentiation by stimulating with agents that act via different intracellular pathways has been shown previously for histamine (which predominantly activates adenylate cyclase and increases intracellular cAMP) with carbachol and gastrin (which act via the Ca²⁺/inositol phospholipid),³⁰ thus it is not surprising that N^α-methyl-histamine also shows such response. Although the recent cloning of the gastrin/CCK_B receptor from parietal cells has

clearly shown that gastrin receptors are expressed in parietal cells³¹ as well as ECL/mast cells, it is typical of in vitro preparations to show relatively weak acid secretory response to gastrin alone, as was exhibited here.

Characteristically this response to gastrin can be enhanced by also increasing the intracellular levels of cAMP, although gastrin itself does not activate adenylate cyclase,³² this may be one basis for the amplification of the acid response to gastrin in vivo produced by gastrin also stimulating the ECL cell to release histamine.

We have used the cultured parietal cell system for studying acid secretion by the parietal cell. This has been used to characterise the responses of the parietal cell to physiological secretagogues and our data confirm those of previous studies that both carbachol and gastrin can directly stimulate acid secretion in a H₂ receptor antagonist insensitive manner and cimetidine had no effect on basal secretion in cultured rabbit parietal cells.²⁶ This suggests that the level of contamination by histamine secreting cells is not significantly changing the acid secretory responses obtained.

The relevance of these findings to the pathophysiology of *H. pylori* infection is apparent in that *H. pylori* infection is usually associated with a reduction in mucosal histamine content and down regulation of histidine decarboxylase (HDC) activity, which is the rate limiting, regulatory step in histamine biosynthesis.^{1–5, 33–34} This reduction could represent depletion due to excessive release but the reduction in HDC actually suggests this is due to reduced synthesis. This reduction may be brought about by N^α-methyl-histamine acting on H₃ receptors to inhibit histamine biosynthesis and release,³ although both inflammatory cytokines³⁵ and *H. pylori* itself³⁶ may be capable of inhibiting histamine release. A reduction in histamine release would be expected to decrease the acid secretion seen after stimulation with gastrin either endogenously (meal or gastrin releasing peptide stimulated) or exogenously infused, however it is apparent that parietal cell sensitivity is usually unimpaired or even increased in *H. pylori* infection.^{10–12} Thus it is possible that N^α-methyl-histamine produced by *H. pylori* and detectable in gastric mucosa and gastric juice is actually providing a degree of stimulation of acid secretion and sensitising the parietal cells to gastrin. N^α-methyl-histamine is not a selective histaminergic agonist and it has been shown to be a potent agonist at the H₃ receptor (K_i for inhibition of [³H] N^α-methyl-histamine binding is 0.4 nM compared with 14 nM for histamine and 1.2 nM for (R)-methyl-histamine² although it is not as selective as (R)-methyl-histamine for the H₃ receptor.²⁹ The second aim of this study was to examine the potential regulation of the parietal cell by the H₃ receptor using N^α-methyl-histamine as the agonist. The results do not support the hypothesis that there is actually an inhibitory H₃ receptor located on the parietal cell itself.⁴ This idea originated following the observation that (R)-methyl-

histamine inhibited carbachol induced acid secretion in gastric glands in a ranitidine insensitive manner, however in our parietal cells there was no inhibitory activity apparent using concentrations of N^α-methyl-histamine, which are known to be effective at the receptor in other tissues,¹⁸ even when any activity at the H₂ receptor was blocked. One theoretical reason why we were unable to demonstrate H₃ receptor activity in our preparation could be due to damage or loss of the receptor during the isolation procedure, this is unlikely to be the case because we have used cells cultured for 40 hours before experimentation, which should have permitted regeneration of functional receptors.²⁶ Thus our findings are consistent with most in vivo data, which demonstrate that H₃ agonists are much more effective in inhibiting indirect stimulants of gastric acid secretion such as gastrin, bombesin, and 2-deoxyglucose than histamine, which acts directly on the parietal cell.¹⁴⁻¹⁷ This would be consistent with the H₃ inhibitory receptor being located on the histamine secreting cell and possibly pre-synaptically on cholinergic neurones.¹⁸ Further study and characterisation of the role of the H₃ receptor in regulating gastric acid secretion is likely to be facilitated by the newest generation of receptor antagonists, even more specific than thioperamide, which are only just becoming available.¹⁸

Therefore it is apparent that N^α-methyl-histamine produced by *H. pylori* in the stomach may have a part to play in the regulation of gastric acid secretion, it is a direct stimulant of parietal cells and sensitises them to other stimulants, equally it can inhibit histamine synthesis and release, which would impair acid secretion.¹⁸ It may also have a role in regulating somatostatin cell function although as yet there are no data confirming this in the gastric body.^{18, 37} The balance of these may determine the different *H. pylori* associated disease outcomes.

The values of N^α-methyl-histamine that are effective in stimulating the H₂ receptor (10⁻⁷ to 10⁻⁴ M) may be higher than those detected in vivo (approximately 10–12/mg protein)¹ and thus the acid stimulatory responses may not be biologically important. However this overall figure may not reflect the values actually found in the exact vicinity of the receptors. Courillon-Mallet *et al* found that mucosal levels of N^α-methyl-histamine were only approximately 10-fold lower than those for histamine in *H. pylori* positive patients and as it can be assumed that a significant proportion of this histamine is intracellular and that the N^α-methyl-histamine is not within the ECL cells and actually bound to specific binding sites,¹ it is clearly not inconceivable that N^α-methyl-histamine may have stimulatory actions on acid secretion as enhancement of acid secretion was found at N^α-methyl-histamine concentrations as low as 10⁻⁷ M. Additionally studies of the role of the H₃ receptor in controlling somatostatin secretion have demonstrated effects with R^α-methyl-histamine at 10⁻⁶ M³⁷ and until more information is available on the active concentrations

of histaminergic agonists within gastric tissues the understanding of the relative roles of these receptors will remain incomplete. As yet there have been no significant studies concerning the pattern of N^α-methyl-histamine concentrations in different *H. pylori* associated disease states, nor on the distribution of N^α-methyl-histamine synthesising ability in different clinical *H. pylori* isolates. Such studies are awaited with interest and will probably further our knowledge of the role of N^α-methyl-histamine and the H₃ receptor in regulation of gastric acid secretion in disease states.

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