

## HELICOBACTER PYLORI

# Effects of *N*-alpha-methyl-histamine on human H<sub>2</sub> receptors expressed in CHO cells

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*Gut* 2002;50:786–789

**Background:** Production of *N*-alpha-methyl-histamine (NAMH), a histamine H<sub>3</sub> receptor (H3R) agonist, is reportedly promoted in *Helicobacter pylori* infected human gastric mucosa. NAMH was suggested to act directly on histamine H<sub>2</sub> receptors (H2Rs) in animals to stimulate acid secretion and to be a H2R agonist. As H2Rs and H3Rs play different roles in gastric acid secretion, it is very important to verify that NAMH is a H2R agonist.

**Aims:** To determine whether NAMH is a H2R agonist, as well as a H3R agonist.

**Methods:** We used a Chinese hamster ovary (CHO) cell line expressing human H2Rs (CHO-H2R) and control CHO cells. Expression of human H2Rs was confirmed by tiotidine binding. cAMP production in CHO-H2R and control cells in response to histamine or NAMH was measured. cAMP production in response to 10<sup>-7</sup> M NAMH was also measured in the presence or absence of the H2R antagonist famotidine and the H3R antagonist thioperamide.

**Results:** NAMH dose dependently stimulated cAMP productions in CHO-H2R cells. This production was inhibited by famotidine but not by thioperamide. Control CHO cells were unresponsive to either histamine or NAMH. In addition, the effect of NAMH, in terms of cAMP production in CHO-H2R cells, was more potent than that of histamine—that is, with a lower EC<sub>50</sub> concentration and higher maximal cAMP production. Both NAMH and histamine, but not *R*-alpha-methyl-histamine, effectively inhibited [<sup>3</sup>H] tiotidine binding to CHO-H2R cells.

**Conclusions:** NAMH, which is produced in the gastric mucosa by *H pylori*, is a potent H2R agonist as well as a H3R agonist.

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Accepted for publication 25 July 2001

**N**-alpha-methyl-histamine (NAMH) content was reportedly increased in *Helicobacter pylori* infected gastric mucosa.<sup>1</sup> NAMH has been, and is still, considered a histamine H<sub>3</sub> receptor (H3R) agonist.<sup>2–6</sup> Activation of H3Rs is reportedly involved in inhibition of gastric acid secretion.<sup>7–10</sup> In contrast, stimulation of H3Rs reportedly increased acid secretion by suppressing somatostatin secretion<sup>11,12</sup> or had no effects on gastric acid secretion.<sup>13</sup> Thus NAMH produced by *H pylori*, as a H<sub>3</sub> agonist, can exert various effects on gastric acid secretion.

Interestingly, Lin *et al* reported that NAMH had stimulatory effects on gastric acid secretion in vivo which were more potent than those caused by histamine.<sup>14</sup> NAMH also had stimulatory effects on acid secretion in isolated rabbit gastric glands and parietal cells in a histamine H<sub>2</sub> receptor (H2R) specific manner.<sup>15,16</sup> This suggests that NAMH can interact directly with the H2R on gastric parietal cells as a H2R agonist. In addition, some evidence implying that NAMH is also a H2R agonist has been presented.<sup>17,18</sup> Despite these reports, NAMH was still used as a H3R agonist in some studies.<sup>2–6</sup> As H3Rs and H2Rs have different effects on gastric acid secretion and NAMH is produced by *H pylori*, it is important to determine whether NAMH is also a H2R agonist.

In this study, we attempted to clarify whether NAMH is a H2R agonist, focusing solely on NAMH and human H2Rs using CHO cells expressing human H2Rs. Herein, we present direct evidence that NAMH is a potent H2R as well as a H3R agonist.

## MATERIALS AND METHODS

### Materials

NAMH was purchased from Calbiochem Co., Ltd (UK). [<sup>3</sup>H] tiotidine was purchased from DuPont-NEN (Boston, Massachusetts, USA). Famotidine, thioperamide, and *R*-alpha-

methyl-histamine were purchased from Sigma (St Louis, Missouri, USA).

### Preparation of the human histamine H2R gene and expression in CHO cells

A polymerase chain reaction was carried out using oligonucleotides corresponding to the full length of the human H2R gene and 100 ng of human genomic DNA.<sup>19</sup> A fragment obtained was subcloned into TA vector and the sequence was confirmed. The fragment in a TA vector encompassing the whole coding region was ligated into the expression vector pCAGGS and transfected into Chinese hamster ovary (CHO) cells by the calcium phosphate precipitation method, as described previously.<sup>20</sup> Several cell lines were selected by their resistance to 600 µg/ml of G418 (Gibco-BRL, Invitrogen Corp, Carlsbad, California, USA), a neomycin derivative. A clone termed CHO-H2R and control CHO cells, transfected with the vector alone, were used for the following experiments.

### Ligand binding assay on intact cells

CHO cells, grown in 24 well plates, were assayed at a density of 1×10<sup>5</sup> cells/well. The cells were incubated for 1.5 hours at 37°C in 200 µl of HEPES-Tyrode's buffer containing 5 nM [<sup>3</sup>H] tiotidine with or without increasing concentrations of unlabelled tiotidine.<sup>21–24</sup> All samples were analysed in triplicate. After incubation, cells were washed three times with ice cold phosphate buffered saline, removed from the wells in 0.1% sodium dodecyl sulphate, and radioactivity was determined by liquid scintillation counting.

**Abbreviations:** CHO, Chinese hamster ovary; H2R, histamine H<sub>2</sub> receptor; H3R, histamine H<sub>3</sub> receptor; NAMH, *N*-alpha-methyl-histamine.

### Measurement of cAMP production

CHO cells on 24 well plates, serum starved for 12 hours, were incubated for 30 minutes at 37°C in 450 µl of HEPES-Tyrodé's buffer containing 0.1% bovine serum albumin and 0.1 mM 3-isobutyl-1-methylxanthine, and 50 µl of histamine or NAMH solution was added to initiate the reaction. After 10 minutes of incubation at 37°C, the reaction was terminated by addition of 500 µl of 12% trichloroacetic acid. Samples were centrifuged for five minutes at 10 000 *g* at 4°C. Following extraction of the supernatants three times with diethylether, cAMP content was measured by radioimmunoassay.<sup>20, 21</sup>

### Statistical analysis

Quantitative values are expressed as mean (SEM). Statistical significance was tested using the unpaired *t* test (two tailed). A value of *p* < 0.05 was considered significant.

## RESULTS

### Expression of human H2Rs in CHO cells

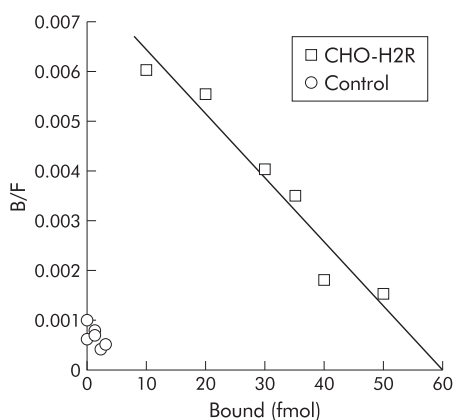
Transfection of CHO cells with an expression vector containing human H2R cDNA resulted in adequate expression of human H2Rs. Scatchard plot analysis of tiotidine binding data showed that CHO-H2R expressed 60 fmol/10<sup>5</sup> cells (fig 1). No specific tiotidine binding was observed in control CHO cells.

### Histamine or NAMH dependent cAMP production in CHO cells

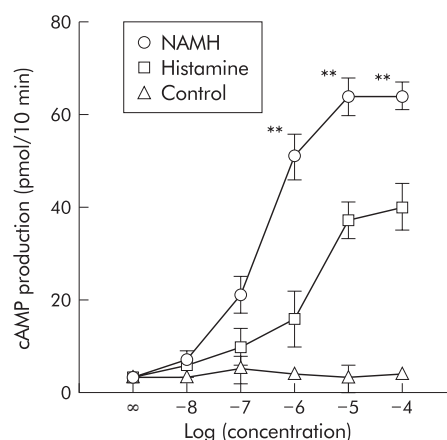
cAMP production in response to histamine or NAMH was measured in intact CHO cells. Interestingly, both histamine and NAMH dose dependently stimulated cAMP production in CHO-H2R cells (fig 2). Furthermore, NAMH stimulated cAMP production in CHO-H2R cells with a lower EC<sub>50</sub> value (10<sup>5.99 ± 0.03</sup> M, *n* = 15; *p* < 0.001 *v* histamine 10<sup>6.48 ± 0.04</sup> M, *n* = 15) and higher maximal cAMP production than histamine (fig 2). Control CHO cells were unresponsive to both histamine and NAMH. Thus it is likely that NAMH acted directly on human H2Rs expressed in CHO cells.

### Effects of a H2R antagonist or a H3R antagonist on cAMP productions in CHO cells

To determine whether the cAMP response induced by NAMH is mediated via H2Rs, NAMH induced cAMP production in the presence of the H<sub>2</sub> receptor antagonist famotidine or the H<sub>3</sub> receptor antagonist thioperamide was measured. CHO-H2R cells were preincubated for 30 minutes with increasing



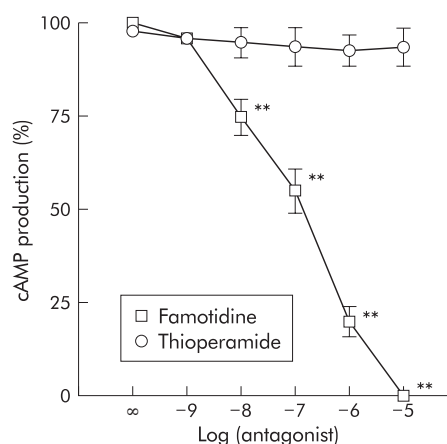
**Figure 1** Expression of human histamine H<sub>2</sub> receptors (H2Rs) in Chinese hamster ovary (CHO) cells; tiotidine binding to CHO-H2R and control CHO cells. CHO cells on 24 well plates were incubated in the presence of 5 nM [<sup>3</sup>H] tiotidine and increasing concentrations of unlabelled tiotidine at 37°C for 1.5 hours. Data are shown as Scatchard plots and expressed as fmol of bound tiotidine per 1 × 10<sup>5</sup> cells. The results were consistent with a single binding site, and the lines were drawn on the basis of linear regression analysis.



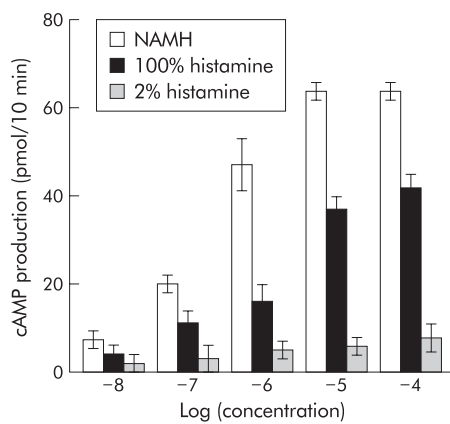
**Figure 2** *N*-alpha-methyl-histamine (NAMH) and histamine stimulated cAMP production in Chinese hamster ovary-histamine H<sub>2</sub> receptor (CHO-H2R) and control CHO cells. CHO cells were incubated with various concentrations of histamine at 37°C for 10 minutes. cAMP content was measured by radioimmunoassay. Measurements were performed in triplicate. Data shown are representative of three independent experiments. Results are expressed as mean (SEM). \*\**p* < 0.01, NAMH compared with histamine.

concentrations of famotidine or thioperamide, and NAMH solution (at a final concentration of 10<sup>-7</sup> M) was then added to initiate the reaction. cAMP produced during the 10 minute incubation period was measured. As shown in fig 3, famotidine but not thioperamide dose dependently inhibited cAMP production in response to 10<sup>-7</sup> M NAMH. It is significant that thioperamide had no inhibitory effect on NAMH dependent cAMP production. Taken together, these results indicate that NAMH has H<sub>2</sub> receptor agonistic activity which is more potent than histamine. Thus NAMH is a H2R as well as a H3R agonist.

The NAMH reagent used in this study had a purity of at least 98%. Thus although unlikely, it is possible that the reagent contained a maximum of 2% impurity. As NAMH is produced from histamine, we were aware that the reagent we used contained histamine and that the above observations could be attributable to residual histamine. To exclude this



**Figure 3** Effects of famotidine and thioperamide on 10<sup>-7</sup> M *N*-alpha-methyl-histamine (NAMH) induced cAMP production. Chinese hamster ovary-histamine H<sub>2</sub> receptor (CHO-H2R) cells were incubated in the presence of various concentrations of famotidine or thioperamide, and 10<sup>-7</sup> M NAMH for 10 minutes. cAMP content was measured by radioimmunoassay. Measurements were performed in triplicate. Data shown are representative of three independent experiments. Results are expressed as mean (SEM). \*\**p* < 0.01, NAMH compared with thioperamide.

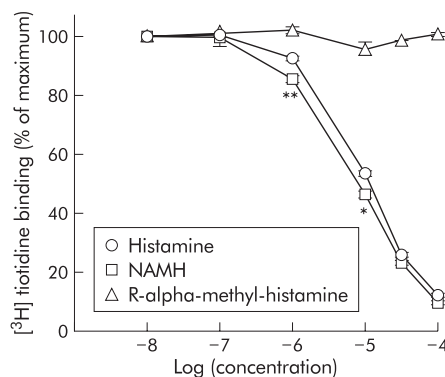


**Figure 4** cAMP productions in response to various concentrations of *N*-alpha-methyl-histamine (NAMH), and 100% or 2% histamine. Measurements were performed in triplicate, and the results are expressed as mean (SEM) of three independent experiments.

possibility, we also performed an experiment in which 2% histamine solution was used to stimulate cAMP production instead of NAMH. As shown in fig 4, the effect of 2% histamine solution on cAMP production was minimal compared with those of NAMH and 100% histamine. If the NAMH reagent does contain 2% histamine, cAMP production attributable to NAMH can be obtained by subtraction of each cAMP value (NAMH–2% histamine), which is far greater in amount than that obtained with 100% histamine. This indicates that NAMH per se has H2R agonistic activity which is more potent than histamine.

#### Effect of histamine, NAMH, or *R*-alpha-methyl-histamine on [<sup>3</sup>H] tiotidine binding

To confirm that NAMH binds directly to the histamine H<sub>2</sub> receptor, the effect of NAMH on [<sup>3</sup>H] tiotidine binding was examined. CHO-H2R cells were incubated with 5 nM [<sup>3</sup>H] tiotidine and increasing concentrations of histamine, NAMH, or the specific H3R agonist *R*-alpha-methyl-histamine at 37°C for 1.5 hours. As shown in fig 5, both histamine and NAMH but not *R*-alpha-methyl-histamine inhibited [<sup>3</sup>H] tiotidine binding. This demonstrates that NAMH directly interacts with the H2R. At concentrations of 10<sup>-5</sup> and 10<sup>-6</sup> M, histamine was slightly but significantly more potent than NAMH in inhibiting [<sup>3</sup>H] tiotidine. Importantly, it was proved that *R*-alpha-



**Figure 5** Effect of histamine, *N*-alpha-methyl-histamine (NAMH), and *R*-alpha-methyl-histamine on [<sup>3</sup>H] tiotidine binding. Chinese hamster ovary-histamine H<sub>2</sub> receptor (CHO-H2R) cells were incubated in the presence of [<sup>3</sup>H] tiotidine and increasing concentrations of histamine, NAMH, or *R*-alpha-methyl-histamine at 37°C for 1.5 hours. Measurements were performed in triplicate, and the results are expressed as mean (SEM) of three independent experiments. \**p*<0.05, \*\**p*<0.01, NAMH compared with histamine.

methyl-histamine did not interact with the H2R. Similar results were obtained in the presence of 100 μM 5'-guanylylimidodiphosphate, a non-hydrolysable GTP analogue which uncouples G proteins from their receptors (data not shown).

#### DISCUSSION

NAMH has been detected in animal gastric mucosa and has been implicated in gastric acid secretion.<sup>25</sup> As with human gastric juice, NAMH was detected in the gastric juice of some but not all individuals tested. However, the origin of its production was unclear.<sup>26</sup> The report by Courillon-Mallet *et al* first revealed NAMH content to be elevated specifically in the gastric mucosa infected with *H pylori*.<sup>1</sup> Considering the pathogenic role of *H pylori* infection in peptic ulcer, it was also suggested that NAMH produced by *H pylori* affected gastric acid secretion.<sup>1 14 16</sup> H3R is present in enterochromaffin-like cells and is reportedly involved in suppression of histamine release and gastric acid secretion.<sup>7-10</sup> In contrast, H3R agonists reportedly increased acid secretion by indirectly suppressing somatostatin secretion from D cells.<sup>11 12</sup> Therefore, it has yet to be determined how NAMH detected in *H pylori* infected gastric mucosa affects gastric acid secretion as a H3 agonist.

Interestingly, some reports suggest that MAMH is as a H2R as well a H3R agonist.<sup>14-18</sup> Because H3Rs and H2Rs play different roles in terms of gastric acid secretion, it is important to determine whether NAMH is a H2R as well a H3R agonist. Experiments performed in vivo or using cells or tissues from animal models can be affected by various factors. In this study, focusing solely on human histamine H2Rs, we used human H2Rs expressed in CHO cells. We were able to show that NAMH interacts with human H2Rs in a H2R specific manner. Interestingly, NAMH was significantly more potent than histamine in terms of stimulating cAMP production via the H2R with a lower EC<sub>50</sub> value (approximately one third the value of histamine) and higher maximal cAMP production. Thus NAMH appears to be more potent than histamine in terms of stimulating cAMP production. However, these data may not be directly applicable to acid secretion in vivo as in native cells it is likely that other counterregulatory systems are also activated. Indeed, other data using isolated gastric cells suggest that NAMH and histamine have approximately equal effects on the H2R.<sup>16 17</sup> Animal data in vivo or using gastric glands however indicated that NAMH was more potent in terms of acid secretion than histamine.<sup>14 15</sup>

Regarding inhibition of [<sup>3</sup>H] tiotidine binding, NAMH was slightly less potent than histamine. However, it is important that NAMH stimulated cAMP production more potently via the H2R because cAMP production occurs via direct binding of H2R agonists to the receptor and cAMP production is the central function of the H2R. Taken together, these observations indicate that NAMH is also a H2R agonist which is more potent than histamine. Thus NAMH produced by *H pylori*, as a dual H2R/H3R agonist, can affect gastric acid secretion in various ways. Because the effect of NAMH on gastric acid secretion is thus complicated, whether NAMH stimulates or inhibits acid secretion depends on the gastric conditions of each individual. Therefore, the clinical significance of NAMH produced by *H pylori* is that it can exert various effects on gastric acid secretion depending on the gastric conditions. In this study, we have demonstrated the potency of NAMH as a H2R agonist. The potency of NAMH as a H3 agonist may be demonstrable using expressed H<sub>3</sub> receptors in CHO cells.

H3R is expressed not only in the stomach but also in various tissues, such as the central nervous system. NAMH, a strong H3R agonist, has been used in a wide variety of research fields.<sup>2-6 27 28</sup> However, considering the finding that NAMH is a dual H<sub>2</sub>/H<sub>3</sub> agonist, we must be very cautious in using NAMH for studying H3Rs in vivo. More specific H3R agonists such as

R-alpha methyl-histamine may be more appropriate for studying the H<sub>3</sub>R based on the findings of this study.

In conclusion, we have shown that NAMH, which is thought to be a H<sub>3</sub>R agonist, is also a H<sub>2</sub>R agonist and that NAMH is more potent than histamine. This finding is important for understanding the gastric acid secretion mechanism associated with *H pylori* infection as well as for the study of the H<sub>3</sub>R in general.

#### ACKNOWLEDGEMENTS

This research was supported in part by a grant (to T Saitoh, No 10670512) from the Ministry of Education, Science and Culture, Japan.

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