World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/2150-5330office
wjgp@wjgnet.com
doi:10.4291/wjgp.v1.i5.154

World J Gastrointest Pathophysiol 2010 December 15; 1(5): 154-165
ISSN 2150-5330 (online)

ORIGINAL ARTICLES

Histamine 3 receptor activation mediates inhibition of acid secretion during *Helicobacter*-induced gastritis

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Supported in part by Public Health Service Grants R37-DK 45729 (JLM), Michigan Gastrointestinal Peptide Research Center Pilot Feasibility Grant P30- DK34933 (YZ) and National Health and Medical Research Council of Australia Grant 350234 (AS)
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Received: May 25, 2010 Revised: November 24, 2010
Accepted: December 1, 2010
Published online: December 15, 2010

Abstract

AIM: To test the hypothesis that histamine 3 receptor (H3R) activation during *Helicobacter* infection inhibits gastric acid secretion *in vivo* and *in vitro*.

METHODS: *Helicobacter felis* (*H. felis*) infected and uninfected C57Bl/6 mice were infused with either PBS or the H3 receptor antagonist thioperamide (THIO) for 12 wk. After treatment, mice were analyzed for morphological changes and gastric acid content. Total RNA was prepared from the stomachs of each group and analyzed for changes in somatostatin and gastrin mRNA abundance by real time-polymerase chain reaction (RT-PCR). Location of H3 receptors in the stomach was analyzed by co-localization using antibodies specific for the H3 receptor and parietal cell marker H^+, K^+-ATPase β subunit.

RESULTS: Inflammation and parietal cell atrophy was observed after 12 wk of *H. felis* infection. Interestingly, treatment with the H3R antagonist thioperamide (THIO) prior to and during infection prevented *H. felis*-induced inflammation and atrophy. Compared to the uninfected controls, infected mice also had significantly decreased gastric acid. After eradication of *H. felis* with THIO treatment, gastric acidity was restored. Compared to the control mice, somatostatin mRNA abundance was decreased while gastrin gene expression was elevated during infection. Despite elevated gastric acid levels, after eradication of *H. felis* with THIO, somatostatin mRNA was elevated whereas gastrin mRNA was suppressed. Immunofluorescence revealed the presence of H3 receptors on the parietal cells, somatostatin-secreting D-cells as well as the inflammatory cells.

CONCLUSION: This study shows that during *H. felis* infection, gastric acidity is suppressed as a consequence of an inhibitory effect on the parietal cell by H3R activation. The stimulation of gastric mucosal H3Rs increases gastrin expression and release by inhibiting release of somatostatin.

Key words: Gastrin; Somatostatin; Histamine; Parietal

cell; *Helicobacter felis*; Nα-methylhistamine; Rα-methylhistamine; Thioperamide

Peer reviewers: Prabhakar Reddy Veerareddy, Professor and Principal, St. Peter's Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda, Warangal, Andhra Pradesh 506001, India; Sharon DeMorrow, PhD, Digestive Disease Research Center, Department of Internal Medicine, Scott and White Hospital and Texas A&M Health Science Center, 702 SW H.K Dodgen Loop, Temple, TX 76504, United States

Zavros Y, Mesiwala N, Waghray M, Todisco A, Shulkes A, Merchant JL. Histamine 3 receptor activation mediates inhibition of acid secretion during *Helicobacter*-induced gastritis. *World J Gastrointest Pathophysiol* 2010; 1(5): 154-165 Available from: URL: http://www.wjgnet.com/2150-5330/full/v1/i5/154.htm DOI: http://dx.doi.org/10.4291/wjgp.v1.i5.154

INTRODUCTION

Histamine 3 receptor (H3R) activation has been implicated as a mediator for altered acid secretion that is observed during *Helicobacter pylori (H. pylori)* infection[1-4]. Courillon-Mallet *et al* first revealed elevated concentrations of H3R agonist Nα-methylhistamine (Nα-MH) in the gastric mucosa of *H. pylori* infected patients[1,5,6]. Nα-MH has an indirect inhibitory effect on acid secretion by inhibiting the acid secretagogue histamine from the ECL-like cells[3,5]. Stimulation of H3Rs on ECL-like cells eventually inhibits histidine decarboxylase (HDC) activity[3,5]. Down-regulation of HDC causes a decrease in the amount of histamine produced and decreased acid secretion[3,5]. Early *in vivo* experiments using H3R agonist Rα-methylhistamine (Rα-MH) also potently inhibits gastric secretion by a number of indirect stimuli[7,8]. However, experiments using *in vitro* techniques are quite controversial. In isolated rat fundic ECL-like cells, Rα-MH is likely to inhibit acid secretion *via* the suppression of histamine[2,4] whereas increased acid secretion secondary to reduced somatostatin secretion is reported in isolated mouse stomach[9]. Overall, the role of H3Rs in the regulation of hormonal and paracrine influences on acid secretion during *Helicobacter* infection is unclear.

The exact cellular location of the H3R in the stomach is not known since its presence is based on pharmacological studies[9,10], thus making it difficult to understand the direct role of the H3R in physiological function. Here, we show that during *H. felis* infection, gastric acidity is suppressed, a response that is mediated by an inhibitory effect on the parietal cell by H3R activation. The stimulation of gastric mucosal H3Rs increases gastrin expression and release by inhibiting release of somatostatin.

MATERIALS AND METHODS

Helicobacter felis culture and animal treatments

Helicobacter felis (*H. felis*) (American Type Culture Collection, Rockville, MD) was inoculated on *Campylobacter* selective agar supplemented with 5% sterile horse blood (BD Diagnostics, Bedford, MA), trimethoprim (5 μg/mL), vancomycin (10 μg/mL) and nystatin (10 μg/mL)[11]. Cultures were incubated for two days in a humidified microaerophilic chamber (BBL Gas System, with CampyPak Plus packs, BD Microbiology, Sparks, MD). *H. felis* was harvested and used to inoculate mouse stomachs by oral intubation. C57BL/6 mice were orally inoculated with a catheter once daily over 3 d with 10^8 *H. felis* organisms per 200 μL of brain heart infusion. Mice were divided into four groups: PBS (n = 8), thioperamide (n = 8), *H. felis* (n = 8) and *H. felis* plus thioperamide (n = 8). For the thioperamide treatment, mice were injected with thioperamide (100 μg/kg per mouse per day, i.p.) during the 12 wk *H. felis* infection subsequent to a 7 d pretreatment.

Quantification of H. felis colonization

A standard curve was generated by extracting total RNA, using Trizol Reagent (Life Technologies, Gaithersburg, MD), from *H. felis* bacterial cultures with densities ranging from 10^3 to 10^9 total bacteria. Total RNA was also isolated from stomach tissue using Trizol Reagent. Primer pairs C97 and C98 were used to amplify the 16S rRNA species that is specific for *Helicobacter* and generates an amplicon of approximately 400 base pairs[12]. PCR amplifications were performed in a total volume of 25 μL, containing 10 × PCR buffer with 10^{-9} moles/L $MgCl_2$, dNTPs, 200 nmol/L primers, 5 μL of cDNA, 10^{-7} moles/L Taq polymerase GOLD and 2.5 μL of Sybr Green (Molecular Probes). Each PCR amplification was performed in duplicate wells in a Biorad I-Cycler (Biorad Laboratories, I-Cycler IQ Real-Time PCR Detection System, Hercules, CA) using the following conditions: 94℃ for 10 min followed by 35 two-temperature cycles (94℃ for 1 min and 55℃ for 1 min).

Infusion of thioperamide to sheep

Merino-Corriedale cross sheep were administered a local anesthetic (2 mL of 1% xylocaine) in the skin surrounding the jugular vein. The method of cannulation involved the insertion of a hypodermic needle into the jugular vein and directed toward the head. The cannula was passed through the hypodermic needle and into the vein. Following removal of the needle, adhesive plaster was used to secure the cannula to the skin with sutures. The same procedure was followed for the contralateral jugular vein except the cannula was directed toward the heart. Each cannula was flushed with 2 mL heparin, capped, wrapped with sterile gauze and tied to the sheep's neck and secured with elastic bandage. The jugular vein cannula directed up was used for blood sampling (10 min intervals) and the cannula directed down for agonist (pentagastrin, Bachem, 5 μg/kg per hour) and antagonist (thioperamide, Sigma, 100 μg/kg per hour) infusions. A recovery period of 24 h was allowed. Sheep were housed in metabolism cages for the duration of the experiment[13].

Table 1 Primer sequences used for the current study

Primer	Sequence (5′ to 3′)
Somatostatin	FW: TGCTGTCCTGCCGTCTCC
	RV: ATCATTCTCTGTCTGGTTGG
Gastrin	FW: ACACAACAGCCAACTATTC
	RV: CAAAGTCCATCCATCCGTAG
Helicobacter	FW: GCTATGACGGGTATCC
	RV: GATTTTACCCCTACACCA
HPRT	FW: AGTCCCAGCGTCGTGATTAGC
	RV: ATAGCCCCCCTTGAGCACACAG

Radioimmunoassays

During the sheep infusions, blood was sampled every 10 min into tubes containing 100 μmol/L Na_2EDTA (10 μL/mL blood), centrifuged at 3000 rpm for 15 min at 4℃ and plasma somatostatin concentration was measured in ethanol extracted plasma by radioimmunoassay (RIA) as previously described[14]. Mice were euthanized and approximately 1 mL of blood was collected by cardiac puncture, aliquoted into tubes containing 100 μmol/L Na_2 EDTA (10 μL/mL blood) and centrifuged at 3000 rpm for 15 min at 4℃. Plasma was collected immediately and stored at -20℃ until assayed for gastrin by RIA as previously described[15].

Gastric acid concentrations

Gastric acidity was measured as previously published[15]. Briefly, after mice were fasted for 16 h the stomachs were opened along the greater curvature and washed with 2 mL normal saline (pH 7.0). The contents were centrifuged at 3000 rpm for 5 min and supernatant was collected. The supernatant was titrated by using 0.005N NaOH and gastric acidity was expressed as μEq.

RNA extraction

RNA extraction was performed on all antral and fundic tissue samples from the mice. Tissue was homogenized with a polytron PT-2000 (Kinematica, Cincinnati, OH) in 1 mL of TRIzol (Sigma) reagent according to the manufacturer's protocol. Once RNA was extracted, it was re-suspended in 50 μL of DEPC H_2O and stored at -80℃.

Quantitative real time-polymerase chain reaction amplification

RNA from all antral and fundic tissue samples underwent quantitative testing using a spectrometer and then was converted to cDNA using the reverse transcriptase kit (Promega). RNA polymerase chain reaction (PCR) amplifications were performed in a total volume of 25 mL containing 10 × PCR buffer with $MgCl_2$, 10 nmol/L dNTPs, 200 nmol/L primers, 5 mL of cDNA, 100 nmol/L Taq polymerase GOLD and 2.5 mL of Sybr green (Molecular Probes). Each PCR amplification was performed in duplicate wells in a Bio-Rad I-Cycler (Bio-Rad, I-Cycler IQ Real-Time PCR Detection System) with the following conditions: 94℃ for 10 min followed by 35 two-temperature cycles (94℃ for 1 minute and 55℃ for 1 min). Sequences for somatostatin, gastrin, *Helicobacter* and HPRT primers are given in Table 1.

Canine parietal- and D-cell culture

Parietal- and somatostatin-secreting D-cells were isolated from canine oxyntic mucosa based on a modified elutriation method by Soll *et al*[16]. The isolated parietal cells (2×10^6 cells/well) were cultured in Ham's F-12/Dulbecco's modified Eagle's medium (1:1) containing 0.1 mg/mL gentamicin, 50 U/mL penicillin G, 0.01 mg/mL ciprofloxacin and 2% DMSO (Sigma) on either 35 mm culture dishes or chamber slides coated with 150 or 50 μL of growth factor reduced Matrigel (BD Biosciences) respectively[17]. The cultures were 95% to 98% homogenous for parietal cells[18]. The elutriated fraction containing D-cells was plated onto Matrigel (2.0×10^6 cells/well) in culture medium containing Dulbecco's modified Eagle's medium: Ham's F-12 medium (50:50) supplemented with 10% heat-inactivated dog serum, gentamicin, penicillin, insulin and hydrocortisone[19]. D cells were enriched to 50%-60% purity by washing away non-adherent mucous cells with culture medium and analyzed for the presence of H3 receptors by flow cytometry.

Aminopyrine uptake

Gastric acid secretion was measured according to published methods[17,20]. Briefly, accumulation of the weak base [^{14}C]aminopyrine (Amersham Biosciences) was used to detect acid production by canine parietal cells. Cultured parietal cells were washed once with Earle's balanced salt solution and incubated with 0.1 μCi of [^{14}C]aminopyrine. Cells were then treated with either (Veh, DMSO/PEG), (r)-α-methylhistamine (Pα-MH, 100 nmol/L), thioperamide (100 nmol/L) or histamine (100 μmol/L) for 30 min. Parietal cells were then lysed with 500 μL of 1% Triton X-100 and the radioactivity of the lysate was quantified in a liquid scintillation counter.

Immunofluorescence

A longitudinal section of the mouse stomach (spanning both the fundic and antral regions) was fixed in 4% paraformaldehyde/PBS, paraffin-embedded and sectioned (3 mm). Parietal cells were cultured on Matrigel-coated chamber slides overnight, rinsed and fixed in methanol for 20 min. Antigen retrieval was performed on tissue after deparaffinization by heating the slides for 10 min at 100℃ in 0.01 mol/L sodium citrate. Cells and tissue were then hydrated using PBS/0.01% Triton X-100 for 20 min and then blocked in 20% normal goat serum for an additional 20 min. Slides were incubated with a 1: 50 dilution of histamine 3 (H3) receptor (Santa Cruz Biotechnology) anti-rabbit antibody at 4℃ overnight followed by a 2 h room temperature incubation with 1: 800 dilution of H^+, K^+-ATPase (MBL International, Watertown, MA) anti-mouse antibody. Stomach tissue expressing the H3 receptor were then detected by using a 1: 100 dilution of either anti-rabbit conjugated to FITC or anti-mouse conjugated to Texas red secondary antibodies for 1 h at room tem-

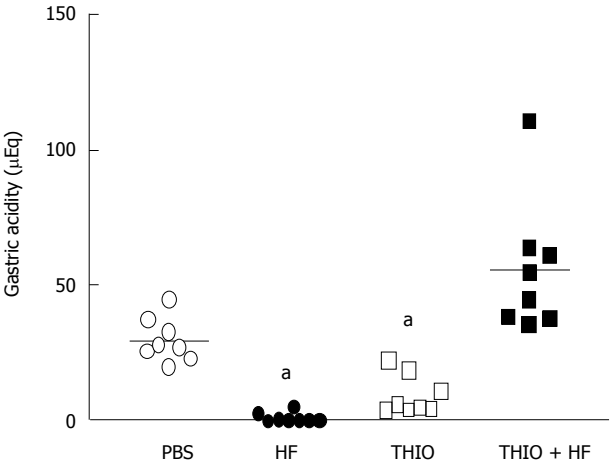

Figure 1 *Helicobacter felis* colonization results in decreased gastric acidity that is resolved with thioperamide treatment. Gastric acidity for PBS, *Helicobacter felis* (HF), thioperamide (THIO) and THIO plus HF infection is shown as a scatter plot for each mouse. $^{a}P < 0.05$ compared to PBS treated control mice (unpaired *t*-test).

perature cover-slipped and viewed under a fluorescence microscope [Olympus (Melville, NY) BX60 with Diagnostic Instruments "Spot" Camera].

Flow cytometry

In a separate experiment, the elutriated fraction containing canine D cells were then double labeled with goat polyclonal somatostatin-FITC (Santa Cruz Biotechnology) and rabbit polyclonal H3 receptor-phycoerythrin (Santa Cruz Biotechnology). FITC-conjugated goat IgG or phycoerythrin-conjugated rabbit IgG was used as the isotype controls. Labeled cells were then analyzed by cytometry using a Coulter Elite ESP Cell Sorter (Bechman-Coulter Electronics, Hialeah, FL).

Statistical analysis

The significance of the results were tested using the unpaired *t*-test or one-way ANOVA where appropriate, using a commercially available software (GraphPad Prism, GraphPad Software, San Diego, CA). A P value < 0.05 was considered significant.

RESULTS

H. felis infection results in inhibition of gastric acid secretion through the H3R

The H3R antagonist thioperamide was used to determine the role of the H3R as a mediator of the decreased gastric acidity during *H. felis*-induced gastritis. Gastric acidity was significantly reduced in 12 wk *H. felis*-infected mice compared to the PBS treated animals (Figure 1). Thioperamide alone caused a significant reduction in gastric acidity compared to PBS-treated mice (Figure 1). Thioperamide treatment during *H. felis* infection reversed the decrease in gastric acidity (Figure 1). These results indicate that *H. felis* has an inhibitory effect on gastric acid secretion *in vivo* and that this inhibitory pathway may be mediated by H3R activation.

H. felis-induced gastritis is reduced with thioperamide treatment

Hematoxylin/eosin (H&E)-stained secretions were examined for changes in histology in response to *H. felis* infection and thioperamide treatment. After 12 wk of *H. felis* infection, mice developed significant inflammation and atrophy compared with the PBS controls (Figure 2A, B). *H. felis*-infected mice treated with thioperamide showed resolution of the inflammation observed with the infection alone (Figure 2D). Minimal inflammation was observed with the thioperamide alone treatment (Figure 2C).

We considered that reduced colonization with *H. felis* might account for the reduced gastritis with the thioperamide treatment. Thus, bacterial numbers per gram of stomach tissue collected from each group of mice was analyzed using a *H. felis* specific quantitative RT-PCR assay. The data revealed that *H. felis* colonization was significantly reduced with thioperamide treatment in both fundic and antral stomach tissue (Figure 2E, F). The resistance to colonization with *H. felis* contributed to the reduced gastritis and restoration of normal gastric acidity with thioperamide treatment.

Reciprocal changes in gastrin and somatostatin with Helicobacter infection

Chronic gastritis and reduced gastric acidity is accompanied by reciprocal changes in gastrin and somatostatin[21]. Analysis by qRT-PCR showed significantly higher gastrin mRNA expression in *H. felis* infected mice compared to untreated PBS controls (Figure 3A). Thioperamide treatment of *H. felis* infected mice resulted in a reversal of the increase in gastrin mRNA expression (Figure 3A), a finding consistent with the reversal of the reduced gastric acidity. Changes in circulating gastrin paralleled that of gastrin mRNA with an increase in response to *H. felis* infection that was resolved with thioperamide treatment (Figure 3B).

The H3 receptor antagonist thioperamide has been shown to stimulate somatostatin release *in vitro*[9,10,22]. In addition, prior studies have shown that somatostatin regulates the immune system, down-regulating a number of functions including lymphocyte proliferation, IFNγ production as well as a direct anti-proliferative effect on *Helicobacter*[21,23-25]. Given that we observed a significant reduction in *H. felis*-induced gastritis with thioperamide treatment, we then measured changes in somatostatin mRNA expression by qRT-PCR in both the fundic and antral mucosa of treated mice (Figure 4). Significantly decreased somatostatin mRNA expression in both the fundic and antral mucosa was observed in the stomachs of *H. felis* infected mice (Figure 4A, B). Although thioperamide alone had a minimal stimulatory effect on fundic somatostatin mRNA expression (Figure 4A), thioperamide treatment significantly increased antral somatostatin levels

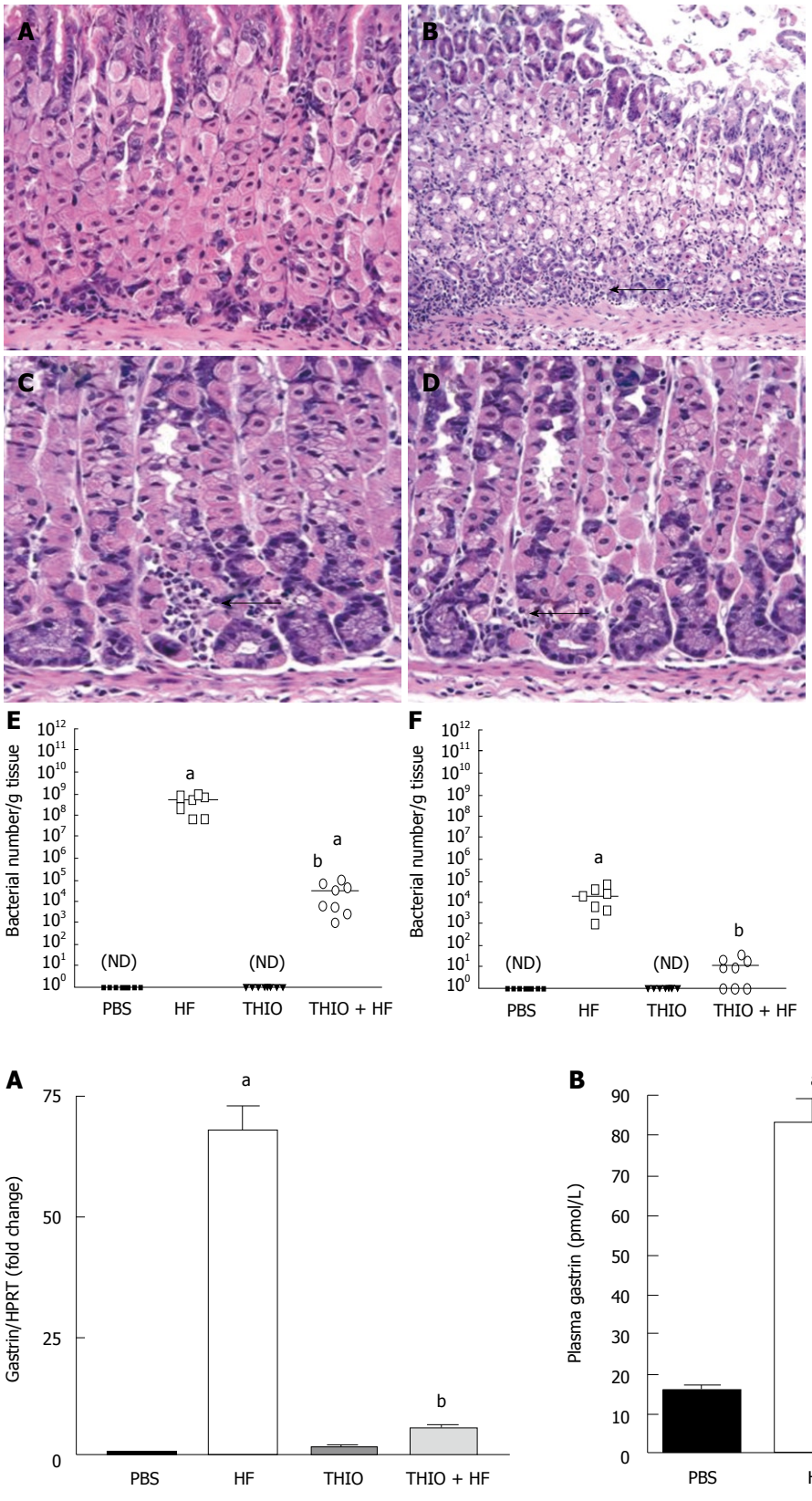

Figure 2 Thioperamide prevention of *Helicobacter felis* gastritis is mediated by blockade of the H3 receptor. H&E stains of (A) PBS, (B) *H. felis* (HF), (C) thioperamide (THIO) and (D) THIO plus HF treated mice (magnification 400 ×). Quantification by qRT-PCR of the amount of *H. felis* colonizing PBS, HF, THIO and THIO plus HF treated mice. Quantitative RT-PCR was performed on RNA isolated from (E) fundic and (F) antral gastric tissue. The log fluorescent emission measured continuously during PCR per cycle was determined. Early amplification showed increased 'starting' fluorescent emission and was used to compute the number of *Helicobacter*/g tissue for each mouse using a standard curve generated from known quantities of *H. felis*. Scatter plots showing the bacterial count for each mouse is shown. The mean indicated by a horizontal bar for $n = 8$ mice. $^{a}P < 0.05$ *vs* PBS mice; $^{b}P < 0.05$ *vs* *H. felis* mice (unpaired *t*-test); ND: non-detectable.

Figure 3 Hypergastrinemia induced by *Helicobacter felis* infection is resolved with thioperamide treatment. A: Quantitative RT-PCR was performed on RNA isolated from the antral mucosa. Shown is the ratio of gastrin to HPRT mRNA in PBS, *H. felis* (HF), thioperamide (THIO) and THIO plus HF treated mice; B: Changes in plasma gastrin were determined by radioimmunoassay. Data are the mean + SEM for $n = 8$ mice; $^{a}P < 0.05$ *vs* PBS control mice; $^{b}P < 0.05$ *vs* THIO treated mice.

(Figure 4B). Reduced fundic and antral somatostatin m RNA expression observed with *H. felis* infection was restored to baseline after thioperamide treatment (Figure 4A, B). Using flow cytometry, we identified a subset of

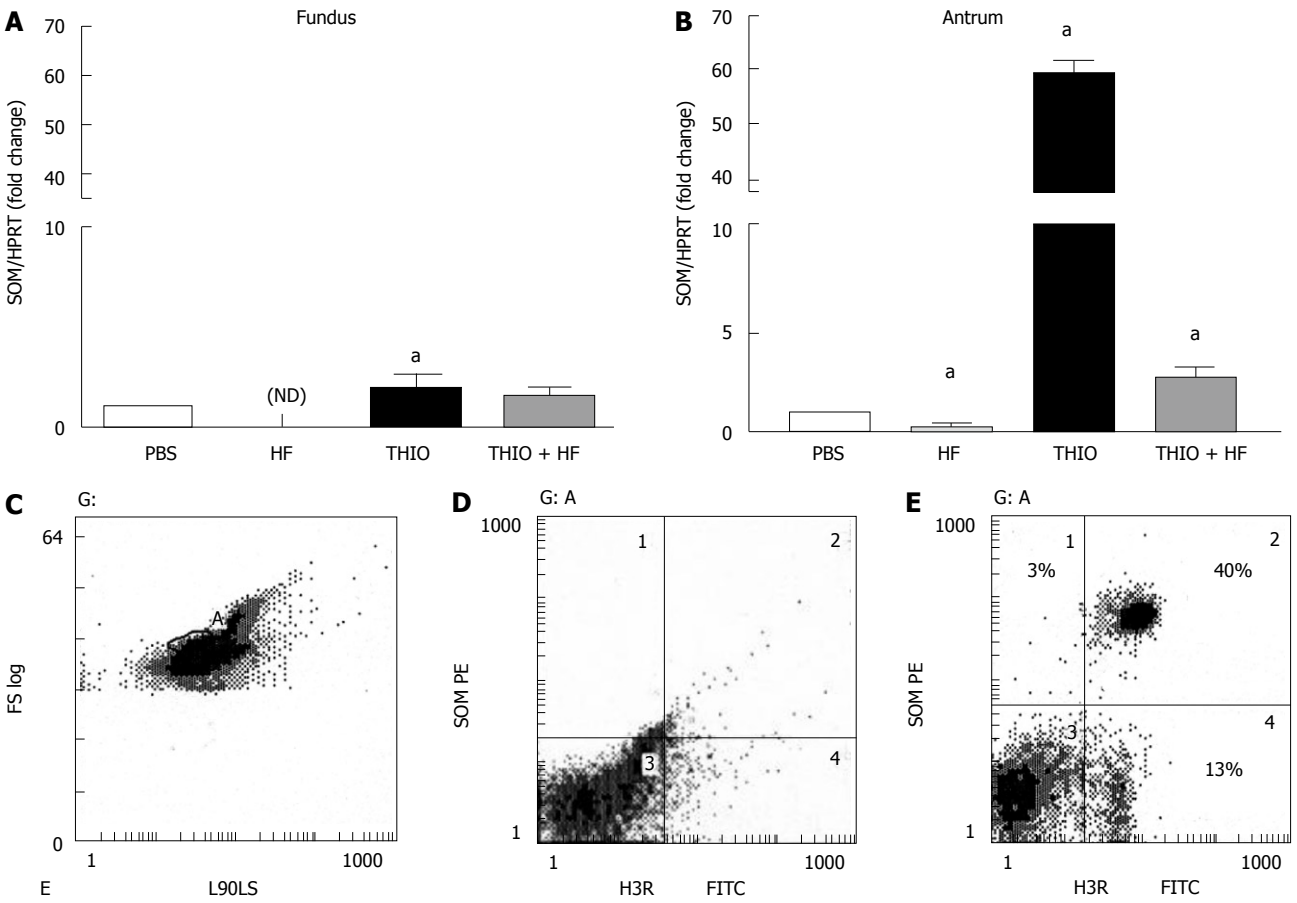

Figure 4 Differential expression of somatostatin mRNA in fundic and antral tissue with thioperamide treatment. Quantitative RT-PCR was performed on RNA isolated from the (A) fundic and (B) antral mucosa. Shown is the ratio of somatostatin to HPRT mRNA in PBS, *H. felis* (HF), thioperamide (THIO) and THIO plus HF treated mice. Data are the mean + SEM for $n = 8$ mice, $^{a}P < 0.05$ *vs* PBS control mice. Identification of H3 receptor on canine D-cells by flow cytometry. D-cells expressing H3 receptors (H3R, FITC) were identified as dual-labeled somatostatin (SOM)-PE and FITC-H3 receptor positive cells. Shown is (C) double-parameter histogram showing forward light scatter (cell size, FS LOG) and right-angle light scatter (cell shape, L90LS), gated cell population (region A) (D) isotype controls and (E) percent of isolated canine fundic D-cells expressing H3 receptors (H3R) (region 2), D-cells not expressing H3R (region 1) and H3R cells not expressing somatostatin (region 3). Results shown are representative of $n = 2$ canine cell preparations.

somatostatin expressing D-cells that also expressed the H3R comprising 40% of the entire cell preparation (Figure 4C-E) supporting a possible direct activation of H3R by thioperamide on D-cells. There was a 13% population of somatostatin expressing cells that were negative for the H3R (Figure 4E).

Infusion of thioperamide to sheep

In order to determine if the thioperamide induced increase in somatostatin mRNA was associated with a functional secretory response in somatostatin, plasma somatostatin was measured in sheep treated with pentagastrin and thioperamide. Previous studies have shown that sheep have a similar reciprocal gastrin/somatostatin response to changes in gastric acidity[13,26,27]. Thioperamide augmented the secretion of somatostatin (Figure 5A, B), indicating a functional relationship between the H3R and the D cell.

H3R is located on mouse parietal- and immune cells

The localization of the H3R in the gastric mucosa has not been fully determined. We used immunofluorescence to determine the expression pattern of the H3R in the stomachs of *H. felis*-infected and uninfected animals. Immunofluorescence showed co-localization of the H3R on mouse H^+, K^+-ATPase positive parietal cells (Figure 6A-E). Using a primary canine parietal cell culture system, immunofluorescence data supported Figure 5 and showed H3R co-localization on H^+, K^+-ATPase expressing parietal cells (Figure 6A-F). Interestingly, in *H. felis*-infected mice, immunostaining for the H3R was also observed within the inflammatory infiltrate (Figure 6F), suggesting a potential direct interaction between thioperamide and the parietal cells and infiltrating immune cells.

H3R located on primary canine parietal cells mediates the inhibitory response to H3R agonist in vitro

We tested the effect of H3R activation on acid secretion from the isolated canine parietal cells, the H3R agonist Rα-MH and the H3R antagonist thioperamide. The H3R agonist or antagonist alone had no effect on radiolabeled aminopyrine uptake (Figures7 and 8). Histamine, which potently stimulates acid secretion through the activation of the H2R, significantly induced aminopyrine uptake

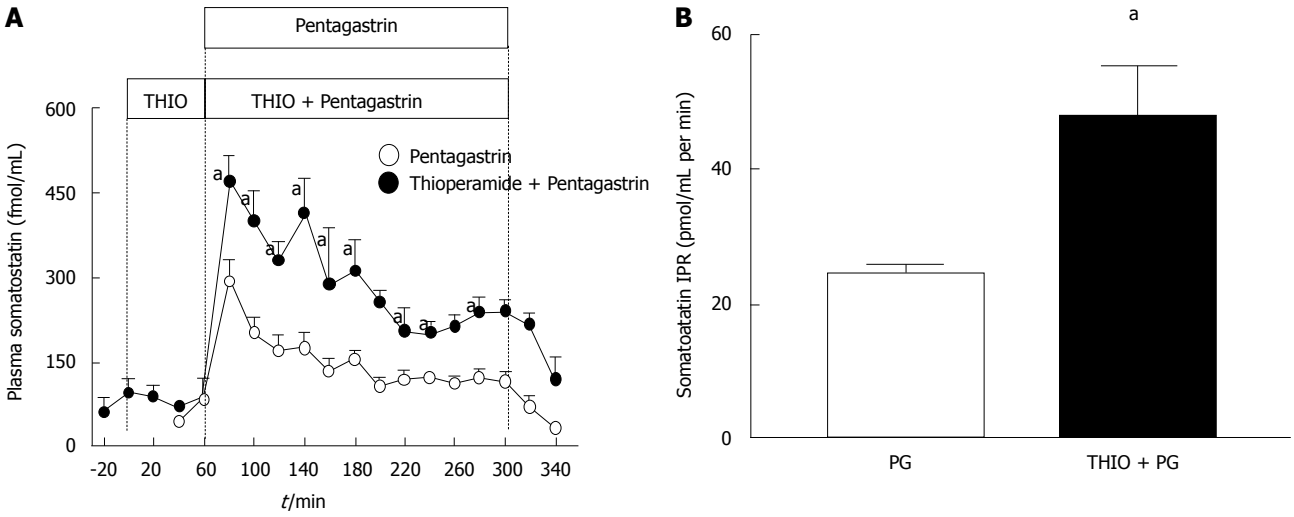

Figure 5 Suppression of somatostatin with chronic infusion of pentagastrin in the sheep model. A: Infusion of pentagastrin for 4 h in the sheep stimulates the release of somatostatin followed by a gradual decrease in concentration. The stimulatory effect of pentagastrin on somatostatin is greater with thioperamide infusion; B: Somatostatin integrated plasma response (IPR, pmol/mL per minute) showing a significant increase in pentagastrin stimulated somatostatin with thioperamide treatment. PG: pentagastrin; THIO: thioperamide. Data shown as mean + SEM, $^{a}P < 0.05$ versus PG, $n = 6$ for PG infusion, $n = 3$ for THIO + PG infusion. The IPR was calculated as the area under the somatostatin time curve as follows: IPR = $DS_0 - DS_1 \times (t_1 - t_2) + \ldots. DS_n - DS_{n-1} \times (t_n - t_{n-1})$; DS: plasma somatostatin-basal somatostatin concentrations; *t*: time of infusion; Subscripts 0, 1, 2…n: times at which samples were collected.

(Figure 8). Rα-MH blocked the stimulatory effect of histamine on aminopyrine uptake agonist (Figure 8). The H3R antagonist thioperamide reversed the inhibitory effect of Rα-MH on histamine-induced aminopyrine uptake (Figure 8). Thus, activation of the H3R on parietal cells results in the inhibition of secretagogue-stimulated acid secretion.

DISCUSSION

We have used *in vivo* and *in vitro* models to identify the physiological function of H3R activation in the stomach. In particular, H3R activation contributed to decreased acid secretion observed during *H. felis* infection. *H. felis*-induced hypochlorhydria was associated with reciprocal changes in gastrin and somatostatin secretion and expression. Findings reported here demonstrate an important role for the H3R in gastric acid and somatostatin regulation during *Helicobacter* infection.

H. felis-induced gastritis was associated with significantly decreased gastric acidity 12 wk after infection, as previously reported[21]. The H3R antagonist thioperamide reversed suppressed gastric acidity observed during *H. felis* infection. Our data was consistent with other *in vivo* observations. In humans it has been reported that gastric acid secretion increases 4-8 wk after eradication therapy, suggesting that *Helicobacter* inhibits acid secretion[28]. In cats, acid secretion was inhibited within 3 wk of *H. pylori* infection[3]. The inhibition of gastric acid secretion may also be accounted for by suppression of histidine decarboxylase (HDC) activity that is important in the production of the major acid secretagogue histamine[5,29,30]. The inhibition of HDC is brought about by the production of the potent H3R agonist Nα-MH secreted from the bacterium itself[5,31]. This is supported by previous evidence showing H3R located on histamine-producing cells that reduce excess acid[1]. Our *in vitro* and immunofluorescence data demonstrating H3R on the parietal cells are consistent with previous observations made in the rabbit[1]. The expression of the H3R on the gastric parietal cell supports the hypothesis of a direct inhibitory effect of the *Helicobacter* metabolite Nα-MH on parietal cell acid secretion (Figure 9A).

The H3R antagonist thioperamide has been shown to stimulate somatostatin release *in vitro*[32] and this may account for the decreased gastric acidity observed. *H. felis* alone also caused a significant decrease in gastric acidity perhaps due to the parietal cell atrophy observed with *Helicobacter* infection. Thioperamide treatment during *H. felis* infection reversed the decrease in gastric acidity observed with the bacterial infection alone. Given that thioperamide is a H3R antagonist, these results may indicate that *H. felis* has an inhibitory effect on gastric acid secretion *in vivo* and that this inhibitory pathway may be mediated by H3R activation. Thioperamide treatment also suppressed bacterial colonization and this may contribute to the normalization of gastric acidity observed with the thioperamide and *H. felis* group. It may be that with continued thioperamide treatment a further decrease in gastric acidity would have been observed in the *H. felis*-infected group.

In the gastrointestinal tract, H3R are located in cholinergic and NANC neurons of the myenteric plexus, in endocrine and paracrine cells of the gastric mucosa and on rabbit parietal cells[1]. The cellular location of H3Rs in the stomach mucosa is uncertain. Our data suggests that in addition to parietal cells, H3Rs are located on gastric D cells. This finding confirms previous pharmacological studies where it has been assumed that the H3R exists on the D-cell[9,10]. Consistent with our findings *in vivo*

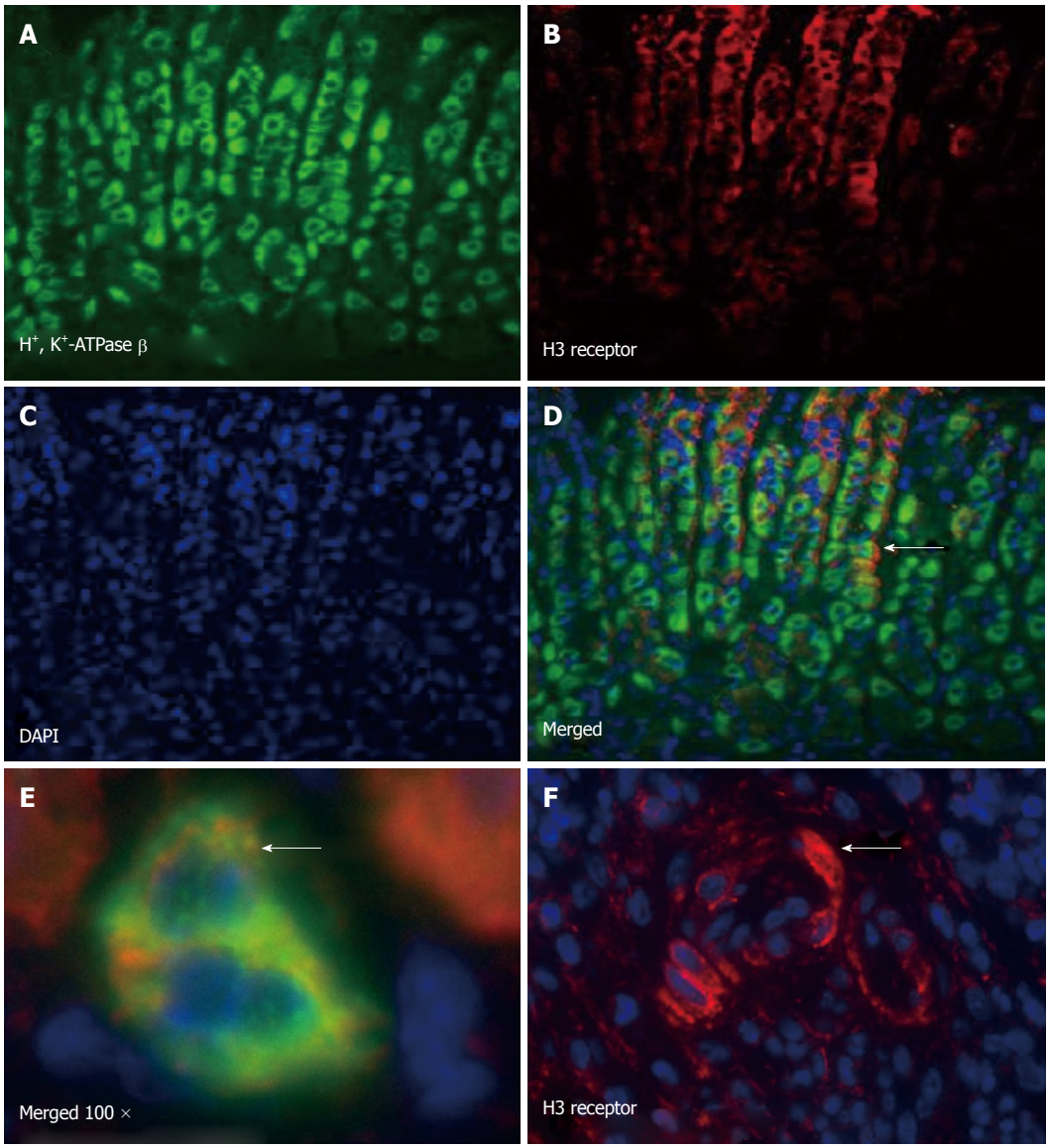

Figure 6 H3 receptor expression of mouse parietal and immune cells. Immunofluorescence of paraffin sections of mouse stomach stained with (A) H^+, K^+-ATPase β subunit (FITC), (B) H3 receptor (Texas red), (C) DAPI, and (D) merged image showing colocalization; E: High power image showing parietal cells expressing the H3 receptor; F: H3 receptor expressing cells within the inflammatory infiltrate in *H. felis* infected mice.

in mouse and sheep where H3R antagonism stimulated somatostatin mRNA and somatostatin secretion, *in vitro* studies using isolated mouse antral mucosa demonstrated a similar increase with thioperamide[32]. *In viv*o, stimulation of somatostatin expression with thioperamide during *H. felis* infection resulted in suppression of hypergastrinemia. Collectively, our study extends these *in vitro* findings by demonstrating the expression of the H3R on the D-cell and suggests a direct inhibitory effect of Nα-MH on somatostatin secretion during *H. pylori* infection (Figure 9A).

Treatment of *H. felis* infected mice with thioperamide abolished the *H. felis*-induced gastritis consistent with a reduction in bacterial colonization. Thus, we considered that resistance to colonization with *Helicobacter* might account for the reduced gastritis with the thioperamide treatment. Infected mice were also hypergastrinemic and exhibited suppressed gastric somatostatin mRNA expression. Reciprocal changes in gastrin and somatostatin expression are characteristic of *Helicobacter* infection or bacterial overgrowth in the stomach and this has been explained by elevation of IFNγ secreted by inflammatory cells within the mucosa[15,33]. With thioperamide treatment, both gastrin and somatostatin levels returned to baseline. In particular, thioperamide alone caused a significant increase in antral somatostatin mRNA expression. Antral somatostatin mRNA expression remained significantly higher compared to the untreated mice. Interestingly in our previous study, we show that somatostatin is both necessary and sufficient to prevent colonization and subsequent development of *Helicobacter*-induced gastritis[21]. Somatostatin is a known modulator of the immune system that is capable of down-regulating a number of functions including lymphocyte proliferation, IFNγ, TNFα and IL-1β production[25,34]. Perhaps the stimulation of

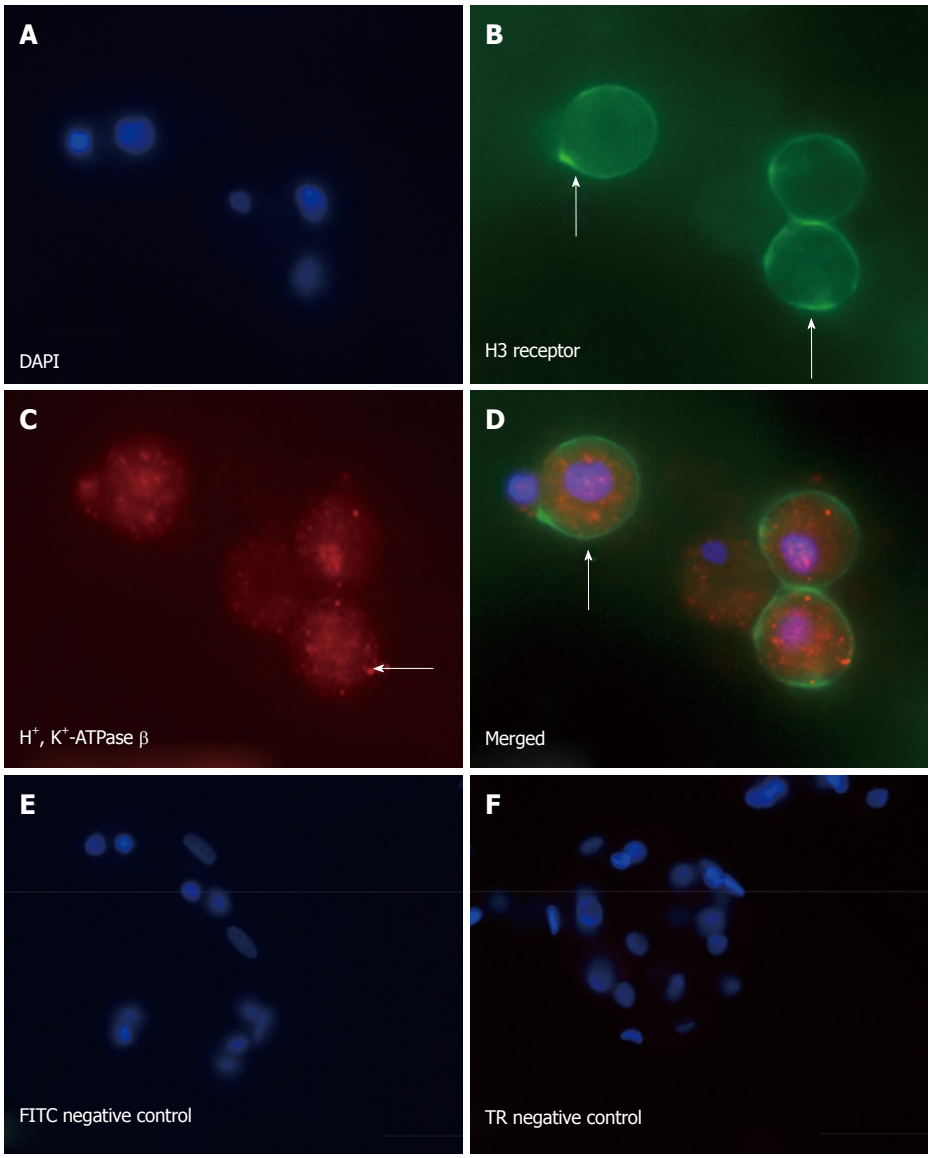

Figure 7 Primary canine parietal cells express the H3 receptor. Immunofluorescence of isolated parietal cells stained with (A) 4',6-Diamidino-2-Phenylindole (DAPI), (B) H3 receptor (FITC), (C) H^+, K^+-ATPase β subunit (Texas red), and (D) merged image showing colocalization of the H3 receptor on the parietal cell. Negative IgG controls for (E) FITC and (F) Texas red (TR) secondary antibodies are shown.

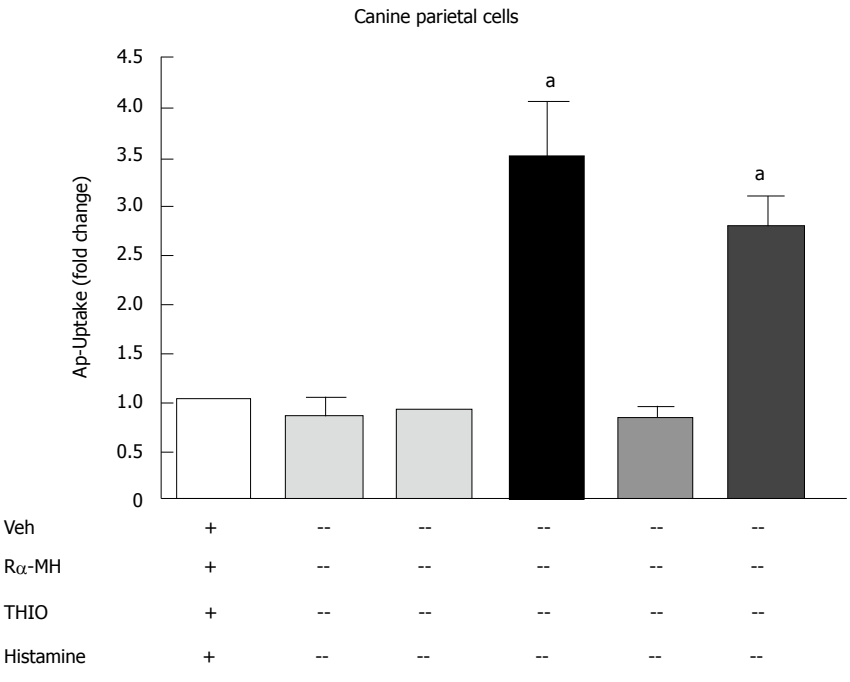

Figure 8 Acid production from canine parietal cells in response to H3 receptor agonist and antagonist. Aminopyrine (AP)-uptake in response to vehicle (Veh), H3 receptor agonist R alpha methylhistamine (RαMH), H3 receptor antagonist (thioperamide, THIO) and histamine was performed. The data are expressed as the mean fold induction over Veh treated cells + SEM, n = 3 experiments, $^a P < 0.05$ *vs* Veh.

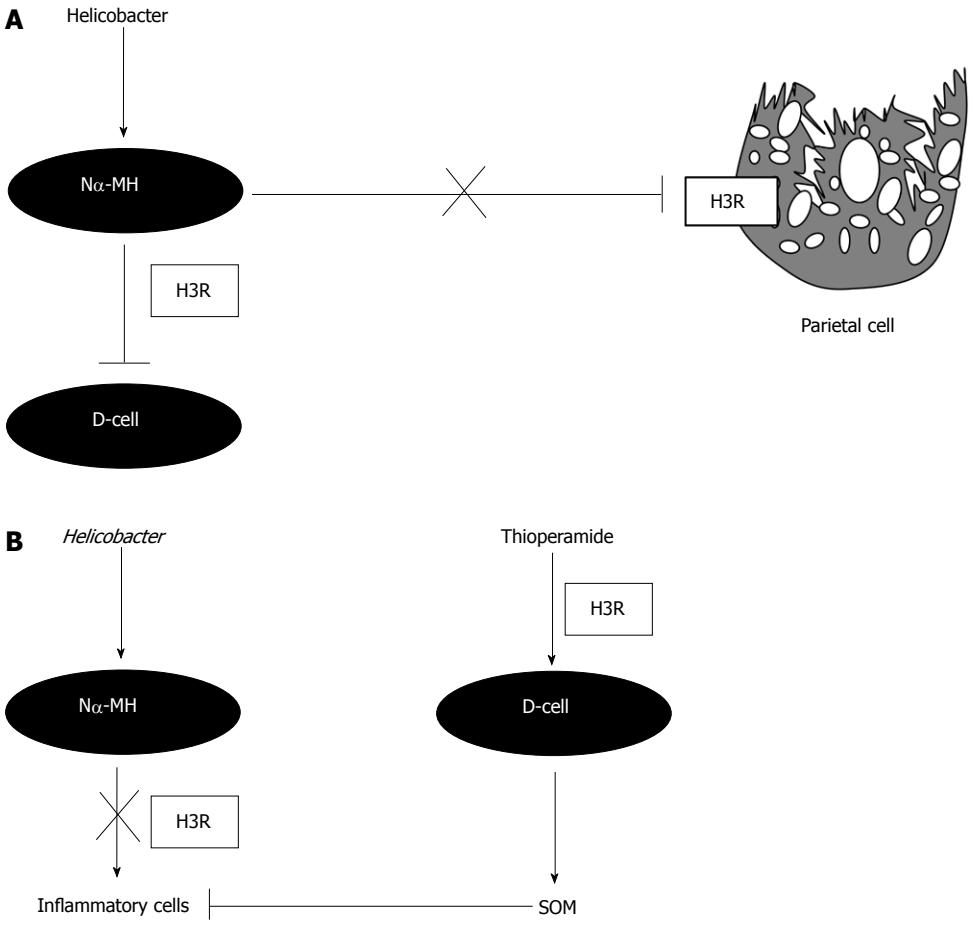

Figure 9 Proposed role for the H3R in gastric function during *Helicobacter* infection. A: H3Rs localized on parietal cells and D-cells supports a direct inhibitory effect of the *H. pylori* metabolite Nα-MH on acid and somatostatin secretion respectively during infection; B: The stimulatory effect of thioperamide on somatostatin expression and secretion regulates the host immune response during *H. pylori* infection; H3R: histamine 3 receptor; SOM: somatostatin.

somatostatin by thioperamide reduced the colonization of *H. felis* in addition to directly block the gastritis (Figure 9B). It is also important to note that H3Rs are located on inflammatory cells within the gastric mucosa of *H. felis* infected animals. This raises the question of whether Nα-MH is stimulating proliferation of the inflammatory cells and whether this response is blocked with thioperamide.

Most patients with peptic ulcer disease are currently treated with proton pump inhibitors or H2R antagonists. The long-term use of these compounds has been associated with potential problems such as ECL-cell hyperplasia, bacterial overgrowth and persistent inflammation of the gastric mucosa[15,35,36]. Thus, there is a rationale for the development of new antisecretory agents. The H3R antagonist thioperamide decreases acid secretion by elevating somatostatin release. The elevated somatostatin concentrations may be of benefit due to the potent inhibitory effect that somatostatin has on gastritis and bacterial colonization[21]. The therapeutic implications of H3R antagonists deserve further study.

ACKNOWLEDGMENTS

We would like to acknowledge the valuable assistance of Kathy McClinchey (McClinchey Histology Lab, Inc., Stockbridge, MI, USA) for paraffin embedding and sectioning tissue.

COMMENTS

Background

Histamine is produced by enterochromaffin-like (ECL) cells and by mast cells in the setting of gastric inflammation such as observed with *Helicobacter* infection. Prior studies have shown that the histamine 3 receptor (H3R) is present on endocrine cells specifically on ECL cells. It is known that inhibitors of the H3R will inhibit acid secretion but the mechanism has been poorly understood. The current article demonstrates that blocking H3Rs will increase somatostatin which in turn can then suppress gastric acid secretion through a paracrine mechanism.

Research frontiers

Important areas in this field are to better understand how inflammation in the stomach regulates parietal cell acid production. Also to identify new targets for the development of acid suppressing therapies given the emerging side effects with current proton pump inhibitors in particular.

Innovations and breakthroughs

Prior studies have focused on the cellular location of the H3R and the development of agonists and antagonists for this receptor. Apparently, antagonists inhibit acid secretion but the mechanism has not been well-described. Most of the studies have focused on the effect of H3R activation on ECL cells which have been reported to express 90% of the H3Rs. However, the study here shows that D and parietal cells as well as immune cells also express H3Rs. This novel finding should make one rethink the mechanism by which the H3R affects acid secretion, especially during *Helicobacter infection*.

Applications

Most human subjects with peptic ulcer, esophageal reflux disease or bleeding from the stomach are treated with H2R antagonists or proton pump inhibitors. However, chronic use of these drugs is now found to have side effects, e.g. fundic gland hyperplasia, bacterial overgrowth and metabolic disturbances such as hip fractures related to calcium absorption. Therefore, other mechanisms should be explored to block acid secretion such as increasing tissue levels of somatostatin that in turn would inhibit gastrin-producing (G) cells. The study here demonstrates that blocking the H3R can directly increase somatostatin and provide a different mechanism to block acid.

Peer reviews

The manuscript by Zavros *et al* demonstrates an inhibitory effect of H3R activation induced by *Helicobacter* infection on gastric secretion *in vivo* and *in vitro*. The mechanism involves decreased expression of somatostatin and increased expression of gastrin. It can be accepted after minor revision.

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