

## Autoregulation of Enterochromaffin-Like Cell Histamine Secretion Via the Histamine 3 Receptor Subtype

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**Introduction:** The neuroendocrine histamine-secreting cell of the gastric fundus, the enterochromaffin-like cell, is the principal regulator of parietal cell acid secretion. We have proposed that histamine may regulate its own synthesis and release via an autocrine mechanism. The purpose of this study was to evaluate the role of the histamine receptor subtypes H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> in the regulation of this phenomenon.

**Methods:** Purified ECL cells were isolated by pronase digestion and EDTA exposure of the rat stomach, followed by particle size and density separation using counterflow elutriation and Nycodenz gradient centrifugation. 24-hr cultured cells were pretreated for 30 min with the agents; H<sub>1</sub> receptor agonist (2-[(3-trimethyl)-diphenyl] histamine) (TMPH), H<sub>1</sub> receptor antagonist (terfenadine); H<sub>2</sub> receptor agonist (dimaprit) or antagonist (cimetidine or loxidine); or H<sub>3</sub> receptor agonist (imetit) or antagonist (thioperamide) (all tested, 10<sup>-10</sup>-10<sup>-6</sup> M). Gastrin was then used to stimulate histamine secretion. Histamine secretion was quantified by specific enzyme-immunoassay.

**Results:** Basal histamine secretion was 2.7 ± 0.14 nmol/10<sup>3</sup> cells. Gastrin-stimulated (10 nM) levels were 4.6 ± 0.4 nmol/10<sup>3</sup> cells (p < .01). TMPH inhibited both basal and gastrin driven histamine secretion with a maximal effect (34 percent) (1.78 ± 0.08 nmol/10<sup>3</sup> cells) and an IC<sub>50</sub> of > 5 × 10<sup>-7</sup> M. H<sub>1</sub> receptor antagonism did not alter histamine secretion alone or in combination with gastrin. Neither H<sub>2</sub> receptor stimulation nor antagonism had any effect on histamine secretion alone or in combination with gastrin. Gastrin-induced histamine secretion was dose-dependently inhibited by imetit (H<sub>3</sub> agonist) with a maximal effect (2.4 ± 0.6 nmol/10<sup>3</sup> cells) (p < .05) and an IC<sub>50</sub> of 10<sup>-9</sup> M. Conversely, Thioperamide (H<sub>3</sub> antagonist) dose-dependently augmented gastrin-stimulated histamine secretion with a maximum effect (5.7 ± 0.5 nmol/10<sup>3</sup> cells) (p < .05) at 10<sup>-8</sup> M and an EC<sub>50</sub> of 7 × 10<sup>-10</sup> M.

**Conclusion:** These data are consistent with the presence of an H<sub>3</sub> receptor on the ECL cell which modulates gastrin-stimulated histamine secretion. Our observations support the proposal that a histamine-mediated short-loop autocrine regulatory mechanism of ECL cell secretion exists.

### INTRODUCTION

Histamine is present in the stomach in higher concentrations than in any other body tissue. Its major source is the enterochromaffin-like (ECL)<sup>b</sup> cell of the oxyntic mucosa [1, 2]. The ECL cell has been identified as the principle regulator of acid secretion, and thus plays

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<sup>b</sup> Abbreviations: ECL, Enterochromaffin-like; CCK, Cholecystokinin; TMPH, 2-[(3-trimethyl)-diphenyl] histamine; EIA, Enzyme immuno-assay; PBS, Phosphate buffered saline; FCS, Fetal calf serum; ITS, Insulin-transferrin-sodium selenite; HDC, Histidine decarboxylase; R-MHA, R-α methyl histamine.

a critical role in gastric physiology [3-5]. Regulation of histamine secretion is complex and is primarily by gastrin activation of the ECL cell gastrin/CCK-B receptor, which stimulates both histamine synthesis and DNA synthesis [2]. Increased plasma gastrin levels result in ECL cell proliferation and increased histamine secretion [6]. The effects of elevated local histamine concentrations are unknown but of considerable relevance since histamine has protean biological effects including a substantial role in the genesis of inflammation and as a known mitogen in a number of cell systems [3].

The specific physiological effects of histamine appear to be mediated by at least three subclasses of cell surface histamine receptors: H<sub>1</sub> receptors were initially identified in the brain and noted to regulate the postsynaptic response to histamine [7]. The second subtype, the H<sub>2</sub> receptor, and the identification of its relevance to gastric parietal cell secretion, revolutionized the therapy of acid-peptic disease [8]. Arrang et al. in 1983 demonstrated feed-back inhibition of histamine synthesis and release in histaminergic neurons in the brain through a third histamine receptor subtype [9]. The subsequent development of selective H<sub>3</sub> receptor agonists and antagonists has led to the identification of this receptor subtype on a number of peripheral tissues including perivascular nerve terminals, the enteric ganglia of the ileum and the lung [10-13]. *In vivo*, H<sub>3</sub> receptor stimulation has been reported to inhibit gastrin, bombesin, 2-deoxyglucose and meal-stimulated acid secretion in cats and dogs [14-17]. Additional studies have noted that H<sub>3</sub> receptor stimulation inhibits histamine release from the rat stomach [18], rabbit gastric gland [19], rabbit gastric mucosal cells [20] and isolated rat ECL cells [2].

The possibility that histamine could autoregulate its own synthesis and release was initially postulated in 1964 by Kahlson. He proposed that the labile histamine forming capacity observed in the gastric mucosa of cats, mice and frogs could be explained if there were to exist a feedback control of histamine forming capacity by the histamine content of the mucosa [21]. The subsequent identification of the ECL cell as a major source of mucosal histamine provided support for the erroneous suggestion that the ECL cell, via an H<sub>2</sub>-receptor, might negatively mediate its histamine secretory response to gastrin [22]. This postulate could not be adequately tested in an intact stomach or mucosal preparation.

The recent development of a preparation of pure, isolated ECL cells has allowed exploration of the regulatory mechanisms of acid secretion, histamine release and proliferation that have heretofore been impossible [2]. Since the ECL cell is the major physiologic site of histamine synthesis and secretion in the stomach, we have examined the proposal that its secretory function may be modulated by histamine autoreceptors. The purpose of this study was to investigate the effects of histamine and histamine subtype receptor ligands on ECL cell secretory function.

## MATERIALS AND METHODS

Human gastrin-17 and dimaprit was purchased from Research Plus, Inc., Bayonne, New Jersey. Terfenadine was obtained from Sigma Chemical Co., St. Louis, Missouri; Cimetidine and Loxitidine were kind gifts from Glaxo, UK.; TMPH was a kind gift of W. Schunack, Berlin, Germany; imetit and thioperamide were kind gifts from J.C. Schwarz, Paris, France. Nycodenz was from Accurate Chemical & Scientific Corp., Westbury, New York. Pronase E was purchased from Boehringer-Mannheim, Indianapolis, Indiana. Histamine EIA kit was obtained from Immunotech International, AMAC, Inc., Westbrook, Maine. Forty-eight well collagen-I-coated plates were from Becton-Dickinson, Bedford, Massachusetts. All other reagents were obtained from the highest quality source available.

### *Cell isolation*

Rat ECL cells were prepared by a modification of the method of Prinz [2]. The National Research Council's guide for the care and use of laboratory animals was followed.

For each condition studied, the stomachs from four animals ( $n = 1$ ) were excised and everted to make mucosal-side-out sacs. Following a thorough rinse with PBS, pronase E was injected into the stomach sacs. The stomachs were then incubated in an oxygenated calcium-free medium containing 1 mM EDTA for 30 min, followed by a 10-min incubation in a calcium-respiration medium. The stomachs were then transferred back to calcium-free-EDTA medium for an additional 30-min incubation. The mucosal cells were harvested by gentle stirring for 10 min in calcium-respiration medium and collected by centrifugation (100 X g, 5 min). This crude cell fraction was subjected to counterflow elutriation (JE-6 rotor, Beckman Instruments). Small cells with diameter of 8 to 10  $\mu\text{m}$  were collected at 2,000 rpm and a flow rate of 20 ml/min. This fraction was subsequently layered on a step Nycodenz density gradient and centrifuged at 1100 rpm for 8 min. The ECL cell enriched fraction was collected at the interface of density 1.040 g/ml.

#### *Cell viability*

Cell viability was demonstrated by trypan blue exclusion using light microscopy. Quantitation was performed by counting the number of cells that excluded trypan blue (> 95 percent) and dividing by the total number of cells in five randomly selected fields at 40 X magnification.

#### *Cell culture*

Isolated cells were washed in culture medium (Dulbecco's Minimal Essential Medium/F12 with two percent bovine serum albumen, pH 7.4) by centrifugation and resuspension. Thereafter, cells were resuspended in growth medium (culture medium plus two percent FCS, ITS 0.5 mg/100 ml, hydrocortisone 10 nM, gentamicin 0.1 mg/100 ml) at a final concentration of  $10^6$  cells/ml, and 200  $\mu\text{l}$  added to each well of a 48-well Collagen-I plate. The cells were cultured overnight at 37°C under standard conditions.

#### *Cell secretion*

*Stimulation of Histamine Secretion:* After 24 hr, the growth medium was removed and replaced with 200  $\mu\text{l}$  of culture medium. After the addition of either culture medium alone (control) or secretagogues at one percent of the final incubation volume, the cells were incubated at 37°C for 60 min. Then, half of the medium was removed from each well and centrifuged at 15,000 X g for 30 sec. Supernatant was diluted 1:10 and stored at -20°C for measurement of histamine content by specific EIA.

*Histamine EIA:* The basis of this assay is competition between the histamine in the sample and the enzyme conjugate histamine acetylcholinesterase, used as a tracer, for binding to monoclonal antibody coated tubes using a commercially available kit. Bound enzymatic activity is measured by the addition of a chromogen substrate, and the intensity of colour development is measured by a spectrophotometer (microplate reader model EL 312e, Biotek Instruments, Vermont). Sample histamine concentrations are calculated from a negative spline-curve. This assay has been adjusted (dilution and non-specific activity) for use over a range from 50 to 0.5 nmol/ $10^3$  cells and is sensitive (0.2 nmol detection level) and specific (interassay variation 9.4 percent, intra-assay variation 8.0 percent). Histamine levels were expressed as nmol/ $10^3$  cells. All agents were tested to ensure no cross-reactivity in the assay.

#### *Statistical analyses*

Statistics was calculated using the Excel 4.0 (Macintosh) program. Results are expressed as mean  $\pm$  SE. Statistical significance was evaluated using either ANOVA or the two tailed Student's test for paired and unpaired values as appropriate, with a probability

of  $p < .05$  representing significance. The number of ECL cell preparations is represented by "n" (four animals per preparation).

## RESULTS

A 90 to 95 percent pure preparation of ECL cells was developed from rat fundic mucosa as determined by chromogranin and histamine immunofluorescence, markers for ECL cells. Cell viability was  $> 95$  percent as demonstrated by trypan blue exclusion.

### Secretion

**Gastrin:** Basal histamine secretion was  $2.7 \pm 0.14$  nmol/ $10^3$  cells. A dose response to gastrin was evident ( $EC_{50}$   $1 \times 10^{-10}$  M). Maximum stimulation ( $4.6 \pm 0.4$  nmol/ $10^3$  cells) was noted at  $10^{-8}$  M (Figure 1).

**Histamine:** No accurate estimate of secretion was possible since stimulation with histamine interfered with the histamine assay.

### Histamine-1 receptor ligands

**TPMH:** The  $H_1$  receptor agonist ( $10^{-10}$ - $10^{-6}$  M) alone inhibited basal histamine secretion (34 percent) at  $10^{-6}$  M, and an estimated  $IC_{50}$  of  $> 5 \times 10^{-7}$  M (Figure 2). Gastrin-stimulated (10 nM) histamine secretion was inhibited in a similar fashion (Figure 3). TPMH in a model of guinea pig ileal contraction was originally characterized with an  $EC_{50}$  of  $\sim 1.5 \times 10^{-7}$  M [23]. In addition, this molecule has been demonstrated to have a substantial cross reactivity with the  $H_3$  receptor subtype ( $EC_{50} \sim 10^{-5}$  M) [23]. It is difficult to interpret the pharmacological effect of TPMH in our model because of the presence of substantial amounts of endogenous histamine which has a similar affinity ( $2 \times 10^{-7}$  M) and efficacy (78 percent compared with TPMH) for the  $H_1$  receptor subtype. The high concentration required to inhibit secretion in our model suggests that this may be a non-pharmacological event or else represent cross-selectivity with other histamine receptor subtypes.

**Terfenadine:** The  $H_1$  receptor antagonist terfenadine ( $10^{-10}$  to  $10^{-6}$  M) had no effect on basal or gastrin-stimulated (10 nM) histamine secretion.

In a separate set of studies (data not shown) we have shown that terfenadine at 1 nM inhibits gastrin-stimulated DNA synthesis and that this effect is completely reversed by TPMH in a dose dependent fashion with an estimated  $EC_{50}$  value of  $10^{-9}$  M. It would thus seem more likely that the  $H_1$  receptor exhibits a proliferative rather than a secretory role.

### Histamine-2 receptor ligands

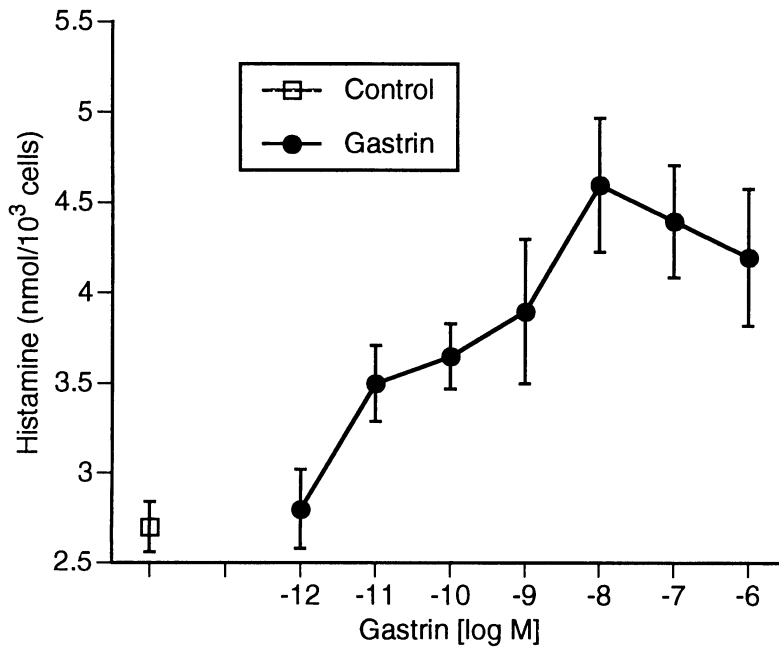
**Dimaprit:** The  $H_2$  receptor agonist dimaprit ( $10^{-10}$  to  $10^{-6}$  M) did not alter basal or gastrin-stimulated (10 nM) histamine secretion.

**Cimetidine and loxidine:** Neither the competitive  $H_2$  receptor antagonist cimetidine ( $10^{-10}$  to  $10^{-6}$  M) nor the irreversible  $H_2$  receptor antagonist loxidine ( $10^{-10}$  to  $10^{-6}$  M) altered basal or gastrin-stimulated (10 nM) histamine secretion.

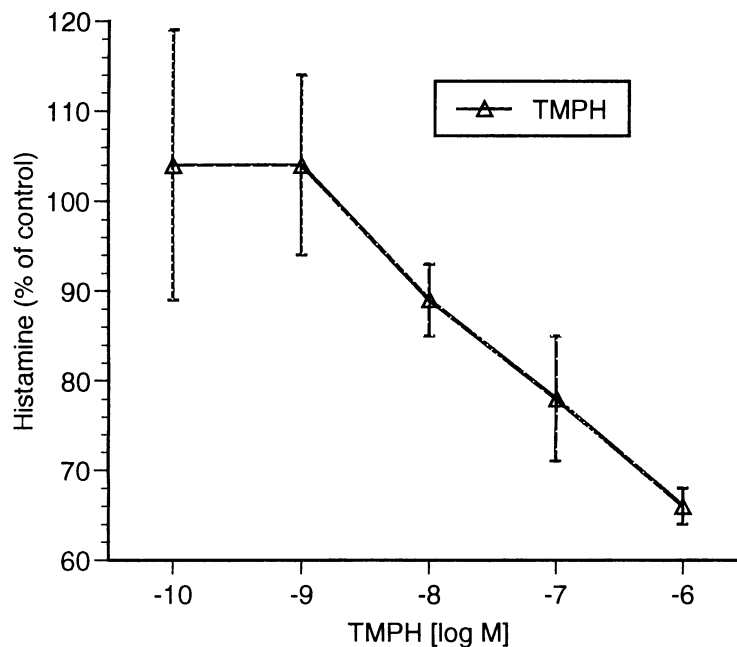
Histamine has been demonstrated to have a similar affinity and efficacy as these agents for the  $H_2$  receptor subtype [7]. The observation that the ECL cell does not respond to these agents suggests that under equivalent conditions, histamine has no direct effect on the ECL cell. Since the parietal cell expresses the  $H_2$  receptor subtype, it is possible that the ECL cell does not exhibit the  $H_2$  receptor.

### Histamine-3 receptor ligands

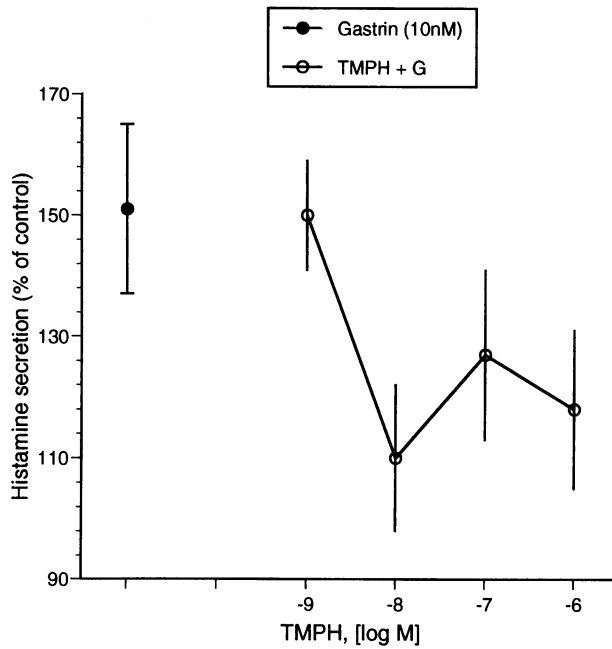
**Imetit:** The  $H_3$  receptor agonist imetit ( $10^{-10}$  to  $10^{-6}$  M) did not alter basal histamine secretion. However, pretreatment with imetit inhibited a sub-maximal dose of gastrin-stimulated (1 nM) histamine secretion (38 percent) at  $10^{-6}$  M, and an  $IC_{50}$  of  $\sim 10^{-9}$  M



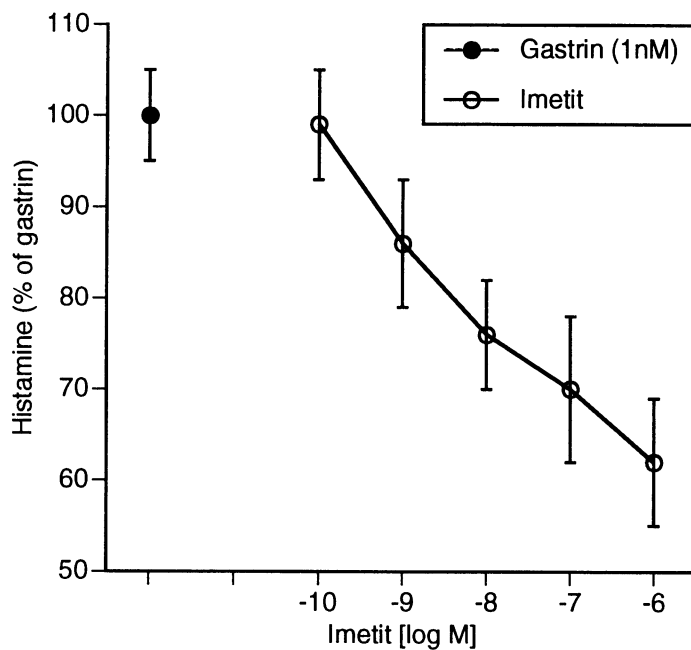
**Figure 1. The gastrin dose response for ECL cell histamine secretion.** Control levels were  $2.7 \pm 0.14$  nmoles/ $10^3$  cells. Gastrin-stimulated histamine release with an estimated  $EC_{50}$  of  $1 \times 10^{-10}$  M ( $3.65 \pm 0.18$  nmoles/ $10^3$  cells,  $p < .01$  vs. control) and an  $EC_{max}$  of  $10^{-8}$  M ( $4.6 \pm 0.37$  nmoles/ $10^3$  cells,  $p < .005$  vs. control) ( $n = 7$ ).



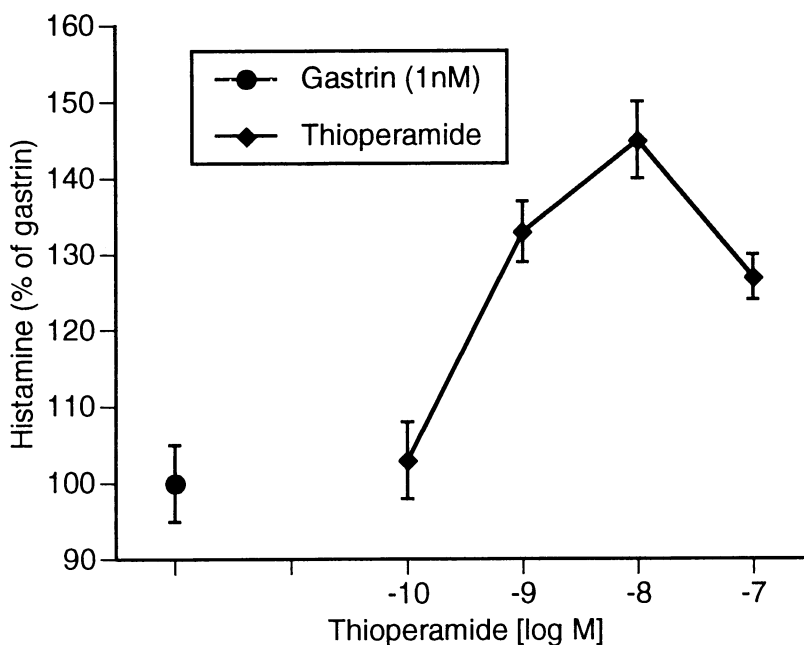
**Figure 2. The inhibitory effect of the  $H_1$  receptor agonist, TMPH, on basal histamine secretion.** Control levels are  $2.5 \pm 0.27$  nmoles/ $10^3$  cells. TMPH dose-dependently inhibited histamine release with an  $IC_{50}$  of  $> 5 \times 10^{-7}$  M and a maximal effect at  $10^{-6}$  M ( $1.94 \pm 0.34$  nmoles/ $10^3$  cells). Maximum inhibition was 34 percent ( $p < .05$  vs. control) ( $n = 3$ ).



**Figure 3.** The inhibitory effect of the  $H_1$  receptor agonist, TMPH, on gastrin-stimulated histamine secretion. Gastrin levels are  $3.8 \pm 0.4$  nmoles/ $10^3$  cells. TMPH inhibited histamine release with an estimated  $IC_{50}$  of  $5 \times 10^{-8}$  M. Maximum inhibition was approximately 26 percent ( $p = .05$  vs. gastrin) ( $n = 3$ ).



**Figure 4.** The inhibitory effect of the  $H_3$  receptor agonist, imetit, on a sub-maximal dose of gastrin-stimulated ( $10^{-9}$  M) histamine secretion ( $3.9 \pm 0.4$  nmoles/ $10^3$  cells). Control levels are  $3.0 \pm 0.2$  nmoles/ $10^3$  cells. Imetit dose-dependently inhibited histamine release with an  $IC_{50}$  of  $10^{-9}$  M. Maximum inhibition was 38 percent ( $2.4 \pm 0.6$  nmoles/ $10^3$  cells) ( $p < .05$  vs. gastrin) ( $n = 3$ ).



**Figure 5.** The effect of the  $H_3$  receptor antagonist, Thioperamide, on gastrin-stimulated ( $10^{-9}$  M) histamine secretion ( $3.9 \pm 0.4$  nmoles/ $10^3$  cells). Control levels are  $2.9 \pm 0.18$  nmoles/ $10^3$  cells. Thioperamide dose-dependently augmented gastrin driven release with an  $EC_{50}$  of  $7 \times 10^{-10}$  M and an  $EC_{max}$  of  $10^{-8}$  M ( $5.7 \pm 0.5$  nmoles/ $10^3$  cells). The maximal augmentation was 146 percent vs. gastrin ( $p < .05$ ) ( $n = 3$ )

(Figure 4). The higher dosage of imetit was not used due to the concern of non-specific effects.

**Thioperamide:** The  $H_3$  receptor antagonist thioperamide ( $10^{-10}$  to  $10^{-6}$  M) did not alter basal histamine secretion. However, pretreatment with thioperamide augmented a sub-maximal dose of gastrin-stimulated (1 nM) histamine secretion with a maximal effect (146 percent) at  $10^{-8}$  M and an  $EC_{50}$  of  $7 \times 10^{-10}$  M (Figure 5). This value is consistent with the reported affinity of thioperamide for the  $H_3$  receptor subtype ( $4 \times 10^{-9}$  M) [10].

**Thioperamide then imetit, then gastrin:** To determine the specificity of the effect of  $H_3$  receptor on gastrin-stimulated histamine secretion, cells were pretreated with  $H_3$  receptor antagonist thioperamide (10 nM) followed by the  $H_3$  receptor agonist imetit ( $10^{-10}$  to  $10^{-6}$  M) and then stimulated with gastrin (1 nM). This abolished the previously observed imetit mediated inhibition of gastrin-stimulated histamine secretion (data not shown).

Histamine has been demonstrated to have a substantially lower affinity (18-fold less) but a similar efficacy for the  $H_3$  receptor subtype compared with the specific  $H_3$  receptor agents [9]. The low concentrations of  $H_3$  agents required to alter histamine secretion support the presence of an  $H_3$  receptor on the ECL cell. It is probable that histamine, albeit at a higher concentration, has a similar effect. Pharmacological manipulation of the  $H_3$  receptor with highly specific agents, therefore, demonstrates unequivocally that receptor activation of the ECL cell results in inhibition of histamine release.

## DISCUSSION

Although the role of histamine in normal physiology has been widely investigated, its biological relevance is incompletely understood. Nevertheless, compounds that selectively antagonize the actions of histamine at these specific cell surface receptors are of considerable clinical utility. Histamine has been determined to exert its complex physiologic and pathologic effects through interaction with at least three receptor subtypes ( $H_1$ ,  $H_2$  and  $H_3$ ) [7, 24] identified on numerous different cell systems including neural and gastrointestinal. Message transduction via the  $H_1$  receptor has been shown to involve alterations in calcium homeostasis and increased production of inositol phosphate, while  $H_2$  receptors are reported to act through elevation of cAMP [3]. The mode of action of  $H_3$  receptors is unknown but it is thought to be negatively coupled to phosphatidylinositol turnover [25].

A role for the  $H_1$  receptor in the regulation of gastric histamine secretion is both unclear and controversial but has been suggested from the results of experiments with anesthetized dogs with gastric fistulas. In these experiments, infusion of an  $H_1$ -receptor agonist (2', 2-pyridyl ethylamine) had no effect on dimaprit stimulated acid secretion [26]. However, infusion of the specific receptor antagonist, hydroxyzine dihydrochloride, enhanced both histamine and dimaprit stimulated gastric acid secretion. Removal of an autoinhibitory effect of histamine on histamine secretion from the ECL cell by an  $H_1$  receptor antagonist might explain these data but the precise interpretation of such studies in intact animals has been difficult. The presence of  $H_1$  receptors in the gastric mucosa has been suggested by binding studies. In preliminary studies, [ $^3$ H]Mepyramine binding in gastric tissue samples from the African rodent *Praomys natalensis* indicated low levels of  $H_1$ -receptors in both antrum and fundus, ~15 fmol/mg protein [27]. The further possibility that the receptor sites were present on ECL cells was supported by an increased degree of binding in ECL cells isolated from fundic ECL carcinoid tumors (Schwartz and Modlin, unpublished data).

In order to evaluate the role of the  $H_1$  receptor directly, we used the agents TMPH and terfenadine. TMPH is a 3-trifluoromethyl derivative of histamine, which is a specific  $H_1$  receptor agonist, and has been reported to be similarly potent to histamine in the guinea pig ileum assay [23]. Terfenadine is a selective, slow-associating  $H_1$  receptor antagonist, which forms a stable complex and has been demonstrated to have a  $IC_{50}$  of 0.2  $\mu$ M for ileal  $H_1$  receptors [28]. In an isolated purified preparation of ECL cells, the  $H_1$  receptor agonist, TMPH (at high dosage), significantly inhibited both basal and a maximal dose of gastrin-stimulated (10 nM) histamine secretion with  $IC_{50} > 0.5 \mu$ M. However, the  $H_1$  receptor antagonist, terfenadine, had no effect on either basal or gastrin driven histamine release. Although these results suggest that ECL cell histamine secretion can be modulated by the  $H_1$  receptor subtype, the high concentrations required are of concern. In addition, since the  $H_1$  receptor clearly modulates proliferation both *in vivo* and *in vitro*, it seems more likely that the observed effects reflect a degree of non-selectivity or cross-reactivity with the  $H_3$  receptor subtype generated by high dosages of a pharmacological compound.

The histamine-2 receptor has been identified on the parietal cell, and pharmacological blockade has proved efficacious in the treatment of patients with acid related disorders. The presence of an  $H_2$  receptor and evidence for an  $H_2$  mediated feed-back inhibition on ECL cell histamine release was initially proposed in 1978 [22]. Histamine has been demonstrated both to inhibit HDC activity *in vivo* and to decrease the half-life of the enzyme [22]. Intravenous infusion of histamine in rats treated with the  $H_2$  receptor blocker, metiamide, resulted in a significantly reduced suppression of HDC activity and in addition, pentagastrin-induced HDC activity in the fundic mucosa was significantly increased. The effect of these agents on histamine secretion was not determined. Based on this information,



Håkanson postulated that H<sub>2</sub> receptor blockade might be mediated at the level of the ECL cell rather than the parietal cell. Our studies, utilizing a purified population of ECL cells, demonstrated that the specific H<sub>2</sub> receptor agonist, dimaprit, had no effect on either basal or gastrin-stimulated histamine secretion. Similarly, the specific H<sub>2</sub> antagonists, cimetidine and loxidine, had no effect on histamine secretion. In separate studies, we have reported that neither H<sub>2</sub> receptor activation nor blockade altered basal or gastrin-stimulated histamine secretion or DNA synthesis [27], suggesting that there is no evidence for a role of the H<sub>2</sub> receptor subtype in the regulation of the ECL cell.

The histamine-3 receptor was initially described in the brain, where it inhibits histamine synthesis and release [29]. H<sub>3</sub> receptors were subsequently identified in the enteric ganglia of the gastrointestinal tract, and evidence provided to support a role in the modulation of cholinergic neurotransmission [30]. More recently, it has been reported that this class of histamine receptor subtype is involved in the control of gastric acid secretion. Evidence for the existence of a gastric H<sub>3</sub> receptor was initially derived from *in vivo* studies utilizing fistula cats [14, 31]. Acid secretion stimulated by meal or pentagastrin was potently and dose-dependently inhibited by the specific H<sub>3</sub> agonist, R-MHA. This activation could be reversed by the specific antagonist, thioperamide. Similar findings were observed in a denervated Heidenhain pouch, suggesting that extrinsic cholinergic vagal nerves were not involved in this process. In addition, in the isolated rat stomach, R-MHA inhibited both basal and gastrin-stimulated vascular histamine release in a ranitidine-insensitive manner [18], suggesting direct modulation of histamine receptors on histamine secreting cells. Indeed to date, no evidence exists for the presence of H<sub>3</sub> receptors on the parietal cell. Furthermore, histamine inhibited HDC activity in isolated rabbit mucosal cell preparations can be augmented by R-MHA and reversed by thioperamide [20]. Histamine thus appears to inhibit its own synthesis via H<sub>3</sub> autoreceptors within the gastric mucosa. Evidence for the functional presence of an H<sub>3</sub> receptor in isolated rat ECL cells was reported by Prinz et al. [2]. Their results indicated a specific inhibition of maximal CCK-8 (10<sup>-8</sup> M) stimulated histamine release by R-MHA with a maximum effect at 10<sup>-6</sup> M. In addition, the effects of a sub-maximal dose (10<sup>-10</sup> M) of CCK-8 could be augmented by thioperamide maximally at 10<sup>-8</sup> M. In order to evaluate the role of the H<sub>3</sub> receptor directly, we used the agents imetit and thioperamide. Imetit is a potent, selective H<sub>3</sub> receptor agonist, which is equipotent to R-MHA. Both agents exhibit ~3x the inhibitory effect of histamine for [<sup>3</sup>H]-histamine release from rat cortical slices [29]. Thioperamide is a competitive H<sub>3</sub> receptor antagonist with little cross-reactivity for either the H<sub>1</sub> or H<sub>2</sub> receptors [10]. Using similarly isolated cells that were allowed to recover following overnight culture, we have demonstrated that imetit specifically inhibits a submaximal dose of gastrin (10<sup>-9</sup> M) driven histamine secretion with an IC<sub>50</sub> of 10<sup>-9</sup> M and a maximal degree of inhibition of ~40 percent. Thioperamide dose-dependently augmented histamine secretion with an EC<sub>50</sub> of 7x10<sup>-10</sup> M. Maximal augmentation (~50 percent) occurred at a dose of 10<sup>-8</sup> M. These values are consistent with previous *in vivo* and *in vitro* studies. The specificity of this effect was demonstrated by pretreatment of the ECL cells with the receptor antagonist (thioperamide), which abolished the inhibitory effect of Imetit on gastrin driven histamine secretion. The demonstration of both incomplete attenuation and augmentation of histamine release by these agents suggests that gastrin-mediated histamine release may be effectively modulated or fine tuned by histamine 3 receptor subtype activation.

Since a receptor may present binding sites with differing affinity states for a hormone, and we have previously demonstrated that histamine effectively modulates ECL cell proliferation *in vitro* [27], it is probable that the H<sub>3</sub> receptor functions in different affinity states which variously alter DNA synthesis or modify histamine secretion. Both the gastrin/CCK-B receptor and the CCK-A receptor are known to exhibit such phenomena [32].

In the ECL cell the EC<sub>50</sub> for gastrin mediated ECL cell DNA synthesis is an order of magnitude less than for histamine secretion [33, 34]. Both high and low affinity states for H<sub>3</sub> receptors have been demonstrated using radiolabelling of cerebral membranes [29]. High affinity binding has been proposed to modulate HDC activity, while low affinity binding is thought to regulate histamine secretion. Low levels of basal histamine release are postulated to down-regulate histamine synthesis, while high concentrations of histamine consequent upon stimulated secretion inhibit histamine release [20]. Our results demonstrate that H<sub>3</sub> receptor activation results in substantial inhibition of histamine release. It is probable that histamine released by gastrin would remain in very close proximity to the ECL cell and result in accumulation of histamine at a high local concentration. Under these circumstances, it is feasible that histamine induces a local feedback inhibitory effect via the H<sub>3</sub> receptor and thus downregulates the initial systemic hormonal signal. It is, therefore, possible that in the gastric mucosa a component of the physiologic function of histamine receptor subtypes may be to exert tonic inhibition of acid secretion via regulation of ECL cell histamine secretion and synthesis.

The utility of pharmacological compounds to define specific receptor subtype activity is limited by the specificity of such agents. Thus, it cannot be excluded that the observed effects of TMPH at high dosage reflect an effect on the H<sub>3</sub> receptor. Nevertheless, the effect of the various histamine receptor subtype probes clearly indicates a role for the histamine receptor subtype 3 in the modulation of histamine secretion. The validity of this proposal is further supported by the autoradiographic demonstration of the H<sub>3</sub> receptor on the isolated ECL cell [27]. Given the critical position of the ECL cell in the regulation of acid secretion, the existence of a histamine-activated autoregulatory secretory mechanism is of considerable physiological and possibly clinical relevance.

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