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• MAST CELL AND INFLAMMATORY BOWEL DISEASE •

# Modulation of tryptase secretion from human colon mast cells by histamine

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## Abstract

**AIM:** To investigate the ability of histamine to modulate tryptase release from human colon mast cells and the potential mechanisms.

**METHODS:** Enzymatically dispersed cells from human colons were challenged with histamine, anti-IgE or calcium ionophore A23187 (CI), and the cell supernatants after challenge were collected. Tryptase release was determined with a sandwich ELISA procedure.

**RESULTS:** Histamine at concentrations from 1 ng/mL was able to induce a "bell" shape dose related release of tryptase from colon mast cells. The maximum release of tryptase was approximately 3.5 fold more than spontaneous release. As little as 10 ng/mL histamine showed a similar potency to 10 μg/mL anti-IgE in induction of tryptase release. Histamine induced release of tryptase initiated at 10 s when histamine (100 ng/mL) was added to cells, gradually increased thereafter, and completed at 5 min. Both pertussis toxin or metabolic inhibitors were able to inhibit histamine induced tryptase release. When histamine and anti-IgE were added to colon mast cells at the same time, the quantity of tryptase released was similar to that induced by anti-IgE alone. The similar results were observed with CI. However, when various concentrations of histamine were incubated with cells for 20 min before adding anti-IgE or CI, the quantity of tryptase released was similar to that was induced by histamine alone.

**CONCLUSION:** Histamine is a potent activator of human colon mast cells, which represents a novel and pivotal self-amplification mechanism of mast cell degranulation.

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## INTRODUCTION

It has been reported that mast cells and their inflammatory mediators are closely associated to a number of intestinal diseases including idiopathic inflammatory bowel disease[1], chronic ulcerative colitis[2], Crohn's disease[3] and collagenous colitis[4]. Through release their proinflammatory mediators including histamine, tryptase, chymase, heparin and some cytokines[5], mast cells actively participate in the pathogenesis of these intestinal diseases.

Tryptase is a tetrameric serine proteinase that constitutes some 20% of the total protein within human mast cells and is stored almost exclusively in the secretory granules of mast cells[6] in a catalytically active form[7]. Relatively higher secretion of tryptase has been detected in ulcerative colitis[8], implicating that this mediator is involved in the pathogenesis of intestinal diseases. Evidence is emerging that tryptase may be a key mediator of allergic inflammation and a promising target for therapeutic intervention[9] as it has been found to be able to induce microvascular leakage in the skin of guinea pig[10], bronchoconstriction[11] in allergic sheep airways, inflammatory cell accumulation in peritoneum of mouse[12] and release of IL-8 from epithelial cells[13]. Moreover, tryptase has long been recognised as a marker of mast cells[14,15], and an indicator of mast cell degranulation *in vivo*[16,17]. However, the application of tryptase as a unique marker of mast cell degranulation in mast cell challenge study *in vitro* was only started recently[18]. This was largely due to the lack of adequate assay to detect this mast cell product.

Histamine, on the other hand, has been widely employed as a marker of mast cell degranulation in mast cell challenge studies over the last four decades. But as a activator of mast cells it has hardly been examined. Since increased levels of histamine or enhanced histamine metabolism have been observed in collagenous colitis, food allergy[19], Crohn's disease[20], ulcerative colitis[20,21] and allergic enteropathy[21], this proinflammatory mediator is likely to participate in the pathogenesis of these diseases. In the current study, we investigated the potential of histamine to activate human colon mast cells *in vitro* in order to understand further the role of histamine in inflammatory bowel diseases.

## MATERIALS AND METHODS

### Reagents

The following compounds were purchased from Sigma (St. Louis, Mo., USA): CI, histamine dihydrochloride, collagenase (type I), hyaluronidase (type I), antimycin A, 2-deoxy-D-glucose, pertussis toxin, bovine serum albumin (BSA, fraction V), penicillin and streptomycin, extravidin peroxidase, *o*-phenylene diamine, biotin conjugate sheep anti-mouse immunoglobulins, extr-avidin peroxidase. Minimum essential medium (MEM) containing 25 mM *N*-2-hydroxylethylpiperazine-*N*' -2-ethane sulphonic acid (HEPES) and FCS was from Gibco (Paisley, Renfrewshire, UK). Goat anti-human IgE (inactivated) was from Serotec (Kidlington, Oxford, UK). A polyclonal antibody and a monoclonal antibody against tryptase were donated by Dr. Andrew F. Walls (University of Southampton, UK). HEPES and all other chemicals were of analytical grade.

### Dispersion of mast cells

The mast cell dispersion procedure was similar to that described

previously[21,22]. Human colon tissue was obtained from patients with carcinoma of colon at colectomy. Only macroscopically normal tissue was used for the study. After removal of fat, the tissue was washed and chopped finely with scissors into fragments of 0.5-2.0 $mm^3$, and then incubated with 1.5 mg/mL collagenase and 0.75 mg/mL hyaluronidase in MEM containing 2% fetal calf serum (1 g colon/10 mL buffer) for 70 min at 37 ℃. Dispersed cells were separated from the undigested tissue by filtration through a nylon gauze (pore size 100 μm diameter), washed and maintained in MEM (containing 10% FCS, 200 U/mL penicillin, 200 μg/mL streptomycin) on a roller overnight at room temperature. Mast cell purity, as determined by light microscopy after staining by Alcine blue, ranged from 3.6% to 5.8%.

### *Mast cell challenge*

Dispersed cells were resuspended in HEPES buffered salt solution (HBSS, pH 7.4) with 1.8 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (complete HBSS), and 100 μL aliquots containing 4-6×$10^3$ mast cells was added to 50 μL histamine, anti-IgE, CI or buffer alone and incubated for 15 min at 37 ℃. The reaction was terminated by the addition of 150 μL ice cold incomplete HBSS and the tubes were centrifuged immediately (500 g, 10 min, 4 ℃). All experiments were performed in duplicate. For the experiments with pertussis toxin, cells were incubated with 0.1 or 1.0 μg/mL pertussis toxin for four hours at 37 ℃, and then washed with HBSS before adding stimulus. Similarly, for the experiments with metabolic inhibitors, cells were incubated with 2-deoxy-$_D$-glucose (10 mmol/L) and antimycin A (1 μmol/L) for 40 min at 37 ℃ before challenged with stimulus. Supernatants were stored at -20 ℃ until use. As reported previously that 10 μg/mL anti-IgE and 1 μg/mL CI were the optimal concentrations for the induction of tryptase released from human colon mast cells[18], therefore they were selected as the standard concentrations throughout the study.

### *Tryptase measurement*

Tryptase concentrations were measured using a sandwich ELISA procedure with a specific polyclonal antibody against human tryptase as the capture antibody and AA5 a monoclonal antibody specific for human tryptase as the detecting antibody[23].

### *Statistical analysis*

Data were shown as mean±SEM for the number of experiments ($n$) indicated, and the paired Student' s $t$ test was applied to evaluate two independent samples. In all analyses, $P<0.05$ was taken as statistically significant.

## RESULTS

### *Induction of tryptase release by histamine*

Histamine at the concentration of 1 ng/mL was able to induce a ' bell' shape dose related release of tryptase from colon mast cells. The maximal release of tryptase was approximately 3.5 fold more than spontaneous release provoked by 100 ng/mL histamine. Relatively less tryptase was released when 1 000 ng/mL and 10 000 ng/mL histamine were incubated with mast cells. As little as 10 ng/mL histamine showed a similar potency to 10 μg/mL anti-IgE in induction of tryptase release from colon mast cells (Figure 1).

### *Time course for histamine induced tryptase release*

Histamine induced release of tryptase initiated at 10 s when histamine (100 ng/mL) was added to cells, gradually increased thereafter, and completed at 5 min (Figure 2).

### *Effects of pertussis toxin and metabolic inhibitors on histamine induced tryptase release*

Tryptase release induced by histamine was reduced to the baseline level by pretreatment of colon mast cells with pertussis toxin or metabolic inhibitors. The same treatment was also able to slightly decrease the spontaneous tryptase release from mast cells (Table 1).

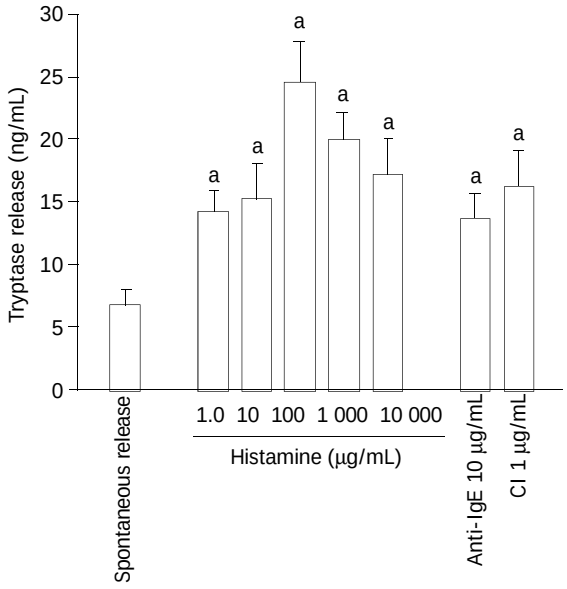


**Figure 1** Histamine, anti-IgE and calcium ionophore (CI) induced tryptase release from colon mast cells. The values shown are mean±SEM for six separate experiments. Stimulus or HBSS alone was incubated with cells for 15 min before termination of the reactions. [a]$P<0.05$ compared with spontaneous release group (paired Student's $t$ test).

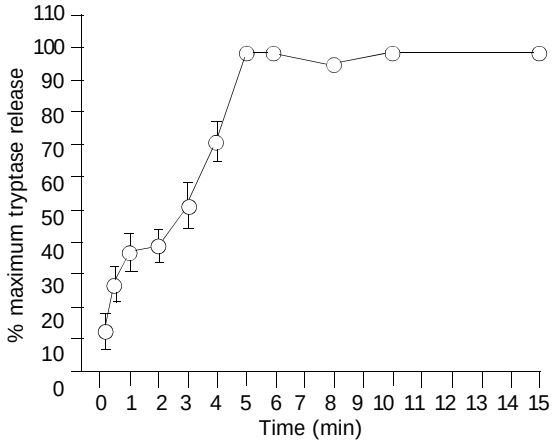


**Figure 2** Time course for histamine (100 ng/mL) induced release of tryptase from colon mast cells. Data shown are mean±SEM of five separate experiments.

**Table 1** Effects of pertussis toxin (1.0 μg/mL) and metabolic inhibitors on histamine induced tryptase release from colon mast cells

| Treatment | Tryptase release (ng/mL) | | |
|---|---|---|---|
| | Hist 100 μg/mL | Hist 1 000 μg/mL | Buffer alone |
| Untreated | 32±5.5 | 30.5±3.9 | 20.5±2.8 |
| Pertussis toxin | 17.1±1.8[a] | 16.7±1.7[a] | 17.6±2.7 |
| Metabolic inhibitors | 18.8±3.5[a] | 17.5±1.9[a] | 16.3±1.9 |

The values shown are mean±SEM of five separate experiments performed in duplicate. [a]$P<0.05$ compared with the untreated group (paired Student's $t$ test). Metabolic inhibitors=1 μM antimycin A+10 mM 2-deoxy-$_D$-glucose. Hist=histamine.

### *Effect of histamine on anti-IgE and CI induced tryptase release*

When 100 ng/mL or 1 000 ng/mL histamine and anti-IgE were added to colon mast cells at the same time, tryptase release was significantly less than that induced by addition of the corresponding concentration of histamine alone. The similar results were observed when the same concentrations of histamine were added to cells at the same time with CI, though there was no significant difference between histamine alone and histamine plus CI (Figure 3). However, addition of various concentrations of histamine to colon mast cells 20 min before placing anti-IgE or CI, tryptase release was similar to that induced by the corresponding histamine alone, except for 10 ng/mL histamine with CI, indicating a synergistic action between them (Figure 4).

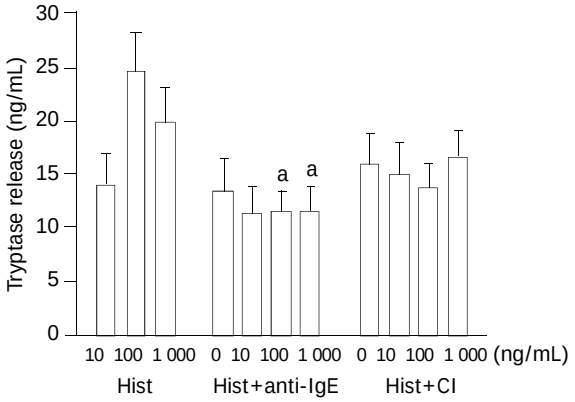


**Figure 3** Effect of co-addition of histamine (hist) and anti-IgE or calcium ionophore (CI) on tryptase release from colon mast cells. The values shown are mean±SEM for six separate experiments. Various concentrations of histamine and anti-IgE or CI were added to cells at the same time, and then incubated with cells for 15 min before termination of the reactions. [a]$P<0.05$ compared with the corresponding histamine alone group (paired Student's *t* test).

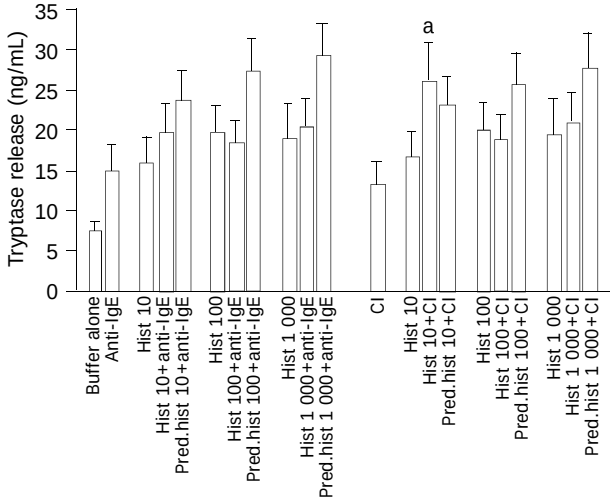


**Figure 4** Effect of histamine (hist) on anti-IgE or calcium ionophore (CI) induced tryptase release from colon mast cells. The values shown are mean±SEM for six separate experiments. Various concentrations of histamine were incubated with cells for 20 min before addition of anti-IgE or CI. [a]$P<0.05$ compared with the corresponding histamine alone group (paired Student's *t* test). Pred.hist + anti-IgE or CI=the sum of tryptase release induced by histamine alone and anti-IgE or CI alone.

## DISCUSSION

The finding that histamine is a potent activator of human colon mast cells demonstrated a novel and pivotal self-amplification mechanism of human mast cell degranulation, that is mast cells release histamine upon degranulation, and then the released histamine activates the adjacent mast cells. Thus, there are at least two self-amplification mechanisms in human mast cells upon degranulation, including tryptase and PAR-2 feedback process previously reported[22,23] and histamine inducing tryptase release.

Histamine appeared a potent secretogogue of tryptase, as little as 10 ng/mL histamine could share a similar potency of 10 μg/mL anti-IgE. This was a surprising result, but it clearly demonstrate a novel self-amplification mechanism of mast cell degranulation as the concentration of histamine inside the mast cell granules was estimated over 100 m mol/mL[24]. Interestingly, histamine concentration at 1 000 ng/mL or above was able to induce less tryptase release from mast cells than 100 ng/mL, which may represent a novel self-protection mechanism of mast cells. Histamine induced tryptase release started slower than that elicited by anti-IgE, CI[18] or tc- LIGRLO[23], but completed 1 min earlier than that induced by anti-IgE and CI, indicating that it has its own activation-degranulation passway in mast cells. Pretreatment of cells with metabolic inhibitors antimycin A which blocks the oxidative phosphorylation process of cells, and 2-deoxy-D-glucose which blocks anaerobic metabolism pathway in cells, abolished the action of histamine, indicating that histamine induced release of tryptase is a non-cytotoxic process depending on cell energy supply. Tryptase release provoked by histamine was also inhibited by pretreatment of cells with pertussis toxin, suggesting that the degranulation process is associated with the activation of G-protein coupled receptors[25].

When histamine and anti-IgE were placed to mast cells at the same time, the quantity of released tryptase was similar to that elicited by anti-IgE alone, much less than that provoked by histamine alone, implicating that tryptase released from colon mast cells was mainly induced by anti-IgE, rather than by histamine. The reason for this was that the initiation of anti-IgE induced tryptase release was quicker than that induced by histamine, and mast cells only accepted one type of stimulation at a time[26,27]. Similar phenomena were observed with CI, and CI provoked mast cell degranulation apparently faster than that induced by histamine. In contrast, addition of various concentrations of histamine to colon mast cells 20 min before placing anti-IgE or CI, tryptase release was similar to that induced by the corresponding histamine alone, which proved further that colon mast cells were only able to respond to one optimal activation at a time, and the desensitized mast cells did not respond to any further stimulation. This behavior is an effective self-protection mechanism of mast cells. However, a synergistic action between 10 ng/mL histamine and CI, but not anti-IgE was observed, suggesting that mast cells experiencing a non-optimal stimulation may still have ability to respond to a further stimulation. In conclusion, histamine is a potent activator of human colon mast cells, and represents a novel and pivotal self-amplification mechanism of mast cell degranulation.

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