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Inhibition of histamine release from human mast cells by natural chymase inhibitors[1]

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ABSTRACT

AIM: To investigate the ability of natural chymase inhibitors to modulate histamine release from human mast cells. **METHODS:** Enzymatically dispersed cells from human lung, tonsil, and skin were challenged with anti-IgE or calcium ionophore A23187 in the absence or presence of the natural chymase inhibitors secretory leukocyte protease inhibitor (SLPI) and α_1-antitrypsin, then histamine release was determined. **RESULTS:** IgE-dependent histamine release from lung, tonsil, and skin mast cells were inhibited by up to 70 %, 61 %, and 62 %, respectively following incubation with α_1-antitrypsin (5000 nmol/L). SLPI 5000 nmol/L was also able to inhibit anti-IgE-dependent histamine released from lung, tonsil and skin mast cells by up to approximately 72 %, 67 %, and 58 %, respectively. While neither α_1-antitrypsin nor SLPI by themselves altered histamine release from lung, tonsil and skin mast cells, they were able to inhibit calcium ionophore-induced histamine release from lung and tonsil mast cells. **CONCLUSION:** Both α_1-antitrypsin and SLPI could potently inhibit IgE-dependent and calcium ionophore-induced histamine release from dispersed human lung, tonsil, and skin mast cells in a concentration-dependent manner, which suggested that they were likely to play a protective role in mast cell associated diseases including allergy.

INTRODUCTION

Mast cell degranulation is a key event in the pathogenesis of allergic disease. When allergens cross-linking their specific IgE on the surface of sensitized mast cell degranulation occurs. Upon degranulation, a range of mediators is released from mast cell including histamine, tryptase, chymase, heparin, and some cytokines[1].

Histamine is a proinflammatory mediator, which is selectively located in the granules of human mast cells and basophils and released from these cells upon degranulation. Increased level of histamine has been observed in a number of diseases including chronic urticaria[2], ischaemic heart disease patients undergoing coronaroangiography[3], allergic conjunctivitis[4], chronic otitis media and rhinitis[5], and asthma[6], indicating that this mediator is involved in the pathogenesis of these diseases.

For more than four decades, histamine has been widely used as a marker of mast cell degranulation *in vitro*, and numerous anti-allergic drugs such as sodium cromoglycate, lodoxamide, salbutamal, ketotifen, terfenadine, and cetirizine[7,8] and salmeteral[9] were re-

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ported to be able to inhibit anti-IgE induced histamine release from human mast cells. In recent years, inhibitors of tryptase[10] and chymase[11] were discovered to possess the ability to inhibit histamine release from human skin, tonsil, and synovial mast cells[12], suggesting these inhibitors are likely to be developed as a novel class of mast cell stabilizer. However, little is known about natural inhibitors of chymase and tryptase on histamine release from human mast cells. Since only one natural inhibitor of tryptase has been found in man and its effect on histamine release from human mast cell was examined previously[13], we investigated the effects of the natural chymase inhibitors α_1-antitrypsin and secretory leukocyte protease inhibitor (SLPI) on histamine release from human mast cells induced by IgE-dependent or independent stimuli in the current study.

MATERIALS AND METHODS

Dispersion of mast cells Human lung, tonsil, and skin tissue were obtained at lobectomy, tonsillectomy, and circumcision, respectively. Only macroscopically normal tissues were used for the study. The mast cell dispersion procedures employed were similar to that described previously[10]. Briefly, finely chopped tissue was incubated with collagenase 1.5 g/L (Sigma) and hyaluronidase 0.75 g/L (Sigma) in MEM (Gibco) containing 2 % foetal calf serum (1 g tonsil in 10 mL buffer, 1 g lung in 10 mL buffer, and 1 g skin in 15 mL buffer) for 60-70 min at 37 ºC. Dispersed cells were separated from undigested tissue by filtration through nylon gauze (pore size 100 μm diameter), washed and maintained in MEM (containing 10 % FCS, benzylpenicillin 200 kU/L, and streptomycin 200 mg/L) on a roller overnight at room temperature. Mast cell purity, as determined by light microscopy after staining by alcine blue, ranged from 2.5 % to 4.5 % for lung, 0.5 % to 1.1 % for tonsil and 3.5 % to 5.8 % for skin.

Mast cell challenge Dispersed cells were resuspended in HEPES buffered salt solution (HBSS, pH 7. 4) with $CaCl_2$ and $MgCl_2$ (complete HBSS), and 100 μL aliquots containing 4×10^3-6×10^3 mast cells were added to 50 μL anti-IgE (Serotec, UK), calcium ionophore (Sigma), or inhibitor in complete HBSS and incubated for 15 min at 37 ºC. 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 ºC). All experiments were performed in duplicate. For the measurement of total histamine concentration, in certain tubes the suspension was boiled for 6 min. Supernatants were stored at -20 ºC until histamine and tryptase concentrations were determined (in duplicate for each tube). Histamine concentration in supernatant was measured using a glass fibre-based, fluorometric assay[10]. As for our previous experiments, 1 % anti-IgE or calcium ionophore 1 μmol/L were selected as standard concentrations throughout the study.

Inhibition of release of histamine Where added, α_1-antitrypsin (Sigma) or SLPI (a gift from Dr Andrew F WALLS, University of Southampton, UK) were incubated with anti-IgE or calcium ionophore for 20–30 min on ice before addition to cells. Data were expressed as the percentage inhibition of histamine release, taking into account histamine release in the presence and absence of the inhibitor.

Statistical analyses Statistical analyses were performed by SPSS software. Data were expressed as mean±SD. Where analysis of variance indicated significant differences between groups with ANOVA, for the preplanned comparisons of interest, *t*-test was applied. For all analyses, $P<0.05$ was taken as significant.

RESULTS

Effects of α_1-antitrypsin, SLPI, anti-IgE, and calcium ionophore on histamine release from mast cells Anti-IgE was able to induce significant histamine release from mast cells obtained from all three tissues tested. Up to 22.8 %±2.6 % net histamine release was achieved with 1 % anti-IgE in tonsil cells. With the same concentration of anti-IgE, however only some 9.1 %±1.1 % net histamine release was observed with lung cells. Calcium ionophore appeared a much more potent stimulus than anti-IgE in provocation of histamine release from all three sources of mast cells with the maximum of 49.7 %±13.0 % net histamine release being achieved. Neither α_1-antitrypsin nor SLPI alone at the concentrations tested was able to provoke a histamine release from any of three sources of mast cells (Tab 1).

Inhibition of histamine release from mast cells by α_1-antitrypsin The concentration-dependent inhibition of anti-IgE-induced release of histamine from lung, tonsil, and skin mast cells were observed when α_1-antitrypsin and the stimulus were added to cells at the same time. Up to 70 %, 61 %, and 62 % inhibition of IgE-dependent histamine release from lung, tonsil and skin cells were achieved (5000 nmol/L), respectively with α_1-antitrypsin. There was no significant

Tab 1. The effect of α_1-antitrypsin, secretory leukocyte protease inhibitor (SLPI), anti-IgE, and calcium ionophore on histamine release from dispersed human mast cells. *n*=4-6 separate experiments performed in duplicate. Mean±SD. ^{b}*P*<0.05 compared with saline control. ND=not done.

Compound	Concentration	Net histamine release/%		
		Lung	Tonsil	Skin
α_1-antitrypsin	0.5 nmol/L	0.1±0.4	0.8±0.6	ND
	5.0	0.1±0.6	0.8±0.46	-0.2±3.4
	50	1.3±1.6	0.4±0.6	1.0±2.4
	500	0.6±0.5	0.5±0.8	0.5±2.4
	5000	0.7±0.5	0.8±0.3	1.1±1.2
SLPI	0.5 nmol/L	-0.3±1.0	0.7±1.2	ND
	5.0	0.6±0.7	-0.6±1.8	0.4±1.8
	50	0.7±0.8	-0.5±1.2	0.5±1.2
	500	1.1±1.6	-0.9±1.4	0.1±0.8
	5000	0.7±0.5	0.5±1.6	0.2 ±1.4
Anti-IgE 1 %		9.1±2.5^b	22.8±6.0^b	17.2±6.0^b
Calcium ionophore 1 mmol/L		33±20^b	50±13^b	35±16^b

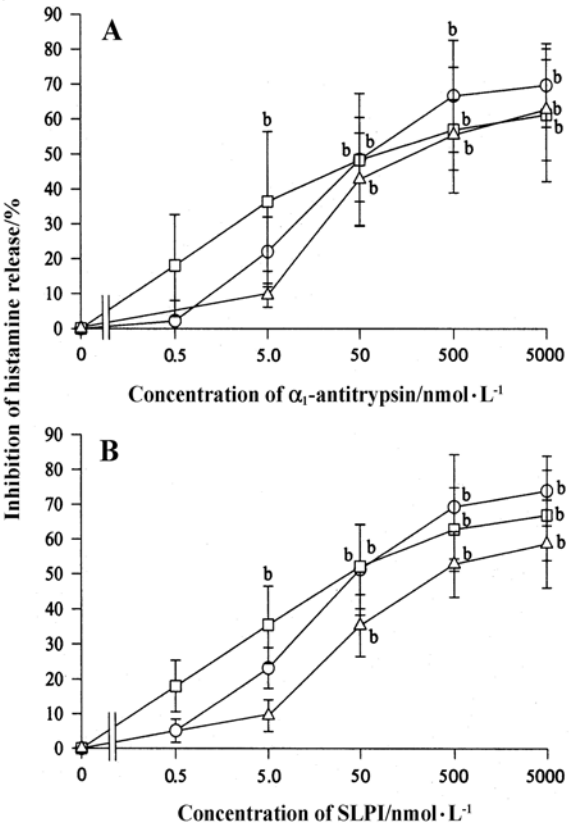

Fig 1. Inhibition of anti-IgE-induced histamine release from dispersed lung (- ○ -), tonsil (- □ -) and skin (- △ -) mast cells by (A) α_1-antitrypsin and (B) secretory leukocyte protease inhibitor (SLPI). The stimulus and inhibitor were incubated on ice for 20-30 min before adding to cells. *n*=4-6 separate experiments performed in duplicate. Mean±SD. ^{b}*P*<0.05 *vs* uninhibited controls.

difference between tissues examined in response to IgE-dependent stimulation (Fig 1A). Histamine release from lung and tonsil cells induced by calcium ionophore were inhibited also by α_1-antitrypsin in a concentration-dependent manner. Up to approximately 56 % and 72 % inhibition of calcium ionophore-induced histamine release from lung and tonsil cells were observed, with α_1-antitrypsin at 5000 nmol/L (Fig 2A).

Inhibition of histamine release from mast cells by SLPI Similar to α_1-antitrypsin, SLPI was also able to inhibit anti-IgE-dependent (Fig 1B) or calcium ionophore-induced histamine release (Fig 2B) from lung, tonsil, and skin (not done with calcium ionophore) mast cells in a concentration-dependent manner. Up to approximately 72 %, 67 %, and 58 % inhibition of anti-IgE-dependent histamine release from lung, tonsil, and skin cells respectively, or 64 % and 70 % inhibition of calcium ionophore-induced histamine release from lung and tonsil cells, respectively were achieved with SLPI at 5000 nmol/L.

DISCUSSION

Histamine has been widely used as a marker of mast cell degranulation *in vitro* as histamine released from basophils in dispersed cell suspension is negligible compared with that released from mast cells[14]. Therefore, inhibition of histamine release from dispersed cells represents the inhibition of mast cell degranulation.

Over the years, many compounds including sodium cromoglycate, lodoxamide, salbutamal, ketotifen, terfenadine, and cetirizine have been recognized as mast cell stabilizers or histamine receptor antagonists, and have been used as anti-allergic drugs in day-to-day clinical practice. However, only less than 40 % inhibition of IgE-dependent mast cell degranulation can be achieved with these compounds, which is much less than that achieved with inhibitors of tryptase and chymase in the similar experimental system[10,11]. The selective tryptase inhibitor APC366 and chymase inhibitor ZIGPFM were able to inhibit up to approximately 90 % or 80 % histamine release from dispersed human mast cells. While some weaknesses of these two compounds prevented them from being developed to anti-allergic agents the natural inhibitor of chymase and tryptase may

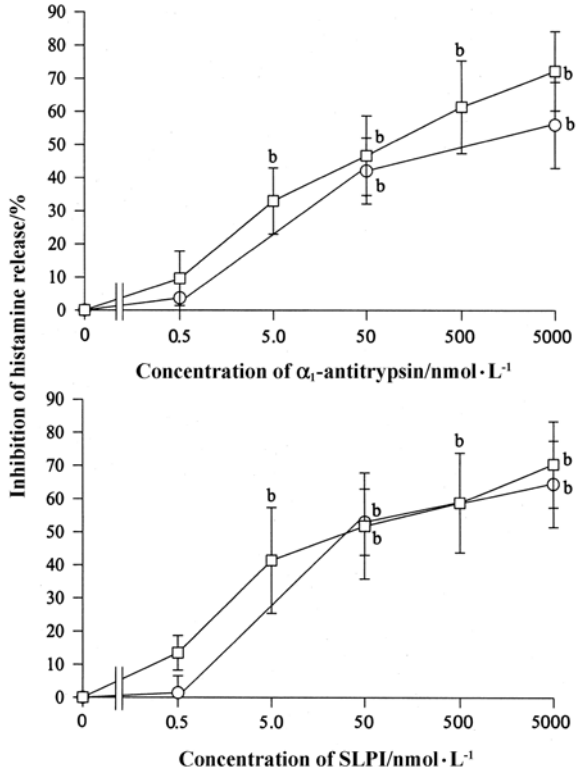

Fig 2. Inhibition of calcium ionophore induced histamine release from dispersed lung (- ○ -) and tonsil (- □ -) mast cells by (A) α_1-antitrypsin and (B) secretory leukocyte protease inhibitor (SLPI). The stimulus and inhibitor were incubated on ice for 20-30 min before adding to cells. *n*= 4-6 separate experiments performed in duplicate. Mean±SD. ^{b}*P*<0.05 *vs* uninhibited controls.

have the potential to be developed as anti-allergic drugs. The ability of SLPI[15] to prevent allergen-induced pulmonary responses in animal models of asthma and the observation that IgE-mediated histamine release from nasal mucosa could be inhibited by SLPI[16] supported the above view.

The concentrations of chymase inhibitors applied in the current study were chosen according to our previous study with α_1-antitrypsin and SLPI[17]. These concentrations of the inhibitors can potently (up to some 72 %) inhibit IgE-dependent histamine release from human mast cells and enzymatic activity of chymase (up to 93 %) suggested that they are likely to play a protective role in the pathogenesis of allergic disease. The previous report that SLPI was able to inhibit calcium ionophore-induced histamine release from tonsil mast cells[18] supported our findings in the current study, but inhibition of histamine release from human mast cells by α_1-antitrypsin seems has not been reported previously.

In conclusion, the finding, that the natural chyamse inhibitors α_1-antitrypsin and SLPI were able to inhibit IgE-dependent histamine release from various sources of human mast cells, demonstrated a novel mechanism, through which allergen-induced mast cell degranulation can be limited in man. Since α_1-antitrypsin is a circulating inhibitor and SLPI is mainly located in the mucosa they are likely to contribute to the local regulatory mechanism of mast cell degranulation both physiologically and pathophysiologically.

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