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Human mast cell tryptase: a stimulus of microvascular leakage and mast cell activation

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Abstract

We have investigated the potential of tryptase to stimulate an increase in microvascular permeability following injection into the skin of guinea pigs. Tryptase was isolated from high salt extracts of human lung tissue by octyl-agarose and heparin-agarose chromatography. Injection of purified tryptase (2.5 ng–2.5 μg/site) into the skin of guinea pigs which had been injected intravenously with Evans blue dye provoked a dose-dependent increase in microvascular permeability. The skin reactions elicited by tryptase were apparent up to 80 min following injection, while histamine-induced microvascular leakage resolved completely by 40 min. Heat-inactivation of tryptase, or preincubating the proteinase with certain proteinase inhibitors, significantly reduced the extent of microvascular leakage, suggesting dependency on an intact catalytic site. No evidence was found for a synergistic or antagonistic interaction between tryptase (2.5 ng–2.5 μg/site) and histamine (1–10 μg/site) when these mast cell products were injected together. Addition of heparin to tryptase (10:1; w/w) prior to injection was without effect on tryptase-induced microvascular leakage. Pretreatment of guinea pigs with a combination of the histamine H_1 receptor antagonist pyrilamine and the histamine H_2 receptor antagonist cimetidine (both 10 mg/kg), partially abolished tryptase-induced microvascular leakage as well as attenuating the reaction to histamine. Reasoning that the microvascular leakage induced by tryptase is likely to involve the release of histamine, we investigated the ability of tryptase to stimulate histamine release from dispersed guinea-pig skin and lung cells in vitro. Tryptase was found to induce concentration-dependent histamine release from both sources of tissue. Mast cell activation stimulated by tryptase in vitro was inhibited by heat treating the enzyme or by addition of proteinase inhibitors, suggesting a requirement for an intact catalytic site. Histamine release was inhibited also by preincubating cells with the metabolic inhibitors antimycin A and 2-deoxy-D-glucose indicating that the mechanism was energy-requiring and non-cytotoxic. We conclude that human mast cell tryptase may be a potent stimulus of microvascular leakage. The activation of mast cells by this proteinase may represent an amplification process in allergic inflammation.

Keywords: Tryptase; Microvascular leakage; Mast cell; Histamine; Heparin; Proteinase inhibitor; Histamine receptor antagonist

1. Introduction

Mast cells are major participants of allergic reactions. Their activation may be all that is sufficient and necessary for the rapid development of microvascular leakage and tissue oedema in sensitized subjects exposed to allergen. The mast cell is a source of potent mediators of inflammation including histamine, neutral proteinases, proteoglycans, prostaglandin D_2, leukotriene C_4 and certain cytokines (Bradding et al., 1995). The pathophysiological role of many of these products has been studied extensively, and several have become targets for therapeutic intervention in allergic disease. However, relatively little is known of the role of the neutral proteinase tryptase, the most abundant product of the human mast cell.

Tryptase, a tetrameric serine proteinase with molecular weight of 134 kDa (Schwartz et al., 1981; Smith et al., 1984) is present in all mast cells (Walls et al., 1990a) and constitutes more than 20% of total cell protein on a weight basis (Schwartz, 1990). There is some 10–35 pg of tryptase per cell, compared with less than 2 pg of histamine per human mast cell (Schwartz et al., 1987a). Tryptase is stored in the secretory granules of mast cells in a fully active form (Glenner and Cohen, 1960) and released on cell degranulation together with other preformed mediators in a complex of 200–250 kDa with proteoglycans (Goldstein et al., 1992).

Following exposure to allergen, tryptase levels may be

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dramatically increased in the bronchoalveolar lavage fluid of asthmatics (Wenzel et al., 1988), nasal lavage fluid of the subjects with allergic rhinitis (Hochstrasser, 1993), skin blister fluid of atopic subjects (Schwartz et al., 1987b), or in the serum of patients with systemic anaphylaxis (Schwartz et al., 1987c). Baseline concentrations of tryptase are elevated in bronchoalveolar lavage fluid from patients with asthma (Walls, 1995) and this has been observed also in patients with various other pulmonary conditions without an allergic aetiology, including cryptogenic fibrosing alveolitis, sarcoidosis, bronchial carcinoma (Walls et al., 1991) and even in smokers (Kalenderian et al., 1988).

An appreciation of the value of tryptase as a discriminating marker of mast cell activation in clinical disease has preceded a detailed understanding of its mediator actions. However, this enzyme has been found to cleave efficiently the potent vasodilator calcitonin gene-related peptide or the bronchodilators vasoactive intestinal peptide and peptide histidine-methionine (Tam and Caughey, 1990; Walls et al., 1992a), and to generate kinins from high and low molecular weight kininogens (Proud et al., 1988; Walls et al., 1992b). Tryptase can also have profound effects on cell behavior acting as a growth factor for fibroblasts (Ruoss et al., 1991) and epithelial cells (Cairns and Walls, 1996), and stimulating interleukin-8 release and upregulating expression of intercellular adhesion molecule-1 (ICAM-1) on epithelial cells (Cairns and Walls, 1996).

Investigating the actions of human tryptase in vivo presents particular difficulties. It is not ethical at present to transfer into man a preparation of this enzyme which has been purified from human tissues. However, useful information could be obtained from injecting human tryptase into laboratory animals. The recent observation that guinea-pig mast cells contain an enzyme which is analogous to human tryptase in its substrate and inhibitor profiles suggests that the guinea pig may be a suitable species for such studies (McEuen et al., 1996). We report here that tryptase is a potent stimulus of microvascular leakage when injected into the skin of guinea pigs and that the activation of mast cells may be important in mediating this response.

2. Materials and methods

2.1. Materials and drugs

The following compounds were purchased from Sigma (Poole, UK): α_1-antitrypsin, α_2-macroglobin, leupeptin, aprotinin, soybean trypsin inhibitor, antipain, *N*-benzoyl-DL-Arg-*p*-nitroanilide (BAPNA), *N*-succinyl-L-Ala-L-Ala-L-Ala-*p*-nitroanilide, *N*-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide, porcine heparin glycosaminoglycan, histamine diphosphate salt (for injection), histamine dihydrochloride (for histamine analysis), pyrilamine maleate, cimetidine, collagenase (type I), hyaluronidase (type I), bovine serum albumin (fraction V), penicillin and streptomycin, minimum essential medium (MEM) containing 25 mM *N*-2-hydroxylethylpiperazine-*N*′-2-ethane sulphonic acid (HEPES), heparin-agarose, Evans blue dye, goat anti-mouse immunoglobulin (Ig) G kit, calcium ionophore A23187, dimethyl sulphoxide (DMSO), 2-(*N*-morpholino)ethane-sulphonic acid (MES), Tris-base, antimycin A, 2-deoxy-D-glucose, goat anti-human IgE (inactivated). HEPES and other common chemical reagents were of analytical grade and were purchased from BDH (Poole, UK). Phthaldialdehyde was obtained from Fluka (Dorset, UK); Coomassie protein assay reagent from Pierce (Rockford, IL, USA); tissue kallikrein from Calbiochem (Beeston, UK); octyl-agarose from Pharmacia (Milton Keynes, UK); 3,3-diaminobenzidine tetrahydrochloride (DAB) from Dako (Glostrup, Denmark); pentobarbitone sodium from Sanofi Animal Health (Watford, UK) and Hypnorm from Janssen (Oxford, UK); ^{125}I-human serum albumin from Amersham (Little Chalfont, UK).

2.2. Purification and characterisation of tryptase

Tryptase was purified and characterised essentially as described previously (Walls et al., 1990b). Approximately 500 g quantities of chopped human lung tissues collected post mortem were blended and centrifuged first with distilled water and then three times with a low salt buffer (0.15 M NaCl, 10 mM MES and 0.02% sodium azide; pH 6.1). The homogenate was then extracted using a high salt buffer (2 M NaCl, 10 mM MES and 0.02% sodium azide; pH 6.1) and a salt fractionation step was employed with 2 M ammonium sulphate. The tryptic activity of the supernatant was filtered and purified by octyl-agarose and heparin-agarose chromatography. Tryptase fractions were concentrated in C-30 centrifugal concentrators (Amicon, Stonehouse, UK) and stored at −80°C until use in a buffer of 1.2 M NaCl, 10 mM MES, pH 6.1.

Tryptic activity was measured by monitoring spectrophotometrically at 410 nm, the hydrolysis of BAPNA, 20 mM in 0.1 M Tris-HCl, 1 M glycerol, pH 8.0 containing 1 mg/ml bovine serum albumin at 25°C. In order to investigate the possible presence of contaminating proteases in the final preparation, elastolytic activity was assayed using 1.4 mM *N*-succinyl-L-Ala-L-Ala-L-Ala-*p*-nitroanilide in the same buffer employed with BAPNA (Nakajima et al., 1979), and chymotryptic activity using 0.7 mM *N*-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide in 1.5 M NaCl, 0.3 M Tris, pH 8.0, and 5% ethanol. In all assays, 50 μl sample was added to a total reaction volume of 1 ml in a 1 cm pathlength cuvette. Multiple readings were taken and reaction rates calculated assuming a molar extinction coefficient of 8800 M^{-1} cm^{-1} for *p*-nitroanilide.

Protein concentrations were determined using Coomassie brilliant blue G (Bradford, 1976) with bovine serum albumin as standard. Sodium dodecyl sulphate-poly-

acrylamide gel electrophoresis (SDS-PAGE) with silver staining (Bio-Rad silver stain kit) revealed a single band of approximately 32 kDa (corresponding to that of disassociated monomers of tryptase). The identity as tryptase was confirmed by Western blotting with monoclonal antibody AA5 specific for tryptase (Walls et al., 1990b). The specific activity of tryptase was 1.6 U/mg where one unit of enzyme was taken as the amount that catalyzed the cleavage of 1 μmol of BAPNA per minute at 25°C. The endotoxin level was very low in the purified tryptase preparation employed, being 56 pg/mg protein measured with the Toxicolor system (Seikagaku, Tokyo, Japan) performed according to the manufacturer's instructions.

2.3. Animals and skin testing

Dunkin Hartley guinea pigs (600–1000 g) were anaesthetized by intraperitoneal injection of pentobarbitone saline (21 mg/kg) and intramuscular injection of Hypnorm (0.35 ml/kg containing 0.11 mg fentanyl citrate and 3.5 mg fluanisone). Skin testing was performed as described previously (Walls et al., 1987). The dorsal hair of the guinea pigs was shaved and 1% Evans blue dye in normal saline was injected intravenously (into the dorsal vein of a hind paw or ear vein) or into the heart (4 ml/kg). Tryptase and other compounds (50 μl) were injected intradermally into randomised sites placed 2–3 cm apart. Animals were kept warm, and 20 min following the final injection were killed by cervical dislocation and the skin removed. In separate experiments, the time-course of the response was investigated by injecting compounds intradermally into anaesthetised guinea pigs, and then at time points ranging from 0 to 360 min, the animals were injected with Evans blue dye as described above, and killed 20 min thereafter. Two perpendicular diameters were recorded for the blueing reaction on the inside of the skin, and multiplied to give a measure of the cutaneous oedema. In order to obtain further validation of the experimental procedures, for an additional group of animals, ^{125}I-albumin (1 μCi ^{125}I/kg) was included with the indicator dye, and after measurement of the blueing reaction, a 15 mm diameter circle of skin containing the injection site was excised, and radioactivity counted in a gamma counter. The cutaneous oedema in each injection site expressed as the radioactivity relative to that in plasma (^{125}I counts in skin at the injection site/ ^{125}I counts in 1 μl plasma) was found to correlate closely with the measurements of the blueing reaction, whether provoked by tryptase ($n = 8$, $r = 0.727$, $P = 0.0392$) or histamine ($n = 8$, $r = 0.738$, $P = 0.0345$; data not shown).

As tryptase is enzymatically unstable in physiological solutions (Schwartz and Bradford, 1986), considerable care was taken in its preparation. Purified tryptase stored in high salt buffer in the presence or absence of heparin was diluted immediately prior to its injection first with sterile distilled water, adjusting the NaCl concentration to 0.15 M, and then with normal saline to obtain the required tryptase concentration. Histamine, bradykinin and tissue kallikrein in saline acted as positive controls. Where added, protease inhibitors or the buffer alone were incubated for 30 min on ice before injection.

For certain guinea pigs a combination of 10 mg/kg pyrilamine plus 10 mg/kg cimetidine was injected, i.p., 30 min before intradermal injection of compounds. In preliminary experiments we found that administration of either of these antihistamines alone did not block completely the plasma extravasation induced by histamine (data not shown), an observation made previously by Owen et al. (1980).

2.4. Mast cell dispersion and activation

Naive Dunkin Hartley guinea pigs were killed by cervical dislocation and the shaved dorsal skin and the lungs removed and placed in Eagle's MEM containing 2% foetal calf serum. Mast cell dispersion was performed employing a procedure similar to that employed previously with human lung tissue (Church et al., 1982) and human skin tissue (Benyon et al., 1987). Lung or skin tissue was chopped finely with scissors into fragments of 0.5–2.0 mm^3, washed twice with MEM (500 × g, 5 min, 4°C), and incubated with 1.5 mg/ml collagenase and 0.75 mg/ml hyaluronidase in the same buffer (1 g lung tissue/10 ml buffer; 1 g skin tissue/15 ml buffer) for 75 min at 37°C. Dispersed cells were separated from undigested tissue by filtration through nylon gauze (pore size 100 μm diameter) and washed twice with MEM containing 2% foetal calf serum. Mast cell numbers were determined by light mi-

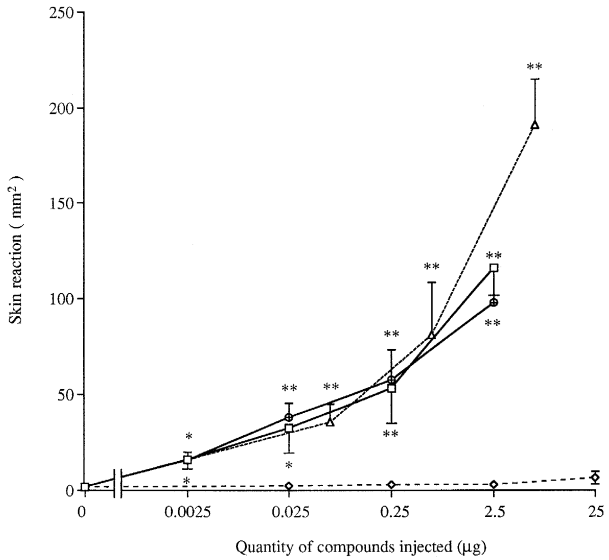

Fig. 1. Microvascular leakage in guinea pig skin 20 min following injection of tryptase alone (□), tryptase with heparin (1:10, ⊕), histamine (△) or heparin alone (◇). Mean ± S.E.M. are shown for seven experiments. * $P < 0.05$ and ** $P < 0.005$ compared with the reaction with saline (paired Student's t-test).

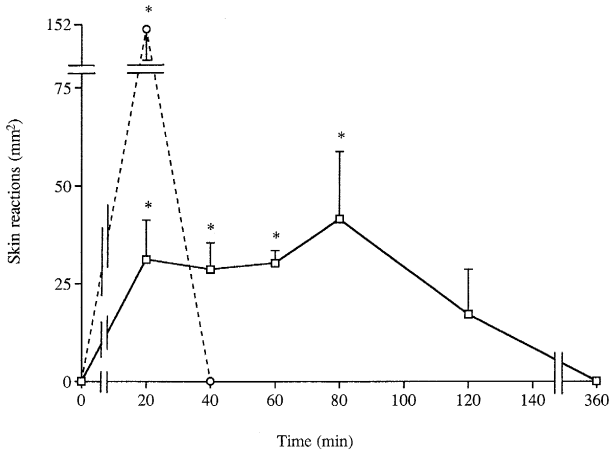

Fig. 2. Time-course of microvascular leakage in guinea-pig skin induced by tryptase (2.5 μg, □) and histamine (5 μg, ○). Values shown are the mean ± S.E.M. for three animals. * $P < 0.05$ compared with the reaction with saline control (paired Student's *t*-test).

croscopy after staining by the procedure of Kimura et al. (1973). Dispersed cells were maintained on a roller overnight at room temperature with MEM containing 10% FCS, 200 U/ml penicillin, 200 μg/ml streptomycin, and were washed twice with HEPES buffered salt solution (HBSS, pH 7.4) without added calcium or magnesium ($500 \times g$, 10 min, 20°C; Okayama et al., 1994).

Following resuspension in HBSS with 1.8 mM $CaCl_2$ and 0.5 mM $MgCl_2$, and warming at 37°C for 5 min, 100 μl aliquots containing $4–6 \times 10^3$ mast cells were added to either 50 μl purified tryptase, control secretagogue or inhibitor in HBSS, and incubated for 20 min, 37°C. Reactions were terminated by the addition of 150 μl ice-cold calcium and magnesium free HBSS and the tubes centrifuged immediately ($500 \times g$, 10 min, 4°C). All experiments were performed in duplicate. In certain tubes the suspension was boiled (100°C, 6 min) for subsequent measurement of total cellular histamine concentrations. Supernatants were stored at −20°C until histamine concentrations were determined.

A glass fibre-based, fluorometric assay for histamine was employed as described previously (Nolte et al., 1987, 1989). This procedure relies on the selective binding of histamine to a glass fibre matrix coated to wells of 96-well microtitre plates. Histamine release was expressed as a percentage of the level of total cellular histamine, and corrected for the spontaneous release which was measured in tubes in which cells had been incubated with the HBSS diluent alone.

2.5. Statistical analysis

Data were expressed as the mean ± S.E.M. for the number of experiments indicated (n), and analysed by paired Student's *t*-test. $P < 0.05$ was taken as significant.

3. Results

3.1. Microvascular leakage

Intradermal injection of human mast cell tryptase into the skin of guinea pigs induced a dose-dependent increase in microvascular permeability (Fig. 1). Even with quantities of tryptase as low as 2.5 ng, there was a significant increase in permeability compared with the saline control. Heparin was added routinely to tryptase (10:1, w/w) in order to reduce the rate of spontaneous inactivation of tryptase activity in vitro (Nolte et al., 1987). However, when tryptase was injected in the absence of heparin, the degree of microvascular leakage evoked was not signifi-

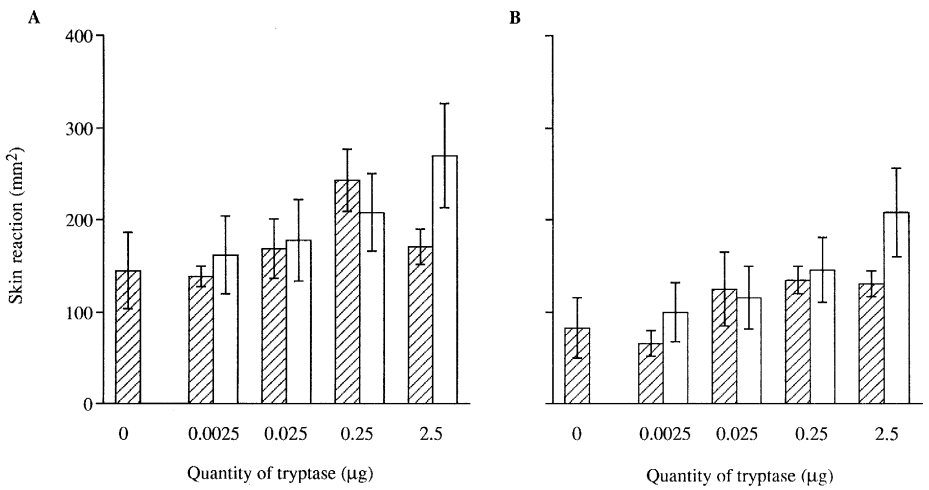

Fig. 3. Microvascular leakage induced by tryptase co-injected with (A) 5 μg or (B) 0.5 μg histamine. Mean ± S.E.M. are shown for experiments with six guinea pigs, involving injection of both histamine and tryptase together (hatched bars) or histamine and tryptase separately. In all cases the size of the skin reaction obtained with both mediators injected together did not differ significantly from the sum of the separate injections (open bars) (paired Student's *t*-test).

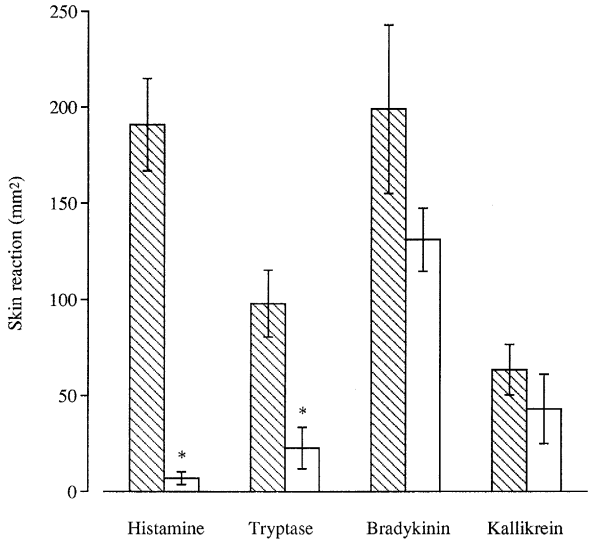

Fig. 4. Inhibition of skin reactions to histamine (5 μg, $n = 6$) and tryptase (2.5 μg, $n = 8$) by antihistamine pretreatment of guinea pigs. Antihistamine pretreatment showed no significant effect on the skin oedema induced by bradykinin (2.5 μg, $n = 5$) or tissue kallikrein (2.5 μg, $n = 7$). Data are shown as mean ± S.E.M. * $P < 0.005$ compared with uninhibited control (paired Student's *t*-test). Hatched bars: untreated; open bars: antihistamine treated.

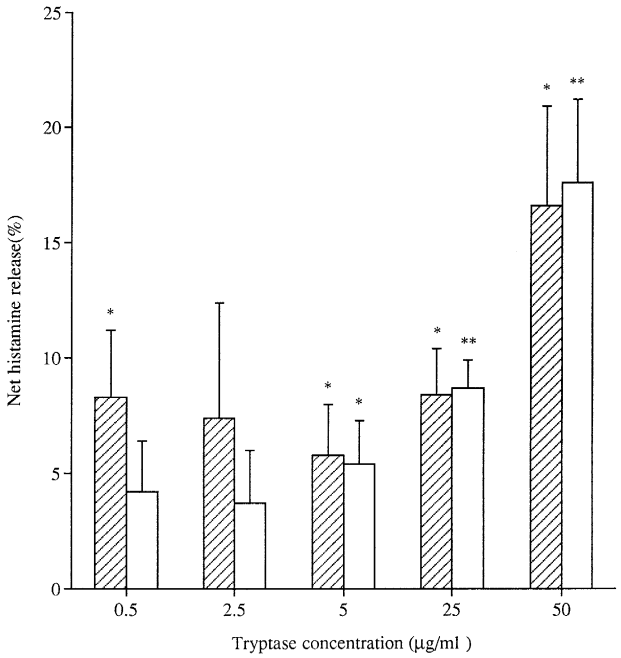

Fig. 5. Net histamine release from dispersed guinea pig lung (hatched bars) or skin (open bars) cells in response to tryptase. Values shown are mean ± S.E.M. for $n = 4–6$ separate experiments. * $P < 0.05$ and ** $P < 0.01$ in comparison with baseline control (paired Student's *t*-test).

cantly different from that obtained with the heparin added. Injection of heparin alone over the range 25 ng–25 μg did not stimulate an increase in microvascular permeability. The response to tryptase was prolonged, still being apparent 80 min following injection. However, there was complete resolution by 360 min (Fig. 2).

On a weight basis, tryptase had a potency similar to that of histamine in inducing microvascular leakage. No evidence was found for a synergistic interaction between these mast cell mediators. Co-injection of 5 μg histamine with tryptase in a range of different concentrations resulted in a skin reaction of a magnitude not significantly different from the sum of the responses to tryptase and histamine alone (Fig. 3A). A similar additive response was obtained when the same range of tryptase concentrations was injected with 0.5 μg histamine (Fig. 3B).

Pretreatment of guinea pigs with the antihistamines pyrilamine and cimetidine abolished the skin reaction to both tryptase and histamine, but not to tissue kallikrein and bradykinin (Fig. 4). When tryptase (2.5 μg) was preincubated with proteinase inhibitors (10 μg for each inhibitor) prior to injection there was a significant reduction in the skin response with leupeptin, soybean trypsin inhibitor and α_1-antitrypsin (Table 1). All proteinase inhibitors tested in the present study had no effect on microvascular leakage when injected alone into the skin. Heat treatment of tryptase

Table 1
Inhibition of tryptase-induced microvascular leakage by proteinase inhibitors or by heat treatment of tryptase

Inhibitors/treatment	Percentage inhibition of skin reaction	
	Tryptase	Histamine
None	0	0
Aprotinin	−13 ± 34	−33 ± 35
α_2-Macroglobulin	29 ± 31	−130 ± 69
Antipain	35 ± 20	−140 ± 120
SBTI	60 ± 15 [a]	−96 ± 80
Leupeptin	60 ± 12 [a]	−9.3 ± 25
α_1-Antitrypsin	67 ± 15 [a]	−84 ± 63
Heat inactivation	97 ± 2.8 [b]	nd

Results are shown as mean ± S.E.M. for five experiments with 2.5 μg tryptase or with 5.0 μg histamine. [a] $P < 0.05$ and [b] $P < 0.005$ in comparison with the response with the untreated tryptase alone (paired Student's *t*-test). SBTI, soybean trypsin inhibitor; nd, not done.

Table 2
The effect of proteinase inhibitors and heat treatment on tryptase-induced histamine release from dispersed guinea pig lung and skin cells

Inhibitor/ treatment	% Inhibition of BAPNA cleavage	% Inhibition of histamine release	
		Lung	Skin
Leupeptin	98 ± 2 [a]	74 ± 7.1 [a]	44 ± 12
SBTI	0.4 ± 0.7	86 ± 7.3 [a]	14 ± 6.7
Heat inactivation	101 ± 3.1 [a]	82 ± 13 [a]	86 ± 13 [a]

Tryptase (25 μg/ml) was preincubated with 10 μg/ml inhibitor before addition to dispersed lung or skin cells. Histamine release was expressed as percentage inhibition of that stimulated with tryptase alone. Mean ± S.E.M. are shown for 3–5 experiments performed in duplicate. [a] $P < 0.05$ in comparison with uninhibited tryptase (paired Student's *t*-test). SBTI, soybean trypsin inhibitor. Addition of inhibitors alone did not induce significant histamine release.

Table 3
Tryptase-induced histamine release from dispersed cells of guinea pig lung and skin tissue in the presence or absence of the metabolic inhibitors antimycin A and 2-deoxy-D-glucose

Tryptase concentration (μg/ml)	Net histamine release (%)			
	Without metabolic inhibitors		With metabolic inhibitors	
	Lung	Skin	Lung	Skin
0.5	8.3±2.9	4.2±2.2	1.6±0.9 [a]	0
2.5	7.4±5.0	3.7±2.3	1.6±1.0	1.1±0.8
5.0	5.8±2.2	5.4±1.9	0.7±0.6 [a]	0.9±0.8 [a]
25	8.4±2.0	8.7±1.2	1.1±0.8 [a]	2.9±1.2 [b]
50	17±4.3	18±3.6	2.6±1.6 [a]	6.5±2.6 [b]

Mean±S.E.M. are shown for 4–6 experiments performed in duplicate. [a] $P < 0.05$ and [b] $P < 0.005$ compared with responses with uninhibited controls (paired Student's *t*-test).

abolished its ability to increase vascular permeability. Incubation of these proteinase inhibitors with histamine under the same conditions did not alter the skin response to histamine.

3.2. Mast cell activation

Tryptase stimulated histamine release from enzymatically dispersed guinea-pig skin and lung cells (Fig. 5). Significant histamine release was induced with concentrations of tryptase as low as 5 μg/ml. Preincubation of tryptase with proteinase inhibitors prior to its addition to mast cells or heat treating the enzyme significantly inhibited mast cell activation for the lung cells (Table 2), whereas addition of the inhibitors alone did not induce histamine release. Mast cells treated with the metabolic inhibitors antimycin A (1 μM) and 2-deoxy-D-glucose (10 mM) did not release histamine in response to tryptase (Table 3).

4. Discussion

We provide compelling evidence that human mast cell tryptase may be a potent stimulus of microvascular leakage in vivo and that this may depend largely on the ability of this proteinase to activate mast cells. The degree of microvascular permeability induced by tryptase was similar to that induced by histamine when these mast cell products were compared on a weight basis. As the quantity of tryptase present in the secretory granules of human mast cells can be more than 10-fold that of histamine (Schwartz et al., 1987a), our findings strongly suggest that tryptase could be an important mediator of microvascular leakage following its release from mast cells in allergic disease.

The tryptase employed in these studies was of high purity with very little contamination with endotoxin, a recognised stimulus of microvascular leakage (Rubin et al., 1992). Moreover, injection of tryptase which had been heat inactivated at 56°C for 60 min (a procedure likely to be insufficient to alter the biological actions of endotoxin), abolished the ability to induce skin reactions, providing further confirmation that endotoxin was not responsible for the microvascular leakage observed. Certain proteinase inhibitors were able to inhibit skin reactions induced by tryptase, but not by histamine, suggesting that the actions of tryptase were dependent on an intact catalytic site.

Selective, non-toxic inhibitors of tryptase are not available, but the effects of a variety of broad spectrum proteinase inhibitors on tryptase-induced microvascular leakage have been investigated. Of the panel tested, leupeptin, an inhibitor of tryptase activity (Smith et al., 1984), significantly inhibited the actions of tryptase. On the other hand, aprotinin, α_2-macroglobulin and antipain, all of which are weak or ineffective as inhibitors of tryptase, failed to reduce microvascular leakage induced by tryptase. The observation that soybean trypsin inhibitor and α_1-antitrypsin could inhibit tryptase-induced skin oedema is somewhat surprising. It is possible that the cleavage of a substrate by tryptase in skin may be inhibited directly by soybean trypsin inhibitor or α_1-antitrypsin (in contrast to findings with other substrates investigated), or these two inhibitors may act on another proteinase involved in a cascade downstream of the stimulus provided by tryptase.

Tryptase is enzymatically unstable under physiological conditions. A half life of just 6–8 min has been reported for purified tryptase maintained at 37°C in vitro (Schwartz and Bradford, 1986; Schechter et al., 1993). As tryptase activity may be stabilized by heparin or other proteoglycans (Schwartz and Bradford, 1986), heparin was added routinely to the preparations of tryptase in the present study. However, we found that addition of heparin was without effect on microvascular leakage induced by tryptase, possibly because tryptase may be stabilised by the presence of proteoglycans in the tissue. Alternatively the stabilising actions of exogenous heparin on tryptase may be counteracted by its anti-inflammatory actions. Heparin has been reported to inhibit antigen-induced cutaneous and airway responses in vivo and also IgE dependent mast cell degranulation in vitro (Ahmed et al., 1993).

Skin reactions provoked by tryptase were long lived, with blueing reactions observed up to 80 min following injection. In contrast, responses to histamine were no longer apparent 40 min after injection. Despite the difference in time-course, it seems likely that the induction of microvascular leakage by tryptase is mediated at least in part by the local release of histamine in the skin of guinea pigs. Pretreatment of guinea pigs with the histamine H_1 and H_2 receptor antagonists pyrilamine or cimetidine markedly inhibited skin reactions to tryptase as well as to histamine.

We considered also the possibility that kinins may have a role in mediating the skin responses to tryptase. The generation of kinins following mast cell activation has been demonstrated both in vitro with dispersed cells from

lung tissue (Proud et al., 1985) and in vivo in the respiratory tract of patients with asthma (Christiansen et al., 1992) or rhinitis (Naclerio et al., 1985). However, there has been some controversy over the extent to which tryptase may have kininogenase activity. Although early studies suggested that tryptase was unable to generate kinins from low or high molecular weight kininogen (Schwartz et al., 1986; Maier et al., 1983), Proud et al. (1988) found that tryptase could induce bradykinin release from low molecular weight kininogen with an optimal pH of 5.5. However, on re-examining this issue we found that tryptase was able to generate kinins from both low molecular weight kininogen and high molecular weight kininogen with both reactions optimal in the neutral pH range (Walls et al., 1992b). In the present study skin reactions were elicited by injecting tissue kallikrein as well as bradykinin, but in contrast to those with tryptase, the response to neither of these stimuli was inhibited by the antihistamine pretreatment. Thus, although the release of kinins may play a role in tryptase-induced microvascular leakage, it would seem likely that much of that observed in this model may have been the result of histamine release.

Some previous studies have indicated important interactions between tryptase and histamine. Relatively high concentrations of histamine can inhibit tryptase activity in vitro, a function which this amine may fulfill within mast cell granules or in the immediate vicinity of activated cells (Alter and Schwartz, 1989). On the other hand, tryptase of canine origin has been reported to enhance markedly the contractility of dog airway smooth muscle tissue to histamine, and an histamine H_1 receptor antagonist has been found to inhibit the hyperresponsiveness (Sekizawa et al., 1989). Chymase, another major proteinase of the mast cell, has been reported to enhance histamine-induced weal formation in the dog, without inducing a weal when injected alone (Rubinstein et al., 1990). However, when we co-injected tryptase with histamine into the skin we found no evidence for a synergistic or antagonistic interaction between these mast cell mediators.

We reasoned that histamine release following injection of tryptase must be derived from tissue mast cells, and we examined directly the ability of tryptase to stimulate histamine release from dispersed mast cells in vitro. With preparations of dispersed cells from skin and lung tissue, we observed a dose-dependent release of histamine at concentrations similar to those which elicited microvascular leakage following injection in vivo. The response to tryptase was attenuated in the presence of antimycin A and 2-deoxy-D-glucose, suggesting that the process was energy requiring and not a consequence of cytotoxicity. As was the case with microvascular permeability, histamine release in vitro was inhibited by heat inactivation of tryptase, or by adding the proteinase inhibitors leupeptin or soybean trypsin inhibitor.

Direct evidence for tryptase as a stimulus of mast cell degranulation has not been sought previously, but a number of other serine proteinases of the chymotrypsin superfamily have been described as having this potential. Thrombin (Razin and Marx, 1984), chymotrypsin (Nials et al., 1983), rat chymase (Schick, 1990) and an elastase derived from the house dust mite (Stewart et al., 1994) have all been reported to stimulate histamine release from rodent or human mast cells. Moreover, a number of broad spectrum inhibitors are able to inhibit both IgE and non-IgE dependent mast cell degranulation in vitro, including chymostatin (Kido et al., 1985), leupeptin, (Kido et al., 1985), soybean trypsin inhibitor (Kido et al., 1988), diisopropylfluorophosphate (Meier et al., 1985) and peptide boronic acids (Kato et al., 1988). This would suggest that proteolytic mechanisms are involved in mast cell activation, and may help to explain our finding that tryptase-induced mast cell activation and microvascular leakage could be inhibited by soybean trypsin inhibitor.

The ability of diisopropylfluorophosphate and other proteinase inhibitors to suppress microvascular leakage has been noted previously in rat models (Spector and Willoughby, 1960). Moreover, it has been reported recently that administration of the tryptase inhibitor APC366 can reduce microvascular leakage in the airways of allergen-challenged sheep, as well as reducing early and late increases in specific lung resistance, blocking airway hyperresponsiveness and inhibiting tissue eosinophilia (Clark et al., 1995). Further studies are required to determine more precisely the nature of the interaction of tryptase with mast cells and to determine to what extent inhibitors of tryptase may have mast cell stabilising properties. However, our studies suggest that tryptase could be an important mediator of microvascular leakage, and that by stimulating further mast cell activation this major mast cell product could provide an amplification signal in allergic disease.

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