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The induction of a prolonged increase in microvascular permeability by human mast cell chymase

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Abstract

Chymase is a major constituent of the secretory granules of human mast cells, but little is known of the contribution of this serine proteinase in acute allergic reactions. We have purified chymase from human skin tissue, and have investigated its potential to induce microvascular leakage in vivo. Injection of chymase into the skin of guinea pigs provoked an increase in microvascular leakage within 20 min. Although skin reactions were smaller than those elicited with similar quantities of histamine at this time point, they were much longer-lived, and were still apparent 120 min following injection. Chymase induced microvascular leakage was reduced in the presence of soybean trypsin inhibitor, and abolished by heat inactivating the enzyme, indicating dependence on an intact catalytic site. Little evidence was found for synergistic interactions between chymase and either histamine or tryptase. Antihistamine pretreatment of animals did not reduce the magnitude of skin reactions to chymase suggesting that they were not mediated by histamine release. Chymase could contribute to increases in microvascular permeability following mast cell degranulation in allergic disease.

Keywords: Chymase; Histamine; Mast cell; Microvascular permeability; (Guinea pig); Antihistamine

1. Introduction

Mast cell activation is a prominent feature of allergic disease. These cells can release a range of potent mediators of inflammation including proteases, histamine, proteoglycans, eicosanoids and cytokines. It is the proteases, however, which represent the most abundant constituents of the secretory granules on a weight basis, with as much as 60 pg present in human mast cells (Walls, 1995). Chymase, a chymotryptic serine proteinase, and tryptase, the tryptic proteinase with which it is co-stored, have proved valuable as markers for mast cells. Their selective presence in mast cells has allowed two distinct subpopulations to be distinguished according to whether they contain both tryptase and chymase or tryptase but not chymase (Irani et al., 1986). Investigation of the pharmacological actions of these major mast cell products has until recently been neglected.

Several studies now suggest that the mast cell proteinases may participate in the induction of allergic responses. Thus, purified human tryptase can increase microvascular permeability with a potency similar to that of histamine on a weight basis when injected into the skin of guinea pigs (He and Walls, 1997) or sheep (Molinari et al., 1995). Tryptase-induced microvascular leakage can be abrogated by pretreating the animals with antihistamines, and tryptase can itself stimulate histamine release from mast cells in vitro (He and Walls, 1997; He et al., 1998). In addition to being able to degrade certain regulatory peptides such as calcitonin gene related peptide and vasoactive intestinal peptide (Tam and Caughey, 1990; Walls et al., 1992), tryptase can activate stromelysin (Gruber et al., 1989) and urokinase plasminogen activator (Stack and Johnson, 1994), and can interact with several cell types as a growth factor (Ruoss et al., 1991; Cairns and Walls, 1996, 1997; Brown et al., 1995) and as a stimulus for cytokine release (Cairns and Walls, 1996) and collagen deposition (Cairns and Walls, 1997). Moreover, the injection of human tryptase into guinea pigs and mice can provoke the accumulation of granulocytes (He et al., 1997a).

Chymase has been implicated in processes of tissue destruction, being able to activate procollagenase to an active form (Saarinen et al., 1994) and to induce the separation of skin tissue at the basement membrane (Brig-

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gaman et al., 1984). In addition, chymase can generate angiotensin II from angiotensin I, more efficiently than angiotensin converting enzyme itself (Urata et al., 1990). The ability of chymase to activate interleukin-1β (Mizutani et al., 1991) and to degrade interleukin 4 (Tunon de Lara et al., 1994) would be consistent with a role in inflammation. However, the contribution of human chymase in acute inflammation has not been investigated directly.

In an early study with chymase prepared from the granules of rat peritoneal and pleural mast cells, it was reported that this proteinase could provoke a wheal and flare response with itching when injected intradermally into human skin (Hagermark et al., 1972). Subsequently a chymotryptic proteinase prepared from human skin (which in retrospect appears to have been similar to mast cell chymase) was found to induce vascular leakage when injected into the skin of rabbits, and erythema and late onset oedema in the skin of a human subject (Fräki, 1977). In contrast, chymase isolated from dog mastocytoma cells has failed to induce a wheal response when injected into the skin of dogs, though it was able to augment wheal reactions provoked by histamine (Rubinstein et al., 1990). Ethical considerations must prevent the injection of proteinases of human origin into human subjects, but as we have reported recently in studies with tryptase (He and Walls, 1997), useful information on their actions in vivo may be obtained using animal models. In the present study we have investigated the potential of purified human chymase to provoke microvascular leakage in guinea pig skin.

2. Methods

2.1. Materials and drugs

The following compounds were purchased from Sigma (Poole, UK): α_1-antitrypsin, leupeptin, aprotinin, soybean trypsin inhibitor, antipain, *N*-benzoyl-D, L-arginine-*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, pyrilamine maleate, cimetidine, heparin–agarose, Evan's blue dye, bovine serum albumin, goat anti-mouse immunoglobulin (Ig) G, dimethyl sulphoxide (DMSO), 3-(*N*-morpholino) propanesulphonic acid (MOPS), 2-(*N*-morpholino)ethane-sulphonic acid (MES) and Tris–base. Coomassie protein assay reagent was obtained from Pierce (Rockford, IL, USA); octyl-agarose and Sephacryl S-200 from Pharmacia (Milton Keynes, UK); silver staining kits from Bio-Rad (Hemel Hempstead, UK); peroxidase-conjugated antibodies to rabbit IgG and 3,3-diaminobenzidine tetrahydrochloride from Dako (High Wycombe, Bucks, UK); pentobarbitone sodium from Sanofi Animal Health (Watford, UK) and Hypnorm (containing 0.11 mg fentanyl citrate and 3.5 mg fluanisone) from Janssen (Oxford, UK); The other common chemical reagents were of analytical grade and were purchased from BDH (Poole, UK).

2.2. Purification of chymase

Chymase was isolated from human skin using the purification procedure described by Schechter et al. (1983) and by McEuen et al. (1995). Briefly, about 500 g of skin excised from amputated legs was minced finely by passing three times through a meat mincer, and then washed with a low salt buffer (0.01 M MOPS, 0.1 M NaCl, pH 6.8). Chymase was extracted using a high salt buffer (0.01 M MOPS, 2 M NaCl, pH 6.8), and filtered through Whatman No. 1 and GF/F filter paper (pore size 0.7 μm). Following dialysis against 0.4 M sodium chloride, 0.01 M MOPS, pH 6.8, the crude chymase extract was subjected to heparin agarose chromatography in the same buffer, eluting with a 0.4 M to 2 M gradient of sodium chloride in 0.01 M MOPS, pH 6.8. The chymase-rich fractions were pooled and concentrated in C-10 Centricon centrifugal concentrators (Amicon, Stonehouse, UK), and applied to a Sephacryl S-200 column with 2 M sodium chloride, 0.01 M MOPS, pH 6.8 as running buffer. The purified chymase was concentrated and stored at -80°C. Two different preparations of chymase obtained in this way were pooled, and characterised as a single preparation in order to avoid possible problems of batch to batch variation in these studies. Purity was assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% reducing gels and stained with silver staining reagents. Western blotting was performed with rabbit antiserum to human chymase as described previously (McEuen et al., 1995).

2.3. Purification of tryptase

Tryptase was purified and characterised using the procedures reported previously (He and Walls, 1997). Briefly, approximately 500 g quantities of chopped human lung tissues collected post mortem were blended and washed with a low salt buffer (0.15 M NaCl, 10 mM MES, and 0.02% sodium azide; pH 6.1). The homogenate was extracted using a high salt buffer (2 M NaCl, 10 mM MES, and 0.02% sodium azide; pH 6.1), and the tryptic activity of the supernatant was filtered, and purified by sequential octyl–agarose and heparin–agarose chromatography. Tryptase fractions were concentrated in C-30 centrifugal concentrators (Amicon) and stored at -80°C until use. The purity of tryptase was assessed by SDS-PAGE with 10% reducing gels and by Western blotting with monoclonal antibody AA5 (Walls et al., 1990).

2.4. Enzyme assays

Enzymatic activities were determined spectrophotometrically at 410 nm, 25°C, using the substrate 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 for measuring chymotryptic activity (Schechter et al., 1988); 20 mM BAPNA in 0.1 M Tris–HCl, pH 8.0, 1 M glycerol, containing 1 mg/ml bovine serum albumin for tryptic activity (Smith et al., 1984) and 1.4 mM *N*-succinyl-L-Ala-L-Ala-L-Ala-*p*-nitroanilide in the same buffer as used with BAPNA for elastolytic activity (Nakajima et al., 1979). In all assays, 50 μl of 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 the standard.

2.5. Animals and skin testing

Dunkin Hartley guinea pigs (600–1000 g, Harlan, Bicester, UK) were anaesthetized by intraperitoneal (i.p.) injection of pentobarbitone saline (21 mg/kg; Sanofi Animal Health, Watford, UK) and intramuscular injection of Hypnorm (0.35 ml/kg; Janssen, Oxford, UK). Microvascular leakage evoked in guinea pig skin in response to injected compounds was assessed essentially as described previously (Walls et al., 1987). After shaving the back, Evan's blue dye in normal saline was injected intravenously (into the dorsal vein of a hind paw or into the ear vein) or alternatively, into the heart (4 ml/kg). Chymase and other compounds (50 μl) were injected intradermally into randomised sites placed 2–3 cm apart. Animals were kept warm, and at 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 Evan's 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 relative size of the area of cutaneous oedema. We have noted previously close correlations between the results of this skin testing method, and those of a procedure in which ^{125}I-albumin is injected intravenously, and the degree of cutaneous oedema is estimated by determining the radioactivity of the skin at the injection site (He and Walls, 1997).

It was investigated if the actions of chymase were dependent on an intact catalytic site by preincubating the purified enzyme with the proteinase inhibitors soybean trypsin inhibitor, α_1-antitrypsin, leupeptin, antipain and aprotinin (for 20 min on ice) and by heat-inactivating the enzyme (for 2 h at 56°C) before injection. In order to investigate the ability of an antihistamine treatment to block the effects of chymase on microvascular leakage, in pilot experiments the antihistamines pyrilamine and cimetidine were administered singly to guinea pigs (dose range 1 to 10 mg/kg). As neither treatment alone at the doses used was able to block completely the histamine-induced plasma extravasation (data not shown), a combination of 10 mg/kg pyrilamine plus 10 mg/kg cimetidine was injected i.p., 30 min before intradermal injection of compounds.

2.6. Statistical analysis

Statistical analyses were performed using StatView software (Version 4.02, Abacus Concepts, Berkeley, CA). All data were presented as mean ± S.E.M. for the number (n) of experiments indicated. Where analysis of variance (ANOVA) indicated significant differences between groups, for the pre-planned comparisons of interest, the paired Student's t-test was employed. For all analyses, $P < 0.05$ was taken as significant.

3. Results

3.1. Purification of mast cell proteinases

SDS-PAGE of the purified chymase revealed a single diffuse band with an apparent molecular weight of approximately 30 kDa. The identity was confirmed as chymase by Western blotting with specific antibodies. The specific activity of the combined preparations of chymase used for this study was 6.8 U/mg, where 1 U of chymase represents that required to hydrolyse 1 μmole of *N*-Suc-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide per min at 25°C, pH 8.0. No contaminating tryptic or elastolytic activity was detected using chromogenic substrates.

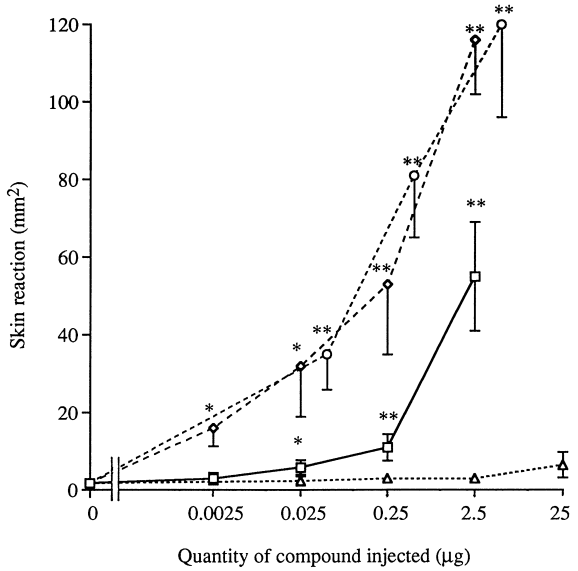

Fig. 1. The effects of injecting chymase (– □ –), histamine (– – ○ – –), tryptase (– – ◇ – –) or heparin (-- △ --) on microvascular permeability in guinea pig skin. The skin reactions were assessed by the size of the blueing area 20 min following intradermal injection of these mast cell products. Mean ± S.E.M. values are shown for seven experiments. * $P <$ 0.05 and ** $P < 0.005$ compared with the reaction with the saline diluent alone (paired Student's t-test).

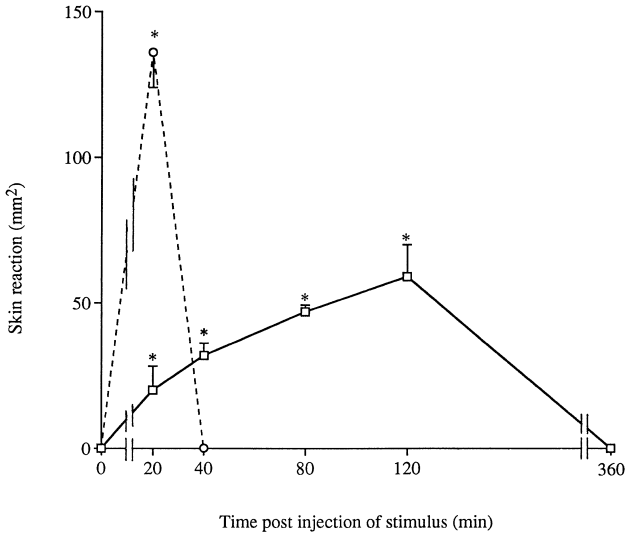

Fig. 2. Microvascular leakage in guinea pig skin at various periods following intradermal injection of chymase (2.5 μg, – □ –) or histamine (5 μg, – – ○ – –). In all cases animals were injected intravenously with Evan's blue dye 20 min before measurement of the skin reaction. Values shown are the mean ± S.E.M. for three animals. * $P < 0.05$ compared with the reaction with saline at 20 min (paired Student's *t*-test).

The tryptase preparation appeared homogenous on SDS-PAGE, bound tryptase- specific antibody on Western blotting, and had a specific activity 1.6 U/mg, where 1 U represents that required to cleave 1 μmole of BAPNA per minute at 25°C, pH 8.0. Chymotryptic and elastolytic activity could not be measured in the purified preparation with the chromogenic substrates used. Contamination with endotoxin was very low, being less than 38 pg/mg for both the chymase and tryptase preparations.

3.2. Induction of microvascular leakage by chymase

Intradermal injection of human mast cell chymase into the skin of guinea pigs provoked a dose dependent increase in microvascular permeability (Fig. 1). As reported previously (He and Walls, 1997) this was also seen in the model when histamine or tryptase were injected, and heparin glycosaminoglycan did not increase microvascular leakage. At 20 min post injection, chymase-induced microvascular leakage was consistently less than that elicited by histamine or tryptase on a weight basis, but the response to chymase was relatively slow, and was much more prolonged than that induced by histamine (Fig. 2). The size of skin reactions at 80 and 120 min was actually greater than that at 20 min. There was complete resolution by 360 min.

3.3. Catalytic site dependency

Heat treatment of chymase abolished almost completely its ability to increase microvascular permeability (Table 1). Co-injection of soybean trypsin inhibitor with chymase also significantly reduced the skin response, though α_1-antitrypsin, antipain, aprotinin and leupeptin (all of which are much less effective as inhibitors of chymase activity) were all without significant effect. None of the proteinase inhibitors tested altered significantly the size of skin reactions elicited by histamine and none provoked microvascular leakage when injected alone.

3.4. Effect of co-injecting chymase with other mast cell mediators

There was little difference between the size of skin reactions provoked by co-injection of chymase and histamine, and the sum of the separate reactions induced by injection of these two mast cell products alone (Fig. 3). There appeared to be a trend towards a synergistic interaction when certain doses of chymase were administered together with 5 μg histamine, but this was not significant by ANOVA. Similar experiments involving injection of

Table 1
The effect of proteinase inhibitors and heat-inactivation on the ability of chymase to induce microvascular leakage or to cleave a chromogenic substrate

Inhibitor/treatment	Percentage inhibition of substrate cleavage	Percentage inhibition of skin reaction	
		Chymase	Histamine
None	0	0	0
Heat inactivation	100 ± 0.3[a]	95 ± 5.0[a]	nd
Soybean trypsin inhibitor	99 ± 0[a]	64 ± 16[b]	−36 ± 27
α_1-antitrypsin	30 ± 4.4[b]	22 ± 25	−43 ± 28
Antipain	18 ± 3.8	−9.0 ± 3.4	−41 ± 27
Aprotinin	7.3 ± 3.8	−12 ± 30	−45 ± 34
Leupeptine	7.3 ± 4.3	−17 ± 26	−19 ± 23

Mean ± S.E.M. values are shown for 8 to 10 separate experiments with 2.5 μg chymase, or five experiments with 5 μg histamine injected into guinea pig skin. A standard quantity of 10 μg of all proteinase inhibitors was co-injected with these stimuli. For enzyme assays, 50 μg/ml chymase was preincubated with 200 μg/ml proteinase inhibitor for 20 min on ice prior to addition of the substrate *N*-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide. The assay was repeated three times.
[a] $P < 0.005$ and [b] $P < 0.05$ compared with the uninhibited control (paired Student's *t*-test).
nd = not done.

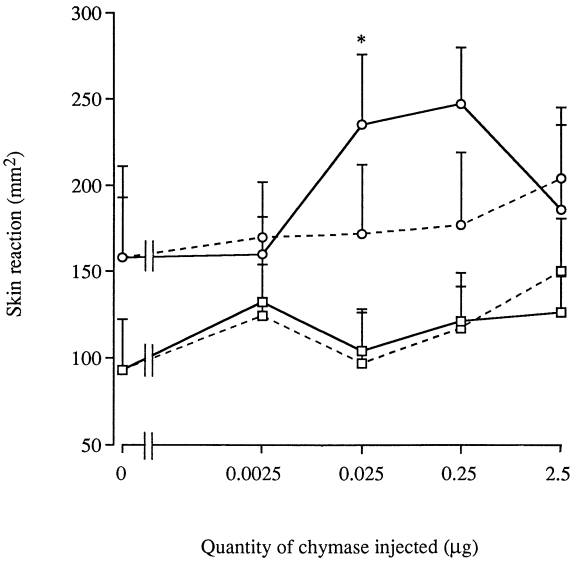

Fig. 3. Microvascular leakage induced by various quantities of chymase co-injected with 0.5 μg (− □ −) or 5 μg (− ○ −) histamine. The sum of the separate reactions elicited with chymase alone and with either 0.5 μg histamine alone (- □ -) or 5 μg histamine alone (- ○ -) are also shown. Values are the mean ± S.E.M. for experiments with six guinea pigs. * $P < 0.05$ compared with the value predicted by adding together reactions induced by separate injections (paired Student's *t*-test).

the same range of doses of chymase together with tryptase (0.0025, 0.025, 0.25 and 2.5 μg) also indicated a purely additive pattern of results, with no evidence for synergistic or antagonistic interactions (data not shown).

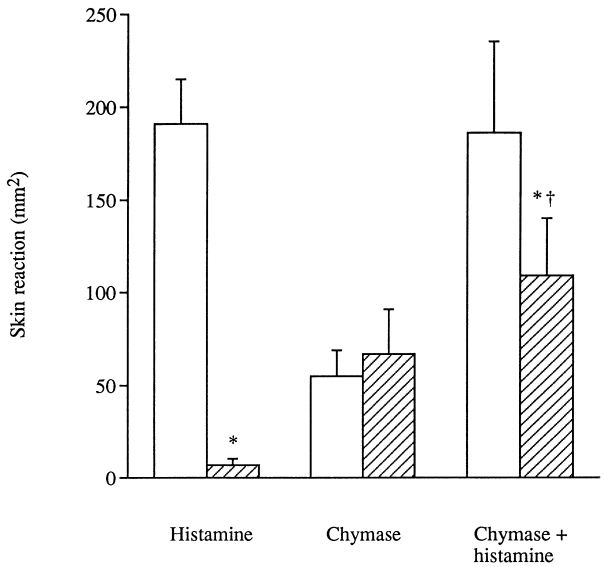

Fig. 4. Skin reactions to histamine (5 μg), chymase (2.5 μg) and histamine with chymase (5 μg + 2.5 μg) in guinea pigs which were untreated (open bars) or treated with antihistamines (hatched bars). Results are shown as mean ± S.E.M. for six animals. * $P < 0.05$ compared with the response in untreated control guinea pigs; † $P < 0.05$ compared with the response to chymase alone in antihistamines treated animals (paired Student's *t*-test).

3.5. Antihistamine treatment

Pretreatment of guinea pigs with the antihistamines pyrilamine and cimetidine prevented the induction of a skin reaction by histamine, but the capacity of chymase to induce a reaction was unaffected (Fig. 4). Microvascular leakage induced by co-injection of 5 μg histamine with 2.5 μg chymase was also reduced in the antihistamine pretreated animals, though not to the size of those elicited by chymase alone.

4. Discussion

Our studies indicate that human mast cell chymase can induce a prolonged increase in microvascular permeability. Chymase, like histamine and tryptase has the potential to contribute to the microvascular leakage following mast cell activation in allergic disease. The mechanisms involved, however, appear quite different from those of the other preformed mast cell mediators.

On a weight basis, chymase was less potent than tryptase or histamine in the degree of microvascular leakage which it could provoke within 20 min of injection. Nevertheless, at this time point, a detectable increase in microvascular permeability was elicited by a quantity of chymase as low as 25 ng. While information is not available on the levels of chymase in human biological fluids or tissues, it was been calculated that there is some 4.5 pg of this proteinase per mast cell, compared with less than 2 pg histamine (Schwartz et al., 1987). With the close spatial relationship between mast cells and small blood vessels, it seems likely that following mast cell degranulation the microvasculature will be exposed to concentrations of chymase at least as great as those employed in the present study. Chymase is secreted from mast cells in a macromolecular complex of 400 to 560 kDa, together with proteoglycans and carboxypeptidase (Goldstein et al., 1992). The large size of this complex will limit the rate of diffusion of chymase away and result in high local concentrations. Moreover, the high affinity of this proteinase for heparin and heparin sulphate of basement membranes and extracellular structures (Sayama et al., 1987) will further serve to localise the actions of chymase.

We examined in particular the ability of chymase to induce microvascular leakage 20 min following injection, as this represents the point when oedema provoked by allergen is maximal in the skin (Voorhorst, 1980). The chymase-induced increase in microvascular leakage, however, was still apparent up to 2 h following injection, though it had resolved completely by 6 h. This contrasts with the relatively short-lived effect of histamine, which was no longer apparent 40 min after its injection. The difference in kinetics of the response could reflect differences in the rates of clearance or inactivation of these two

stimuli, as well as differences in the mechanisms of action on the microvasculature. While allergen-induced microvascular leakage has been noted previously to be much more long-lived than that provoked by histamine (Voorhorst, 1980), it is rare for it to last for as long as 2 h. It is possible therefore that the actions of endogenous chymase may be of shorter duration than those observed with the purified preparation in the present study. Our findings are nevertheless consistent with a previous study with a chymotryptic proteinase from human skin, for which microvascular leakage was measured 60 min following injection into the skin of rabbits or of a human subject (Fräki, 1977). Moreover, we have observed that human tryptase can induce skin reactions in the guinea pig up to 80 min after its injection (He and Walls, 1997); and studies in other animal models with certain other proteases of non-mast cell origin including factor Xa (Cirino et al., 1997) and collagenase (Souza Pinto et al., 1995) have demonstrated their ability to provoke oedema lasting several hours.

The microvascular leakage induced by chymase could be inhibited by preincubation of the enzyme with soybean trypsin inhibitor, an inhibitor of chymase activity (Schechter et al., 1983). On the other hand, no reduction in the size of chymase-induced reactions was observed with α_1-antitrypsin, which is a relatively weak inhibitor of this proteinase (Schechter et al., 1989), or with leupeptin, aprotinin and antipain whose inhibitory actions on chymase are negligible. Thus, as found with tryptase (He and Walls, 1997), the ability of chymase to induce microvascular leakage appears to depend on an intact catalytic site. Consistent with this was the observation that heat inactivation abrogated the effect completely.

Chymase could act on molecular or cellular targets to generate secondary mediators of microvascular leakage. Thus, for example, it has been noted that interleukin-1β, a cytokine which may be activated by human chymase (Mizutani et al., 1991) can increase microvascular permeability when injected into the skin of rats (Martin et al., 1988). Alternatively, chymase could induce alterations in tissue architecture more directly. In the study of Scudamore et al. (1995) involving infusion of a rat chymase (rat mast cell protease II) into the cranial mesenteric artery, it was suggested that the increased translocation of macromolecules into the jejunum may have been a consequence of increased paracellular permeability, as gross morphological changes were not observed. The actions of human chymase on guinea pig skin could also be mediated by cleavage of intercellular proteins, and in keeping with such a mechanism is the ability of this proteinase to cleave type I procollagen (Kofford et al., 1997), to activate interstitial procollagenase (Saarinen et al., 1994) and to degrade human skin in vitro at the basement membrane (Briggaman et al., 1984).

We have reported previously that the induction of increased microvascular permeability by tryptase seems to depend to a large extent on the activation of mast cells, and that it is abolished almost completely by pretreating guinea pigs with antihistamines (He and Walls, 1997). In contrast, we found under similar conditions in the present study, that treating animals with pyrilamine and cimetidine had no effect on skin reactions provoked by chymase. This would suggest that the mechanisms of chymase and tryptase-induced microvascular leakage are different. In the early study of Fräki (1977), it was found that treating rabbits with the antihistamine promethazine reduced the subsequent development of skin responses to the chymotryptic proteinase of human skin. If the presence of chymase was responsible for the actions of that preparation, it is difficult to reconcile those findings with our own. A rat chymase has been reported to stimulate the degranulation of rat mast cells (Schick et al., 1984), but human chymase is not a stimulus for histamine release from human mast cells (He et al., 1997b). It would appear unlikely that the microvascular leakage which we observed in response to chymase was mediated by the activation of mast cells.

As chymase is stored in the same granules of human mast cells as histamine and tryptase (Sayama et al., 1987), and both are released together on mast cell degranulation (Schwartz et al., 1981) we investigated the potential for interaction between these preformed mediators. Over various concentrations, however, co-injection of chymase with histamine or with tryptase elicited skin reactions of approximately the same magnitude as those which were obtained with each of these stimuli alone. Rubinstein et al. (1990) have reported that though chymase of canine mastocytoma origin was unable by itself to induce a wheal reaction when injected into the skin of dogs, it could, nevertheless, potentiate the responses to low doses of histamine. We found little evidence for a synergistic interaction between human chymase and histamine when these were co-injected, though the failure of the antihistamine pretreatment to reduce the response to chymase and histamine to that with chymase alone does suggest that this may occur to a certain degree.

Our studies, indicate that human chymase could be more important as a stimulus for microvascular leakage in its own right than as a cofactor for other preformed mast cell mediators. It seems likely that chymase will contribute to increases in microvascular permeability following mast cell degranulation in inflammatory disease, and this proteinase could contribute to the antihistamine independent component of allergic disease. Inhibitors of chymotryptic proteinases have been found to inhibit microvascular leakage provoked in animal models with allergen and with various other mast cell degranulating agents (Spector and Willoughby, 1960; Rubinstein et al., 1990; Moore and Dannenberg, 1993), and it will be important to investigate in such systems the therapeutic potential of selective inhibitors of chymase when they become available. In man, the contribution of chymase is likely to be greatest follow-

ing mast cell activation in skin and in submucosal tissues of the respiratory and gastrointestinal tracts, as chymase-rich mast cells predominate at these sites (Irani et al., 1986, 1989). However, recent studies have indicated that the majority of human mast cells may contain chymase, even at mucosal sites (Beil et al., 1997), and this proteinase may therefore participate in processes of mast cell-dependent microvascular leakage throughout the body.

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