ELSEVIER

European Journal of Pharmacology 419 (2001) 261–267

ejp

www.elsevier.nl/locate/ejphar

Tryptase-induced airway microvascular leakage in guinea pigs: involvement of tachykinins and leukotrienes

Scott Greenfeder *, Susan Sehring, Nansie McHugh, Michel Corboz, Maria Rivelli, John C. Anthes, Motasim Billah, Robert W. Egan, Richard W. Chapman

Department of Allergy, Schering-Plough Research Institute, MS-1600, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

Received 4 January 2001; received in revised form 22 March 2001; accepted 3 April 2001

Abstract

Tryptase, a serine protease synthesized by and stored in mast cells, is implicated as an important mediator in the pathogenesis of airway inflammation. In this study, tryptase was evaluated for its ability to induce microvascular leakage into the airways of guinea pigs. Dose- and time-dependent increases in airway microvascular leakage were produced by intratracheal tryptase (0.3–3 μg). Intratracheal tryptase (3–30 μg) had no effect on airway tone as measured by pulmonary insufflation pressure. Tryptase-induced airway microvascular leakage was partially blocked by the tachykinin NK_1 receptor antagonist CP 99994 [(+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine] and an inhibitor of leukotriene formation SCH 37224 (1-(1,2-dihydro-4-hydroxy-2-oxo-1-phenyl-1,8-naphthyridin-2-yl)pyrrolidinium, hydroxide inner salt). Neither CP 99994 nor SCH 37224 inhibited tryptase proteolytic activity in-vitro. Pretreatment of guinea pigs with histamine H_1 receptor antagonists or a tachykinin NK_2 receptor antagonist had no affect on the airway microvascular leakage induced by tryptase. It is speculated that tryptase may be important in the pathogenesis of airway inflammation, particularly in disorders that involve increased airway microvascular leakage such as asthma. © 2001 Published by Elsevier Science B.V.

Keywords: Microvascular leakage, airway; Leukotriene; SCH 37224; Tachykinin; Tryptase

1. Introduction

Tryptase is a serine protease made by and stored in the secretory granules of mast cells and has been shown to constitute up to 20% of the total protein content of mast cells (Schwartz et al., 1981). Tryptase, while related to trypsin in its proteolytic activity, is unique among the serine proteases by virtue of its homo-tetrameric structure (Caughey, 1994; Pereira et al., 1998; Schwartz et al., 1990; Welle, 1997). The tetrameric nature of tryptase creates a ring-like structure with active sites positioned in the center pore of the ring. It is this arrangement of active sites that is proposed to give tryptase its high substrate specificity.

It has been suggested that tryptase has a role in the pathogenesis of allergic diseases as well as in chronic inflammatory diseases such as asthma. This comes largely from the elucidation of various in-vitro activities of tryptase. Tryptase cleaves and inactivates vasoactive intestinal peptide (VIP), calcitonin gene related peptide (CGRP) and peptide-histidine methionine (PHM) and also cleaves kininogen to liberate bradykinin. These actions may contribute to the enhanced smooth muscle contractility found in the lungs of asthmatics (Caughey, 1994; Welle, 1997).

The potential role of tryptase in mediating airway hyperresponsiveness is also supported by the effects of tryptase on isolated tissue. Tryptase administered to bronchi isolated from guinea pigs or humans potentiates the contractile response of the tissue to histamine (Barrios et al., 1998; Berger et al., 1999; Johnson et al., 1997). In addition, tryptase mediates hyperresponsiveness to histamine of isolated dog airway smooth muscle (Sekizawa et al., 1989).

Additional activities of tryptase suggest that it may play a role in tissue remodeling and fibrosis in the asthmatic lung. Tryptase cleaves fibronectin and activates matrix metalloproteinases. These active proteinases degrade collagen and may lead to enhanced collagen deposition and airway wall stiffening. Tryptase has been shown to be a potent mitogen for airway smooth muscle cells, bronchial

* Corresponding author. Tel.: +1-908-740-7217; fax: +1-908-740-7175.

E-mail address: scott.greenfeder@spcorp.com (S. Greenfeder).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00960-8

epithelial cells and fibroblasts (Brown et al., 1995a,b; Cairns and Walls, 1995; Hartmann et al., 1992; Ruoss et al., 1991). Hyperplasia of these cell types within the asthmatic lung is a hallmark of airway remodeling. Tryptase has also been shown to be mitogenic and activating for keratinocytes and myocytes (Corvera et al., 1997; Steinhoff et al., 1999). Current evidence suggests that many if not all of these mitogenic responses are due to activation of proteinase-activated receptor-2 (PAR-2) by tryptase.

Tryptase administered directly to the lungs of sheep via inhalation causes a direct bronchoconstriction, induces hyperresponsiveness to carbachol and causes an increase in the histamine content of bronchoalveolar lavage. These effects of tryptase are inhibited by APC 366 (L-prolinamide, *N*2-[(1-hydroxy-2-naphthalenyl)carbonyl]-L-arginyl-, monohydrochloride) (an inhibitor of tryptase, trypsin and thrombin) (Molinari et al., 1996). APC 366 also inhibits antigen-induced carbachol hyperresponsiveness, pulmonary eosinophilia and increases in bronchoalveolar lavage albumin levels in sheep (Clark et al., 1995). Tryptase has also been shown to have direct effects on skin in-vivo. Tryptase causes immediate cutaneous responses in allergic sheep (Molinari et al., 1995) and causes microvascular leakage and the recruitment of inflammatory cells into the skin of guinea pigs (He et al., 1997; He and Walls, 1997; Molinari et al., 1995).

In this study, we have investigated the effect of administering human mast cell tryptase directly to the trachea of guinea pigs. Tryptase induced microvascular leakage into the trachea and bronchi of these animals. The effect of tryptase was inhibited by a tachykinin NK_1 receptor antagonist (CP 99994-(+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine) and an inhibitor of leukotriene formation (SCH 37224-(1-(1,2-dihydro-4-hydroxy-2-oxo-1-phenyl-1,8-naphthyridin-2-yl)pyrrolidinium, hydroxide inner salt). Histamine H_1 receptor antagonists (pyrilamine and chlorpheniramine) and a tachykinin NK_2 receptor antagonist (SR 48968-(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) failed to affect the tryptase-induced microvascular leakage. Additionally, we tested the effect of direct administration of tryptase on airway tone. This was tested in both non-sensitized and sensitized guinea pigs to determine if sensitization rendered the airways more responsive to tryptase. Tryptase failed to induce bronchospasm in guinea pig airways.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Charles River, MA, USA) weighing 350–450 g were fasted overnight before study but given water ad libitum. On the day of study, the guinea pigs were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg) and catheters were placed into the trachea and jugular vein. The tracheal catheter was connected to a rodent respirator and the animals were artificially ventilated (volume = 3.5 ml, frequency = 55 breaths/min) throughout the study. Pulmonary insufflation pressure was measured via a side port in the tracheal cannula and changes in pulmonary insufflation pressure were monitored using a Buxco Biosystem XA (Buxco Electronics, Sharon, CT USA). The jugular venous catheter was filled with heparinized saline to facilitate the injections of drugs.

Sensitized guinea pigs were prepared by intraperitoneal injection of 100 μg of ovalbumin absorbed onto 100 mg of aluminum hydroxide gel (alum) given in a volume of 0.5 ml/animal. Eight days later, the guinea pigs were given a booster injection of the same ovalbumin/alum mixture. The sensitized guinea pigs were studied 4 weeks after the first injection of the ovalbumin/alum mixture.

2.2. Airway microvascular leakage

Airway microvascular leakage was measured using the Evans blue dye technique (Danko et al., 1992). Evans blue (30 mg/kg) was injected intravenously followed 1 min later by the intratracheal (i.t.) administration of tryptase. The tryptase was diluted in phosphate buffered saline (PBS) and delivered into the tracheal catheter in 200 μl volume using a micropipette. At selected times after tryptase administration the thorax was opened and a blunt-ended 13-gauge needle passed into the aorta. An incision was made in the right atrium and blood was expelled by flushing 200 ml of saline through the aortic catheter. The lungs and trachea were removed and the trachea and main bronchi were isolated and stripped of connective tissues. The trachea and bronchi were then blotted dry and weighed. Evans blue dye was extracted by incubation of the tissues at 37°C for 18 h in 2 ml of formamide in stoppered tubes. The amount of dye was calculated by interpolation from a standard curve of Evans blue in the range of 0.5–10 μg/ml in formamide. The dye concentration is expressed as ng dye per mg tissue wet weight.

Test compounds were administered intravenously (i.v.) or orally (p.o.) before tryptase. Pretreatment times for the test compounds were 5 min for i.v. and 2 h p.o.

2.3. In-vitro human tryptase inhibition assay

Compounds were dissolved in dimethyl-sulfoxide (DMSO) and further diluted to the appropriate concentrations in assay buffer (0.1 M Tris–HCl, pH 8.0, 0.1 M NaCl). Compounds were preincubated with 0.5 nM tryptase in assay buffer; total volume 150 μl, in a 96-well microtiter plate for 1 h at room temperature. Enzyme activity was determined by the addition of 50 μl substrate

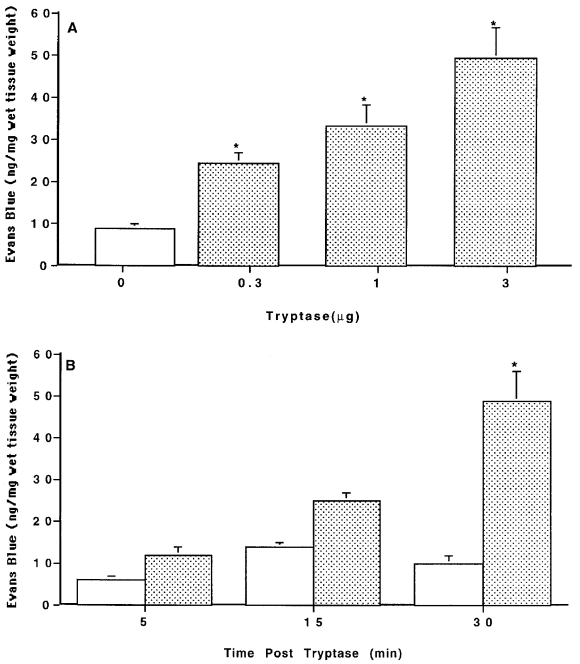

Fig. 1. Dose response (A) and time course (B) of tryptase-induced airway microvascular leakage in guinea pigs. Open bars indicate airway microvascular leakage due to PBS control. Shaded bars indicate the response to tryptase. In panel A, measurements were taken 30 min after administration of tryptase. In panel B, tryptase was dosed at 3 μg. Values are ± S.E.M. ($n = 9$). * $P < 0.05$ compared to PBS.

(*N*-*p*-tosyl-gly-pro-lys-*p*-nitroanilide in DMSO) to a final concentration of 0.5 mM. Wells containing tryptase and substrate with no compound were used to define 100% activity and wells containing tryptase alone were used to define 0% activity. Plates were immediately read in a Spectramax Plus plate reader (Molecular Devices, Sunnyvale, CA, USA) at 410 nm for > 15 min in the kinetic mode. Initial velocity determinations from linear portions of the assay curves were used to evaluate the effect of compounds and heat denaturation on tryptase activity.

2.4. Drugs

The following chemicals were used: Evans blue, histamine, formamide, pyrilamine and ovalbumin (Sigma, St. Louis, MO, USA) aluminum hydroxide gel (Reheis, Berkley Heights, NJ, USA). SR 48968, CP 99994 and SCH 37224 were synthesized at Schering-Plough Research Institute (Kenilworth, NJ, USA). Recombinant human beta-tryptase (tryptase) was purchased from Promega (Madison, WI, USA).

2.5. Statistical analysis

Data are represented as mean ± S.E.M. Statistical differences between means were evaluated using analysis of variance (ANOVA) with post-hoc analysis performed with a Fisher's protected least significant difference program.

2.6. Animal care and use

These studies were performed with the approval of the Animal Care and Use Committee of SPRI which is a facility accredited by the American Association for Accreditation of Laboratory Animal Care.

3. Results

3.1. Effects on airway microvascular leakage

Intratracheal tryptase (0.3–3 μg) administered to normal guinea pigs induced microvascular leakage into the trachea and bronchi (Fig. 1A). The effect at 3 μg tryptase was approximately a fivefold increase in dye content compared to that induced by phosphate buffered saline. The airway microvascular leakage induced by 3 μg of intratracheal tryptase was much less at 5 and 15 min after tryptase compared to values 30 min after tryptase (Fig. 1B). When tryptase (3 μg, i.t.) was subjected to heat denaturation, the increase in dye leakage was completely abolished (Fig. 2).

Pretreatment with a pharmacologically effective dose of the tachykinin NK_1 receptor antagonist, CP 99994 (10 mg/kg, p.o.) (McLean et al., 1992), partially inhibited the tryptase-induced microvascular leakage (26 ± 9% inhibition). Pretreatment with SCH 37224 (5 mg/kg, p.o.), an inhibitor of leukotriene formation, significantly reduced the magnitude of the tryptase-induced microvascular leakage (61 ± 5% inhibition). A lower dose of SCH 37224 (1 mg/kg, p.o.) was inactive. A combination of SCH 37224 (5 mg/kg, p.o.) and CP 99994 (10 mg/kg, p.o.) produced no additional inhibition of tryptase-induced microvascular leakage than SCH 37224 alone (Fig. 3). Pretreatment of normal guinea pigs with the histamine H_1 receptor antagonists, pyrilamine (10 mg/ kg, p.o.) or chlorpheniramine (1

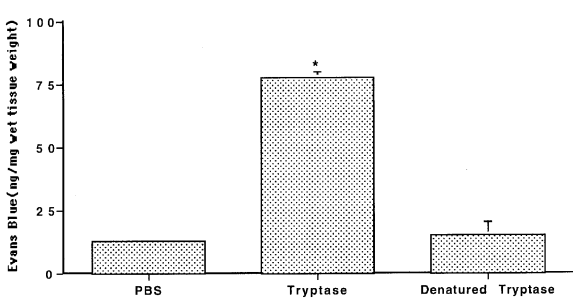

Fig. 2. Effect of heat denaturation on tryptase-induced airway microvascular leakage in guinea pigs. Tryptase was heated to 90°C for 10 min and then cooled to room temperature prior to administration. Values are mean + S.E.M. ($n = 3$). * $P < 0.05$ compared to PBS.

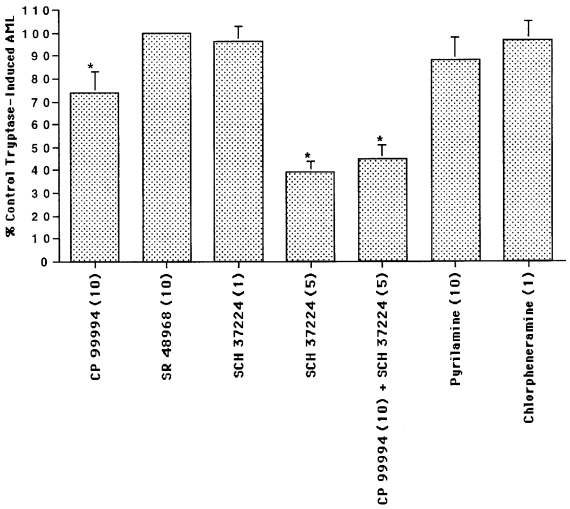

Fig. 3. Effect of drug treatment on tryptase-induced airway microvascular leakage in guinea pigs. Data are presented as percent of airway microvascular leakage induced by 3 μg of tryptase after 30 min. Control values were determined in parallel for each drug treatment tested. All drugs were administered p.o. except for chlorpheniramine, which was administered i.v. The dose of each drug (mg/kg) is denoted in parentheses.

mg/kg, i.v.) had no effect on the magnitude of the airway microvascular leakage induced by tryptase (Fig. 3). Furthermore, pretreatment with the tachykinin NK_2 receptor antagonist, SR 48968 (10 mg/kg, p.o.) had no effect on tryptase-induced microvascular leakage.

3.2. Effects on airway tone

Due to the effect of tryptase on microvascular leakage in the airway, we further evaluated the ability of tryptase to alter airway tone in whole animals. Intratracheal PBS increased pulmonary insufflation pressure by 8 ± 2 cm H_2O over baseline values of 9 ± 1 cm H_2O (89% increase over baseline). Intratracheal tryptase (3–30 μg) had no significant effect on pulmonary insufflation pressure from that induced by PBS in both non-sensitized and ovalbumin-sensitized guinea pigs (Table 1). Consistent with these results no effect on pulmonary insufflation pressure was seen when tryptase was administered to the vasculature of perfused lungs isolated from non-sensitized guinea pigs (data not shown).

Table 1
Effect of tryptase on airway function in-vivo

Tryptase (μg)	ΔPIP[a,b] (cm H_2O)	
	Sensitized	Non-sensitized
0	8.0 ± 1.0	8.0 ± 2.0
3	10.0 ± 1.0	8.0 ± 2.0
10	5.0 ± 1.0	8.0 ± 1.0
30	7.0 ± 2.0	8.0 ± 1.0

[a] Pulmonary insufflation pressure (PIP).
[b] Values are means ± S.E.M. (non-sensitized, $n = 4$; sensitized, $n = 5$).

Table 2
Effect of compounds and heat denaturation on tryptase enzyme activity

Treatment	Concentration (μM)	Velocity[a,b] (m OD/min)
Buffer	–	30.8 ± 3.1
CP 99994	100	32.8 ± 0.1
SCH 37224	100	34.2 ± 0.5
90°C for 10 min	–	0.3 ± 0.1
No substrate	–	0.2 ± 0.2

[a] Velocity of substrate cleavage after incubation with 0.5 nM rh tryptase.
[b] Values are mean ± S.E.M. ($n = 3$).

3.3. Effects on in-vitro tryptase activity

The ability of CP 99994 and SCH 37224 to inhibit the enzyme activity of tryptase was determined as described in Section 2. Neither CP 99994 nor SCH 37224 caused inhibition of tryptase when tested at 100 μM (Table 2). Heating the tryptase to 90°C for 10 min completely inhibited the enzyme activity.

4. Discussion

Tryptase administered intratracheally to normal guinea pigs resulted in microvascular leakage into the trachea and bronchi. This effect was both dose- and time-dependent. The tryptase-induced airway microvascular leakage was not inhibited by the histamine H_1 receptor antagonists pyrilamine and chlorpheniramine, nor was it inhibited by a tachykinin NK_2 receptor antagonist (SR 48968). Tryptase-induced airway microvascular leakage was significantly inhibited by a tachykinin NK_1 receptor antagonist (CP 99994) and by an inhibitor of leukotriene formation (SCH 37224).

We have developed a model of airway microvascular leakage induced by topical administration of tryptase in order to study the mechanism of tryptase-induced airway microvascular leakage. We have confirmed that the airway microvascular leakage is a result of application of enzymatically active tryptase since heat denaturation of the enzyme abolishes the effects on microvascular leakage. Tryptase induced airway microvascular leakage in a dose responsive manner in this model with a maximal response at approximately 3 μg. In addition, the airway microvascular leakage induced by tryptase was time dependent with significant leakage apparent after 30 min. Previous studies assessing the ability of tryptase to induce microvascular leakage in guinea pig and sheep skin have shown that significant responses were obtained as low as 2.5 and 1 ng, respectively (He and Walls, 1997; Molinari et al., 1995). These studies also indicate that maximal responses to

tryptase take at least 20 min to be manifested. While the temporal profile of the airway microvascular leakage is consistent with that found in the skin the doses of tryptase required to induce a significant response are higher. The differences in sensitivity of the two methods are likely due to the routes of administration.

Tryptase has been shown to cause bronchospasm in sheep; however, we found no evidence of bronchospasm in guinea pigs even when tryptase was administered directly to the lung at a dose as high as 30 μg or when tryptase (1 ug/ml) was administered directly to perfused lungs. In allergic sheep doses of tryptase as low a 100 ng caused bronchoconstriction (Molinari et al., 1996). This bronchoconstriction was blocked by pretreatment with the histamine H_1 receptor antagonist chlorpheniramine. The lack of tryptase induced physiological changes in this report may be due to an inherent difference in the reactivity of guinea pig airways to tryptase relative to that of sheep. Also consistent with results in this report are recent results from Carr et al. (2000) in which trypsin was found to induce contraction of guinea pig bronchus while tryptase did not. Additional differences may arise from the different routes of administration (intratracheal in this study vs. inhalation of nebulized material in sheep) or the fact that the sheep used in the previous study were naturally allergic to *Ascaris suum* antigen while the guinea pigs used in the present study were either normal, non-sensitized animals or guinea pigs that were actively sensitized to antigen (ovalbumin).

Previous studies in skin and airways of both sheep and guinea pigs suggest that mast cell mediators, particularly histamine, are responsible for the effects observed with tryptase. We found no role for histamine H_1 receptor activation using doses of H_1 receptor antagonists that have previously been shown to block histaminergic responses in guinea pigs (Kreutner et al., 1988). Tryptase induced bronchospasm in sheep was blocked by treatment with a histamine H_1 receptor antagonist (Molinari et al., 1996). Inhibition of skin responses required a combination of H_1 and H_2 receptor antagonists (He and Walls, 1997; Molinari et al., 1995) although in guinea pig lungs, the majority of the pharmacological effects of histamine, including bronchospasm and airway microvascular leakage are mediated via the H_1 receptor (Saria et al., 1988).

It appears that endogenously released tachykinins contribute to the airway microvascular leakage induced by tryptase because pretreatment of guinea pigs with CP 99994, at a dose previously shown to block capsaicin-induced microvascular leakage (McLean et al., 1992) partially blocked the effect of tryptase. In guinea pigs, microvascular leakage in the trachea and bronchi induced by exogenous or endogenously released tachykinins is mediated by tachykinin NK_1 receptor activation (Rogers et al., 1988) although tachykinin NK_2 receptors contribute to the airway microvascular leakage in peripheral airways (Tousignant et al., 1993). However, in this study SR 48968, a tachykinin NK_2 receptor antagonist (Emonds-Alt et al., 1993; Emonds-Alt et al., 1992) had no effect on the airway microvascular leakage induced by tryptase when administered at a dose previously shown to inhibit tachykinin NK_2 receptor mediated responses (Emonds-Alt et al., 1993). This observation rules out a contribution from activation of this receptor subtype.

Tryptase may stimulate release of endogenous tachykinins by direct activation of C-fiber afferent nerves. Indeed, recent evidence points to a direct action of serine proteases on nerves. Trypsin has been shown to cause bronchoconstriction in guinea pigs through activation of neuronal PAR-2 and subsequent release of tachykinins (Ricciardolo et al., 2000). In addition, both trypsin and tryptase demonstrate proinflammatory properties through activation of PAR-2 in rat primary spinal afferent neurons (Steinhoff et al., 2000). Another plausible explanation is that tryptase stimulates the release of inflammatory mediators which in turn activate C-fiber afferents. In this regard, leukotrienes, histamine and bradykinin potently stimulate tachykinin release from pulmonary C-fibers in guinea pigs (Bloomquist and Kream, 1990; Martins et al., 1991; Saria et al., 1988).

Sch 37224 is an orally active antiallergy compound that has been shown to have a number of pharmacological effects including inhibition of the formation and/or release of leukotrienes (Kreutner et al., 1988; Vigano et al., 1992). Indeed, this pharmacological action is the primary mechanism by which SCH 37224 blocks allergen induced bronchospasm in guinea pigs, rats and sheep (Abraham et al., 1988; Kreutner et al., 1988). In this study, pretreatment with Sch 37224 at a dose (5 mg/kg, p.o.) that inhibits leukotriene-mediated allergic bronchospasm in guinea pigs (Kreutner et al., 1988) attenuated the airway microvascular leakage induced by tryptase suggesting that this effect is mediated predominantly by the release of leukotrienes. The source of the leukotrienes mediating this effect is unknown, but it is unlikely to come from mast cells. We found no evidence of mast cell activation after tryptase by the fact that histamine H_1 receptor antagonists had no effect on the airway microvascular leakage and there was no increase in airway tone after tryptase, an effect which typically occurs in guinea pigs following mast cell activation (Kreutner et al., 1988; Saria et al., 1988). Tryptase stimulates the recruitment and activation of other inflammatory cells such as polymorphonuclear leukocytes, eosinophils and macrophages (He et al., 1997) and has potent effects on resident cells in the lungs such as fibroblasts, myocytes, smooth muscle cells, endothelial cells and epithelial cells (Brown et al., 1995a,b; Cairns and Walls, 1995; Corvera et al., 1997; Hartmann et al., 1992; Ruoss et al., 1991). The contribution of these different cells to the airway microvascular leakage induced by tryptase is currently unclear.

In conclusion, intratracheal administration of tryptase to guinea pigs induced microvascular leakage into the air-

ways. This response involves the actions of endogenous tachykinins and leukotrienes. It is speculated that tryptase may be important in the pathogenesis of inflammatory airway disorders, particularly those involving increased airway microvascular leakage such as asthma.

Acknowledgements

The authors would like to thank Dr. John Hey for helpful critique of the manuscript and Maureen Frydlewicz for preparation of the manuscript.

References

Abraham, W.M., Stevenson, J.S., Garrido, R., 1988. A leukotriene and thromboxane inhibitor (SCH 37224) blocks antigen-induced immediate and late responses and airway hyperresponsiveness in allergic sheep. J. Pharmacol. Exp. Ther. 247, 1004–1011.

Barrios, V.E., Middleton, S.C., Kashem, M.A., Havill, A.M., Toombs, C.F., Wright, C.D., 1998. Tryptase mediates hyperresponsiveness in isolated guinea pig bronchi. Life Sci. 63, 2295–2303.

Berger, P., Compton, S.J., Molimard, M., Walls, A.F., N'Guyen, C., Marthan, R., Tunon-de-Lara, J.M., 1999. Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. Clin. Exp. Allergy 29, 804–812.

Bloomquist, E.I., Kream, R.M., 1990. Release of substance P from guinea pig trachea by leukotriene D4. Exp. Lung Res. 16, 645–659.

Brown, J.K., Jones, C.A., Tyler, C.L., Ruoss, S.J., Hartmann, T., Caughey, G.H., 1995a. Tryptase-induced mitogenesis in airway smooth muscle cells. Potency, mechanisms, and interactions with other mast cell mediators. Chest 107, 95S–96S.

Brown, J.K., Tyler, C.L., Jones, C.A., Ruoss, S.J., Hartmann, T., Caughey, G.H., 1995b. Tryptase, the dominant secretory granule protein in human mast cells, is a potent mitogen for cultured dog tracheal smooth muscle cells. Am. J. Respir. Cell Mol. Biol. 13, 227–236.

Cairns, J.A., Walls, A.F., 1995. Mast cell tryptase is a mitogen for epithelial cells. Stimulation of IL-8 production and intracellular adhesion molecule-1 expression. J. Immunol. 156, 275–283.

Carr, M.J., Schechter, N.M., Undem, B.J., 2000. Trypsin-induced, neurokinin-mediated contraction of guinea pig bronchus. Am. J. Respir. Crit. Care Med. 162, 1662–1667.

Caughey, G.H., 1994. Serine proteases of mast cell and leukocyte granules: a league of their own. Am. J. Respir. Crit. Care Med. 150, S138–S142.

Clark, J.M., Abraham, W.M., Fishman, C.E., Forteza, R., Ahmed, A., Cortes, A., Warne, R.L., Moore, W.R., Tanaka, R.D., 1995. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. Am. J. Respir. Crit. Care Med. 152, 2076–2083.

Corvera, C.U., Dery, O., McConalogue, K., Bohm, S.K., Khitin, L.M., Caughey, G.H., Payan, D.G., Bunnett, N.W., 1997. Mast cell tryptase regulates rat colonic myocytes through proteinase-activated receptor 2. J. Clin. Invest. 100, 1383–1393.

Danko, G., Hey, J.A., Del Prado, M., Kreutner, W., Chapman, R.W., 1992. GABA-B induced inhibition of peptidergic airway microvascular leakage in guinea pigs. Pharmacol. Commun. 1, 203–209.

Emonds-Alt, X., Vilain, P., Goulaouic, P., Proietto, V., Van Broeck, D., Advenier, C., Naline, E., Neliat, G., Le Fur, G., Breliere, J.C., 1992. A potent and selective nonpeptide antagonist of the neurokinin A (NK-2) receptor. Life Sci. Pharmacol. Lett. 50, 101–106.

Emonds-Alt, X., Proietto, V., Van Broeck, D., Vilain, P., Advenier, C., Neliat, G., Le Fur, G., Breliere, J.-C., 1993. Pharmacological profile and chemical synthesis of SR 48968, a non-peptide antagonist of the neurokinin-A (NK2) receptor. Bioorg. Med. Chem. Lett. 3, 925–930.

Hartmann, T., Ruoss, S.J., Raymond, W.W., Seuwen, K., Caughey, G.H., 1992. Human tryptase as a potent, cell-specific mitogen: role of signaling pathways in synergistic responses. Am. J. Physiol. 262, L528–L534.

He, S., Walls, A.F., 1997. Human mast cell tryptase: a stimulus of microvascular leakage and mast cell activation. Eur. J. Pharmacol. 328, 89–97.

He, S., Peng, Q., Walls, A.F., 1997. Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. J. Immunol. 159, 6216–6225.

Johnson, P.R.A., Ammit, A.J., Carlin, S.M., Armour, C.L., Caughey, G.H., Black, J.L., 1997. Mast cell tryptase potentiates histamine-induced contraction in human sensitized bronchus. Eur. Respir. J. 10, 38–43.

Kreutner, W., Sherwood, J., Sehring, S., Rizzo, C., Chapman, R.W., Siegel, M.I., Egan, R.W., 1988. Antiallergy activity of Sch 37224, a new inhibitor of leukotriene formation. J. Pharmacol. Exp. Ther. 247, 997–1003.

Martins, M.A., Shore, S.A., Drazen, J.M., 1991. Release of tachykinins by histamine, methacholine, PAF, LTD4, and substance P from guinea pig lungs. Am. J. Physiol. 261, L449–L455.

McLean, S., Ganong, A., Seymour, P.A., Snider, M.R., Desai, M.C., Rosen, T., Bryce, D.K., Longo, K.P., Reynolds, L.S., Robinson, G., Schmidt, A.W., Siok, C., Heym, J., 1992. Pharmacology of CP-99994: a nonpeptide antagonist of the tachykinin Neurokinin 1 receptor. 1993. J. Pharmacol. Exp. Ther. 267, 472–479.

Molinari, J.F., Moore, W.R., Clark, J., Tanaka, R., Butterfield, J.H., Abraham, W.M., 1995. Role of tryptase in immediate cutaneous responses in allergic sheep. J. Appl. Physiol. 79, 1966–1970.

Molinari, J.F., Scuri, M., Moore, W.R., Clark, J., Tanaka, R., Abraham, W.M., 1996. Inhaled tryptase causes bronchoconstriction in sheep via histamine release. Am. J. Respir. Crit. Care Med. 154, 649–653.

Pereira, P.J.B., Bergner, A., Macedo-Ribeiro, S., Huber, R., Matschiner, G., Fritz, H., Sommerhoff, C.P., Bode, W., 1998. Human beta-tryptase is a ring-like tetramer with active sites facing a central pore. Nature 392, 306–311.

Ricciardolo, F.L.M., Steinhoff, M., Amadesi, S., Guerrini, R., Tognetto, M., Trevisani, M., Creminon, C., Bertrand, D., Bunnett, N.W., Fabbri, L.M., Salvadori, S., Geppetti, P., 2000. Presence and bronchomotor activity of protease-activated receptor-2 in guinea pig airways. Am. J. Respir. Crit. Care Med. 161, 1672–1680.

Rogers, D.F., Belvisi, M.G., Aursudkij, B., Evans, T.W., Barnes, P.J., 1988. Effects and interactions of sensory neuropeptides on airway microvascular leakage in guinea pigs. Br. J. Pharmacol. 95, 1109–1116.

Ruoss, S., Hartmann, T., Caughey, G., 1991. Mast cell tryptase is a mitogen for cultured fibroblasts. J. Clin. Invest. 88, 493–499.

Saria, A., Martling, C.-R., Yan, Z., Theodorsson-Norheim, E., Gamse, R., Lundberg, J.M., 1988. Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperizinium, and vagal nerve stimulation. Am. Rev. Respir. Dis. 137, 86–90.

Schwartz, L.B., Lewis, R.A., Austen, K.F., 1981. Tryptase from human pulmonary mast cells: purification and characterization. J. Biol. Chem. 256, 11939–11943.

Schwartz, L.B., Bradford, T.R., Lee, D.C., Chlebowski, J.F., 1990. Immunologic and physicochemical evidence for conformational changes occurring on conversion of human mast cell tryptase from active tetramer to inactive monomer. Production of monoclonal antibodies recognizing active tryptase. J. Immunol. 144, 2304–2311.

Sekizawa, K., Caughey, G.H., Lazarus, S.C., Gold, W.M., Nadel, J.A., 1989. Mast cell tryptase causes airway smooth muscle hyperresponsiveness in dogs. J. Clin. Invest. 83, 175–179.

Steinhoff, M., Corvera, C.U., Thoma, M.S., Kong, W., Mcalpine, B.E.,

Caughey, G.H., Ansel, J.C., Bunnett, N.W., 1999. Proteinase-activated receptor-2 in human skin: tissue distribution and activation of keratinocytes by mast cell tryptase. Exp. Dermatol. 8, 282–294.

Steinhoff, M., Vergnolle, N., Young, S.H., Tognetto, M., Amadesi, S., Ennes, H.S., Trevisani, M., Hollenberg, M.D., Hollenberg, J.L., Hollenberg, W., Caughey, G.H., Mitchell, S.E., Williams, L.M., Geppetti, P., Mayer, E.A., Bunnett, N.W., 2000. Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat. Med. 6, 151–158.

Tousignant, C., Chan, C.-C., Guevremont, D., Brideau, C., Hale, J.J., MacCoss, M., Rodger, I.W., 1993. NK2 receptors mediate plasma extravasation in guinea pig lower airways. Br. J. Pharmacol. 108, 383–386.

Vigano, T., Crivellari, M.T., Accomazzo, M.R., Monopoli, A., Belloni, P.A., Torre, M., Nicosia, S., Folco, G.C., 1992. Effect of SCH 37224 on anti-IgE-induced formation of sulfidopeptide leukotrienes in human lung parenchyma. Eur. J. Pharmacol. 219, 159–164.

Welle, M., 1997. Development, significance, and heterogeneity of mast cells with particular regard to the mast cell-specific proteases chymase and tryptase. J. Leukocyte Biol. 61, 233–245.