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EXPERIMENTAL ITCH IN HUMAN SKIN ELICITED BY RAT MAST CELL CHYMASE

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Abstract. A chymotrypsin-like enzyme (mast cell chymase), prepared from granules of rat mast cells, was injected intracutaneously into 20 patients with itching dermatoses and 5 healthy subjects. Itch was elicited in 72% of the cases. This raised the possibility that when mast cells are degranulated not only the released histamine but also the concomitantly released chymase may be pruritogenic. However, in contrast to the papain-induced itch, the itch produced by the chymase and by trypsin seemed to be mediated by released histamine, since the two proteases caused redness, wheal and flare, and the itch was markedly reduced by previous depletion of histamine at the injection site. The possibility that chymase in released granules may induce histamine release is discussed but considered less probable, i.e. the chymase of the "intact", secreted mast cell granules seems to be inactive as long as it is bound to heparin.

Histamine and proteolytic enzymes produce itch when injected intracutaneously. Activation or release of these agents has therefore been suggested to be a link in the peripheral itch mechanism (4, 1). Although proteases are considered to be the key agents, histamine is very likely to be involved when there are visible vascular effects with whealing and flare of the skin.

Histamine, stored in the basophil granules of the mast cells, may be released from these cells by various kinds of stimulation, e.g. antigen–antibody reaction, mechanical trauma to the skin, or injection of histamine liberators. In order to release histamine the mast cells excrete these granules (9, 11). Since the granules also store high concentrations of a proteolytic enzyme with properties similar to chymotrypsin, "mast cell chymase" (6, 7), a protease will be released from the cells together with histamine.

In earlier works on substances released from mast cells, the biological effects of histamine and heparin have been stressed, while possible effects of released chymase have not been studied. Thus, it was considered of interest to find out whether the chymotrypsin-like enzyme from mast cells may elicit itching. The effects were compared with those of trypsin and papain, proteases well-known to produce itch following intracutaneous injection (1, 8).

MATERIAL AND METHODS

Mast cell chymase was obtained from granules of rat mast cells. Peritoneal and pleural mast cells were isolated from male Sprague-Dawley rats according to Thon & Uvnäs (10) using density gradient centrifugation in Ficoll. The mast cells were disrupted with hyposmotic shock in distilled water (12) and the mast granules thus liberated were dissolved in 1 M NaCl solution. The solution was then chromatographed on a column of Dowex 1-X2 and the chymase eluted with 1 M NaCl (Bergqvist, Samuelsson & Uvnäs, to be published). The solution was dialysed against distilled water. The desalted solution was then centrifuged and the supernatant containing the enzyme was freeze-dried.

Immediately before use the freeze-dried chymase was dissolved in physiological saline at a concentration of 200 μg/ml and filtered through a Millipore filter (RAWP 013, 1.2 μ, 13 mm).

Trypsin (Trypure®, Novo Industri, Copenhagen, Denmark) was dissolved in saline at a concentration of 100 μg/ml.

Papain (2 × crystallized, Sigma Chemical Co., St. Louis, Miss., USA) was diluted in saline at a concentration of 50 μg/ml.

The hydrolytic activity of the chymase and trypsin preparations was assayed using casein as substrate. The hydrolysed casein was measured in a Zeiss spectrophotometer. The activity of the chymase varied: 1 μg of trypsin corresponded to 1.0–1.2 μg of chymase.

Table I

	Trypsin	Chymase	Saline
No. of persons responding with itch	25 (100%)	18 (72%)	1[a]

[a] In an additional person some tenderness but no itch.

Table II. *The average itch duration ± S.E.M. in those persons responding with itch*

	Trypsin ($n=25$)	Chymase ($n=18$)
Duration	2 min 13 sec ±17 sec	1 min 7 sec ±12 sec

Itch experiments

Comparison of chymase and trypsin. About 0.02 ml of trypsin, chymase and physiological saline was injected intracutaneously in each of 20 patients (11 with atopic dermatitis, 5 with itching psoriasis, 2 with chronic urticaria, 1 with pruritus and 1 with eczema) and 5 healthy volunteers. Thus each person received 2 μg of trypsin and 4 μg of chymase. The experiment was performed as a double blind study, the three solutions given in random order to the different subjects. The duration of the itch was recorded, as described previously (8).

Local depletion of mast cell histamine. Compound 48/80 was injected subepidermally on the lateral part of the arm for three consecutive days, 20 μg in 0.1 ml saline given on each day. Saline was injected on the other arm. On the fourth day about 0.02 ml of one of the itch-producing proteases (trypsin, chymase or papain) was given intra-epidermally on both histamine-depleted and control side. The duration of the itch response was recorded.

RESULTS

Comparison of chymase and trypsin. The enzyme preparations were used immediately after sterile filtration. It appeared that the enzyme activity was decreased during filtration and the final activity was about half that of the original solution. The results of the intracutaneous administrations are summarized in Tables I and II. Chymase elicited itching in 18 out of 25 persons; the sensation of itch was somewhat less than after trypsin administration. There was no difference in reactivity to the enzyme preparation between patients with dermatoses and the small group of healthy controls. One patient reacted not only to trypsin and chymase but—to a lesser degree—even to physiologic saline.

Effect of histamine depletion. At the site of injection of trypsin or chymase there appeared wheal, redness and flare, indicating that the proteases might release mast-cell bound histamine. To establish whether histamine was involved in the itch response, the local histamine stores were depleted by injection of compound 48/80 for 3 days prior to injection of the proteases. On the first day 48/80 produced a marked triple response, but on the second day the response was decreased and on the third day hardly any visible reaction could be observed. When the histamine had thus been released, trypsin, chymase or papain was injected intracutaneously on the fourth day, and the itch duration measured. It was found that the itch produced by chymase and trypsin was significantly decreased at the histamine-depleted site compared with the saline-treated site, whereas the itch response after papain was not influenced by pretreatment with 48/80 (Table III).

Pretreatment with histamine for 3 days—producing a triple response each day—led to some decrease of the itch caused by trypsin on the fourth day, but the decrease was not as marked as after pretreatment with compound 48/80 (Table IV).

Table III. *Effect of local histamine depletion by 48/80*

	Itch duration ± S.E.M. (sec)		
	Control side (NaCl-treated)	48/80 pretreated side	Significance of differences
Trypsin ($n=14$)	98±16	37± 8	$0.001 > p$
Chymase ($n=11$)	51±13	9± 7	$0.001 > p$
Papain ($n=9$)	169±26	162±33	Not sign.

Table IV. *Effect of pretreatment with histamine on itch duration*

	Itch duration ± S.E.M. (sec)		
	Control side (NaCl-treated)	Histamine-pretreated side	Significance of difference
Trypsin ($n=10$)	62±8	37±3	$0.05 > p > 0.01$

DISCUSSION

Mast cell degranulation and histamine release are connected phenomena. Histamine is bound to the granular matrix by ionic forces and is released from the granules by ion exchange when they are exposed to cations, e.g. Na^+. This happens when the granules are discharged from the mast cell; the extracellular sodium ions immediately replace the histamine molecules at the granular binding sites (12). The granular matrix consists of heparin linked to chymase with strong ionic forces which are not disrupted in physiological saline (0.15 M), but at higher concentrations of NaCl, e.g. 1 M (12). However, some observations indicate that even "intact" granules may have a chymase activity (Bergqvist, unpublished observation). If this were correct it might be expected that released granules exert chymase activity on the surroundings.

On the basis of the well-known pruritogenic effect of histamine the role of mast cells in itching has been discussed. Arthur & Shelley (2) could not, however, find correlation between "itch points" and the number of mast cells, although Asboe-Hansen already in 1950 investigated the problem of mast cells in itching dermatoses (3). Among other skin diseases, the number of mast cells was found to be increased in some cases of lichen ruber planus with itching. Furthermore, patients with pruritus may have somewhat increased skin histamine levels (5).

In the present investigation it was possible to demonstrate the itch-producing property of rat mast cell chymase. The results confirm earlier findings on the pruritogenic effect of proteases. As a foreign protein was injected, its possible immunogenic effect should be considered. In the present studies, however, it was the first administration of rat chymase that induced itch. Thus an immunologic mechanism seems unlikely.

It was found that the injected chymase and trypsin caused such visible changes as edema and flare. Thus, it seemed possible that the itch response was—at least to some extent—due to release of histamine from the dermal mast cells. This was strongly supported by the finding that depletion of the mast cell histamine prior to injection of trypsin or chymase markedly abated the itch. However, the papain-elicited itch was not influenced by pretreatment with compound 48/80. These observations agree with those of Arthur & Shelley (1), who describe how trypsin acts as a histamine liberator whereas papain does not. It was also investigated whether injection of histamine may reduce the itch. However, pretreatment with histamine did not decrease the itch response as much as pretreatment with compound 48/80.

In conclusion, chymase and trypsin produce itch that to a large extent is mediated by histamine released from dermal mast cells. This means that the chymase prepared from the mast cell granules may itself induce mast cell degranulation and histamine release. This raises the question whether the chymase of the "intact" granules, which are released from mast cells in connection with histamine liberation, could elicit itch and histamine release. However, this seems unlikely, since there have been no signs of self-inductive histamine release in earlier studies. By contrast, the histamine release from tissues and isolated mast cells is dependent on the concentration of the liberator, and the reaction ceases before all histamine has been released. Probably the chymase of the secreted granules is inactive as long as it is bound to heparin (12). The physiological significance of the chymase in the released granules remains to be established.

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