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Synthetic Peptides Comprising Sequences of the Human Immunoglobulin E Heavy Chain Capable of Releasing Histamine

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On the basis of previous studies on the structure–activity relationship of model polypeptide histamine liberators, a site within the Fc region of immunoglobulin E antibody molecules has been proposed as that responsible for the direct triggering of target mast cells after antigen challenge. Peptides comprising this region of the ϵ -chain have now been synthesized and shown to induce histamine release from normal rat peritoneal mast cells in a selective manner essentially similar to that mediated by anaphylactic antibody–antigen interaction.

Corticotropin ('ACTH') and melittin, together with certain of their derivatives, were used in previous studies (Jasani & Stanworth, 1973; Jasani, 1973) aimed at defining the structural basis of mast-cell triggering by polypeptide histamine liberators. Thus it was established that a cluster of basic amino acid residues was an essential structural requirement for the expression of this type of biological activity.

Moreover, as histamine release induced from normal rat mast cells by such artificial liberators appeared to involve an active secretory process similar to that effected by allergen challenge of antibody-sensitized cells, it was considered that basic polypeptides might serve as analogues in the determination of that part of the antibody molecule concerned with mast-cell triggering in hypersensitivity reactions of the immediate type. This implies that basic amino acid residues comprise an effector site located in the Fc region of anaphylactic antibodies, which, it has been postulated (Stanworth, 1974), is brought into the correct juxtaposition relative to an activation site on the target-cell membrane as a result of cross-linking of cell-bound antibody molecules by specific antigen (i.e. allergen). Contiguous hydrophobic residues within such an antibody effector site would be expected to have a potentiating effect on histamine-releasing activity, since this has been suggested from our previous studies on artificial polypeptide liberators.

On this assumption we have sought amino acid sequences incorporating such characteristics within the recently reported primary structures of those immunoglobulin classes with which anaphylactic

antibody activity is associated; notably IgE. Examination of the human ϵ -chain structure reported by Bennich and associates (Bennich & Von Bahr-Lindstrom, 1974; Bennich *et al.*, 1976) reveals the following sequence spanning residues 496–506 within the C₄ domain, which seems most likely to meet these structural requirements:

-Arg-Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe

We now report the synthesis of peptides comprising sequences representative of this region (including an octapeptide, 497–504; a nonapeptide, 496–504; and a decapeptide, 497–506) and the results of testing the ability of these to induce selective (i.e. non-lytic) histamine release from normal rat peritoneal mast cells *in vitro*.

Materials and Methods

Peptides were synthesized by using classical procedures, the purity of product being checked by amino acid analysis and t.l.c. The octapeptide sequence shown in Table 1 was prepared by using a 4+4 fragment strategy involving azide coupling of protected intermediates:

Boc-Lys(Z)-Thr(Bzl)-Lys(Z)-Gly-N₂H₃

and

Ser(Bzl)-Gly-Phe-Phe-OMe

followed by Boc-cleavage and hydrogenolysis. The nonapeptide (Table 1) was obtained on coupling Z-Arg(Z₂)-ONSu with Boc-cleaved fully protected octapeptide intermediate referred to above, followed by hydrogenolysis. The decapeptide (Table 1) was prepared by a 4+2+4 fragment strategy coupling

Abbreviations used: IgE, immunoglobulin E; Boc, t-butoxycarbonyl; Z, benzyloxycarbonyl; Bzl, benzyl; Me, methyl; ONSu, succinimido-oxy.

Table 1. *Histamine-release values for octapeptide, nonapeptide and decapeptide in the rat mast-cell system*
None of the synthetic peptides induced lactate dehydrogenase release at any concentration tested.

[Peptide] (as methyl ester) (μM)	Histamine release* (%)		
	Octapeptide (497-504)	Nonapeptide (496-504)	Decapeptide (497-506)
100	66.5 $\pm$ 4.5	62.5 $\pm$ 0.5	77.0 $\pm$ 0
50	65.5 $\pm$ 3.5	59.5 $\pm$ 0.5	75.0 $\pm$ 1.0
10	50.0 $\pm$ 1.0	53.0 $\pm$ 2.0	60.0 $\pm$ 2.0
5	40.5 $\pm$ 1.0	45.0 $\pm$ 2.0	46.0 $\pm$ 3.5
1	19.5 $\pm$ 2.5	17.5 $\pm$ 0.5	14.0 $\pm$ 4.0

* Values obtained from two determinations in duplicate.

Boc-Ser(Bzl)-Gly-ONSu to Phe-Phe-Val-Phe-OMe, followed by Boc-cleavage and a further azide coupling of Boc-Lys(Z)-Thr(Bzl)-Lys(Z)-Gly-N₂H₃, Boc-cleavage and hydrogenolysis giving the decapeptide methyl ester. Purification of peptides was by column chromatography on Sephadex LH-20 or Bio-Gel P2; elution with water was followed by freeze-drying. T.l.c. on silica-gel plates [butan-1-ol/acetic acid/water (5:2:2, by vol.)] with ninhydrin identification showed single-component compounds; amino acid analysis, performed on samples hydrolysed with constant-boiling HCl at 140°C for 4 h under N₂, with a Rank-Hilger automatic analyser, gave values consistent with expected structure.

The positive-control histamine-releasing peptide used in the present studies was synthetic corticotropin-(1-24)-tetracosapeptide, marketed under the name 'Synacthen'. It promoted 50% release of histamine from normal rat peritoneal mast-cell preparations *in vitro* at an average concentration of 1 μM .

Disodium cromoglycate was kindly given by Fisons Pharmaceutical Laboratories Ltd., Loughborough, Leics., U.K. The sodium salt of 5,6-dimethyl-2-nitroindane-1,3-dione (BRL 10833) was prepared in our laboratories (Buckle *et al.*, 1973). Drugs were administered simultaneously with peptide challenge.

Mast cells were obtained from the peritoneal washings of male, outbred Wistar rats (250-300 g). In a typical experiment, duplicate portions of cell suspensions were incubated with peptide at 37°C for 5 min; after centrifugation the supernatants were assayed for histamine by an automated spectrofluorimetric method (Evans *et al.*, 1973). Specific histamine release was calculated as a percentage of total histamine available in each test, by taking into account that remaining in the cell pellet.

Mast cells were purified by the procedure described by Cooper & Stanworth (1974). Lactate dehydrogenase activity was measured by the method of King (1960).

None of the peptides tested interfered with these assays.

Results and Discussion

The histamine-releasing activities of synthetic peptides representative of human ϵ -chain sequences determined in the rat mast-cell system *in vitro* are shown in Fig. 1. The basic peptides Lys-Thr-Lys and Arg-Lys-Thr-Lys showed only relatively weak releasing activity. This increased, however, with blockage of the C-terminal carboxy group by methylation to give Arg-Lys-Thr-Lys-OMe, whereas extension of this sequence to Arg-Lys-Thr-Lys-Gly-OMe did not alter activity appreciably. Addition of other ϵ -chain residues to the C-terminus and omission of the N-terminal arginine residue resulted in a substantial increase in activity, which reached a peak in the octapeptide sequence Lys-Thr-Gly-Ser-Gly-Phe-Phe-

Fig. 1. *Dose-response relationships shown by synthetic peptides representative of human ϵ -chain sequences in a purified rat peritoneal-mast-cell assay*

(a) Histamine release from rat mast cells *in vitro* by ϵ -chain sequence:

(b) Histamine/LDH (lactate dehydrogenase) dose-response relationships for all-L and all-D Phe-Phe-OMe.

OMe (as will be noted from Fig. 1a). The C-terminal hydrophobic tetrapeptide of this sequence showed no capacity to release histamine *per se*, but addition of it to the C-terminal end significantly enhanced activity of the basic tetrapeptide sequence. These findings are, in general, consistent with our previous observations on the structural requirements for histamine-releasing activity (Jasani & Stanworth, 1973; Jasani *et al.*, 1979).

Extension of the octapeptide sequence at the N-terminus with arginine to give a more basic nonapeptide (ϵ -chain, 496–504), or at the C-terminus by Val-Phe to give a more hydrophobic decapeptide (ϵ -chain, 497–506), failed to lead to a further marked increase in activity (Table 1), suggesting, perhaps, that the octapeptide sequence is of optimal length for histamine-releasing activity.

Interestingly, the dipeptide ester Phe-Phe-OMe, forming the C-terminus of the octapeptide sequence, itself showed histamine-releasing activity comparable with that of the octapeptide. This activity, however, as shown in Fig. 1(b), is accompanied by evidence of cell lysis as recorded by lactate dehydrogenase release, although this reached a peak at higher peptide concentrations. On the other hand, the all-D form of Phe-Phe-OMe was inactive as a histamine/lactate dehydrogenase releaser, indicating a stereospecific requirement for lysis by this type of compound. Since, however, both enantiomers are physicochemically identical, lysis induced by the all-L form of the dipeptide ester cannot be related to a direct detergent effect (e.g., by Triton X-100).

This finding prompts reconsideration of the meaning of selective and non-selective as ascribed to mechanisms of histamine release (Johnson & Moran, 1969), for it seems that lysis leading to non-selective histamine release can in turn have a stereoselective basis. Activity of all-L Phe-Phe-OMe is also lost on N-terminal extension with glycine, and with Ser-Gly (to complete the C-terminal half of the active ϵ -chain octapeptide), or by the replacement of the methyl ester with an amide group. In this connection it is noteworthy that the ability of aromatic amino acids to release histamine from rat mast cells has been reported previously by Keller & Beeger (1963), and the sensitivity of cells to those esters was considered to be attributable to activation of serine esterases. If this is the case, it would appear that N-terminal elongation of Phe-Phe-OMe in the present study has decreased substrate specificity for the chymotrypsin-like enzyme system that would seem to be associated with cell lysis; yet such an extension leads to a structure in which a capacity to induce histamine-release selectively is retained, with maximal activity being expressed at the level of the octapeptide.

The mechanism of release of histamine from mast cells by octapeptide and nonapeptide methyl esters shows striking similarities to the antigen-induced

release process. There is, for instance, retention of lactate dehydrogenase after incubation of these peptides with purified mast cells (Table 1); and release is blocked by lowering the temperature to 5°C. In the latter case, interaction of octapeptide and nonapeptide with mast cells in the cold led to subsequent desensitization of cells at 37°C, a pattern of behaviour similar to that reported to occur with histamine release from sensitized mast cells induced by reaction with specific antigen (Johnson & Moran, 1970). By contrast, inhibition of all-L Phe-Phe-OMe-induced histamine release brought about by lowering the temperature to 5°C was largely reversed on re-warming cells to 37°C. This process of cooling and re-warming the mast cells had no influence, on the other hand, on the release of histamine effected by the detergent Triton X-100.

Confirmation that the mechanism of release by octapeptide/nonapeptide is indeed by a pathway analogous to that mediated by homocytotropic antibody was obtained from the demonstration of some inhibition of release by disodium cromoglycate and by 4,5-dimethyl-2-nitroindene-1,3-dione (Spicer *et al.*, 1975), two anti-allergic compounds that partially inhibit antigen-induced histamine release from antibody-sensitized rat mast cells *in vitro*.

The observation that considerably less (of the order of 1000-fold on a molar basis) allergen than synthetic ϵ -chain peptide is required to effect a similar extent of histamine release from sensitized rat mast cells is not necessarily contrary to such a conclusion, for it seems likely that IgE antibody molecules prebound to the target mast cells are poised for optional triggering, once having been bridged by specific antigen. By contrast, the synthetic peptides can be expected to be less efficient stimulators, in view of their probable lack of the distinctive cell-binding site assumed to be present in the whole native-antibody molecule, quite apart from the absence of other regions of ϵ -polypeptide chain structure, which might be expected to exert some secondary influence in the immunological triggering process.

Thus it has been demonstrated that synthetic peptides comprising sequences found within the Fc region of human IgE are capable of inducing histamine release from normal rat mast cells in a manner essentially similar to that mediated by anaphylactic antibody-allergen interaction on the surface of pre-sensitized cells. These findings are not inconsistent with the suggestion (Stanworth, 1971) that mast cells are triggered by an effector group within the Fc region of anaphylactic antibodies that is distinct from the target-cell binding site. It seems likely, therefore, that further study of peptide-induced histamine-release processes will throw more light on the immunological mechanism underlying hypersensitivity reactions of the immediate type.

References

- Bennich, H. & Von Bhar-Lindstrom, H. (1974) *Prog. Immunol.* **11**, 49–58
- Bennich, H., Johansson, S. G. C., Von Bhar-Lindstrom, H. & Karlsson, T. (1976) *Nobel Symp.* **33**, 175–194
- Buckle, D. R., Morgan, N. J., Ross, J. W., Smith, H. & Spicer, B. (1973) *J. Med. Chem.* **16**, 1334–1339
- Cooper, P. H. & Stanworth, D. R. (1974) *Prep. Biochem.* **4**, 105–114
- Evans, D. P., Lewis, J. A. & Thompson, D. S. (1973) *Life Sci.* **12**, 327–336
- Jasani, B. (1973) Ph.D. Thesis, University of Birmingham
- Jasani, B. & Stanworth, D. R. (1973) *Int. Arch. Allergy Appl. Immunol.* **45**, 74–81
- Jasani, B., Kreil, G. & Stanworth, D. R. (1979) *Biochem. J.* in the press
- Johnson, A. R. & Moran, N. C. (1969) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **28**, 1716–1720
- Johnson, A. R. & Moran, N. C. (1970) *J. Pharmacol. Exp. Ther.* **175**, 632–640
- Keller, R. & Beeger, I. (1963) *Int. Arch. Allergy* **22**, 31–44
- King, J. (1960) *J. Med. Lab. Technol.* **17**, 1–21
- Spicer, B. A., Ross, J. W. & Smith, H. (1975) *Clin. Exp. Immunol.* **21**, 419–423
- Stanworth, D. R. (1971) *Nature (London)* **233**, 310–316
- Stanworth, D. R. (1974) *Haematologia* **8**, 299–319