Immunoglobulin E decapeptide-induced 5-hydroxytryptamine release from rat peritoneal mast cells

Comparison with corticotropin-(1–24)-peptide, polyarginine, polylysine and antigen

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A synthetic basic decapeptide from the C4 domain of human immunoglobulin E, corticotropin-(1–24)-peptide, polyarginine and polylysine stimulated up to 90% net release of 5-hydroxytryptamine from mast cells in rat peritoneal-cell suspensions. Concentration–response curves to all four polypeptides were parallel. Polyarginine and polylysine ($EC_{50} \approx 0.05 \mu M$) were approximately 100-fold more potent than immunoglobulin E decapeptide and corticotropin-(1–24)-peptide ($EC_{50} \approx 5 \mu M$). Polypeptide-induced release was complete within 5–10 s. Immunoglobulin-E-decapeptide-induced 5-hydroxytryptamine release was additive to that induced by low concentrations of polyarginine, but non-additive to that induced by high concentrations of polyarginine. In contrast, release induced by antigen was additive along the whole length of the concentration–response curve to polyarginine. Benzalkonium chloride inhibited immunoglobulin-E-decapeptide- and polyarginine-induced 5-hydroxytryptamine release but enhanced release induced by immunological stimulation.

The IgE-dependent activation of mast cells to release granule-associated and newly generated inflammatory mediators occurs through polymerization of cell-bound IgE molecules by antigen (Ishizaka & Ishizaka, 1978a; Metzger, 1978). The discovery that a synthetic basic decapeptide from the C4 domain of human IgE is capable of releasing histamine from rat mast cells (Stanworth *et al.*, 1979) provided support for the theory that cross-linking of IgE by antigen reveals an Fc-region-effector site that triggers an activation site on the mast-cell membrane (Stanworth, 1974). A number of other basic polypeptides, including corticotropin, melittin, polymyxin B, protamine, polylysine and substance P (Jasani *et al.*, 1979; Read & Kiefer, 1979), are also capable of inducing mast-cell secretion by a mechanism that shares several features in common with IgE-dependent release: it is non-cytolytic, energy- and temperature-dependent, Ca^{2+} -dependent under certain conditions and inhibited by sodium cromoglycate (Stanworth *et al.*, 1979; Ennis *et al.*, 1980). These characteristics are also shown by human basophil leucocytes in

response to the polypeptides polyarginine and polylysine, and immunological stimuli (Foreman & Lichtenstein, 1980). Structure–activity studies on naturally occurring and synthetic polypeptides have established that mast-cell-degranulating activity is dependent on a cluster of basic residues (arginine and lysine) and on the absence of acidic residues in the same region (Jasani *et al.*, 1979).

The present study compares the kinetics and concentration–response characteristics of mediator release from rat mast cells induced by IgE decapeptide, corticotropin-(1–24)-peptide, polyarginine and polylysine. The response to polyarginine was studied alone and in combination with either IgE decapeptide or antigen. The results of these experiments, together with studies with benzalkonium chloride, a known inhibitor of polyamine-induced mediator release (Read & Kiefer, 1979), were used to compare mechanisms of mast-cell mediator release induced by IgE decapeptide and by other basic polypeptides, and IgE-dependent mechanisms.

Experimental

Materials

IgE decapeptide (Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe) was provided by Dr. D. R. Stanworth

Abbreviations used: IgE, immunoglobulin E; MEM medium, Minimum Essential Medium containing 1.0 mg of bovine serum albumin/ml.

(Department of Immunology, University of Birmingham, Birmingham, U.K.). Corticotropin-(1-24)-peptide was obtained from CIBA-GEIGY, Horsham, Sussex, U.K. Poly-L-arginine hydrochloride (average mol.wt. 60000; 300 residues), poly-L-lysine hydrobromide (average mol.wt. 40000; 180 residues), bovine serum albumin (fraction V), ovalbumin (grade V) and benzalkonium chloride were obtained from Sigma Chemical Co., Poole, Dorset, U.K. *Bordetella pertussis* vaccine (The Lister Institute, Elstree, Herts., U.K.), sheep anti-(rat IgE) serum (Miles Laboratories, Slough, Berks., U.K.), powdered Minimum Essential Medium with Hanks salts containing 20 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.), 5-hydroxy-[G-³H]tryptamine creatinine sulphate (specific radioactivity 11 Ci/mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.) and Liquid Scintillator Miniria 20 (Koch-Light Laboratories, Colnbrook, Bucks., U.K.) were purchased from the suppliers. Hooded Lister rats (200–250 g) were supplied by OLAC 76, Bicester, Oxfordshire, U.K.

Antisera containing IgE antibody to ovalbumin were raised by the two-step procedure described by Jarrett *et al.* (1980), with *Bordetella pertussis* vaccine as adjuvant.

Methods

Rat mast cells were obtained by peritoneal lavage of Hooded Lister rats with Minimum Essential Medium containing 20 mM-Hepes and 1.0 mg of bovine serum albumin/ml and adjusted to give pH 6.8 at 37°C. The cell suspensions were centrifuged at 300 *g* for 5 min at room temperature. The supernatant was discarded and the cell pellet washed twice with MEM medium. Each rat yielded 0.4×10^6 – 1.6×10^6 mast cells, which constituted 1–5% of the total peritoneal-cell population as assessed by light-microscopy after staining with aqueous Toluidine Blue.

Before activation of mast cells by antigen or sheep anti-(rat IgE) serum, cells were suspended in 2.0 ml of anti-ovalbumin serum diluted 1:1 with MEM medium and containing 20 μ Ci of 5-hydroxy[³H]-tryptamine. Incubation was for 90 min at 37°C. Before activation with polypeptides alone the incubation was for 45 min at 37°C in a volume of 10 ml, and the anti-ovalbumin serum was omitted. After incorporation of 5-hydroxy[³H]tryptamine the cells were washed three times and resuspended in MEM medium to give 1×10^4 – 4×10^4 mast cells/ml. Cells and releasing agents were prewarmed for 5 min at 37°C before triplicate cell suspensions (0.5 ml) were challenged with 0.5 ml of releasing agent. In kinetic studies the reaction was terminated by addition of 3 vol. of ice-cold MEM medium, followed by rapid immersion of the tubes in ice. Supernatants were

separated from cell pellets by centrifugation at 300 *g* for 5 min at 5°C.

Mast-cell mediator release was assayed as release of 5-hydroxy[³H]tryptamine, which is taken up by mast-cell granules and released in parallel with endogenous granule histamine (Morrison *et al.*, 1974). The net percentage release of 5-hydroxy[³H]tryptamine was calculated as:

$$\text{Net \% release} = \frac{2(a-b)}{c} \times 100$$

where *a* is the radioactivity (c.p.m.) recovered in 0.5 ml of supernatant from stimulated cells, *b* is the radioactivity (c.p.m.) recovered in 0.5 ml of supernatant from unstimulated cells and *c* is the total radioactivity (c.p.m.) per reaction mixture. Radioactivity was counted in a Packard scintillation spectrometer with an efficiency of 50.7%. Approx. 10 000–20 000 c.p.m. was incorporated per 10^4 mast cells.

The coefficient of variation between treatments performed in triplicate was 2.5–5.0%. The background amount of 5-hydroxy[³H]tryptamine recovered in the supernatant of unstimulated cells was 2–4% of cell content.

Results

Kinetics of release and concentration-response relationships

IgE decapeptide (10 μ M), corticotropin-(1-24)-peptide (10 μ M), polyarginine (0.1 μ M) and polylysine (0.1 μ M) all stimulated rapid release of 5-hydroxy[³H]tryptamine at 37°C, which was complete within 5–10 s after challenge (Fig. 1). In subsequent experiments challenge was for 5 min to ensure completion of the release reaction.

All four polypeptides induced a concentration-dependent release of 5-hydroxy[³H]tryptamine with maximum net release of 90% at the highest concentrations used (Fig. 2). IgE decapeptide and corticotropin-(1-24)-peptide were of equal potency, the concentration giving half-maximal release (EC_{50}) being $\approx 5 \mu$ M. Polyarginine and polylysine were also of equal potency but were approximately 100-fold more potent than the two small polypeptides ($EC_{50} \approx 0.05 \mu$ M). The concentration-response curves for each of the four polypeptides were parallel, as assessed by analysis of co-variance.

Cells passively sensitized with anti-ovalbumin serum released up to 20% of cell 5-hydroxy[³H]tryptamine after antigen challenge with ovalbumin and up to 52% after challenge with sheep anti-(rat IgE) serum. The concentration-response curves to antigen and anti-IgE serum were considerably less steep than those to polypeptides. The response to polypeptide stimulation did not differ

Fig. 1. Kinetics of basic polypeptide-induced 5-hydroxy-³H]tryptamine release from rat mast cells

For experimental details see the text. (a) □, Corticotropin-(1-24)-peptide; ●, IgE decapeptide. (b) ○, Polyarginine; △, polylysine. Results are means ± S.E.M. for three experiments.

between non-sensitized cells and cells passively sensitized with anti-ovalbumin serum.

Interactions between secretory stimuli

To further investigate whether mast-cell secretion induced by IgE decapeptide shares common mechanisms with secretion induced by other polypeptides and antigen, the interactive effects of these stimuli were determined by observing the effects of a fixed concentration of one stimulus on the concentration-response curve to another. Experiments comprised two concentration-response titrations: the titrated stimulus alone and the titrated stimulus in combination with a fixed concentration of the second stimulus. The concentration of the second stimulus was chosen to induce 20% or less net 5-hydroxy[³H]tryptamine release on its own. A concentration of 1 μM-IgE decapeptide alone in-

Fig. 2. Concentration-dependent effect of basic polypeptides on 5-hydroxy[³H]tryptamine release from rat mast cells

For experimental details see the text. ○, Polyarginine; △, polylysine; □, corticotropin-(1-24)-peptide; ●, IgE decapeptide. Results are means ± S.E.M. for three experiments.

Fig. 3. Additive effect of a fixed concentration of IgE decapeptide on the concentration-response to polyarginine

For experimental details see the text. ○, Polyarginine alone; □, polyarginine + 1.0 μM-IgE decapeptide. Results are means ± S.E.M. for three experiments.

duced approx. 20% net 5-hydroxy[³H]tryptamine release, which was additive to the effect of 0.0033–0.01 μM-polyarginine (Fig. 3). At higher concentrations of polyarginine the additive effect of IgE decapeptide progressively diminished, and above 0.1 μM-polyarginine no additive effect was observed.

Table 1. *Effect of benzalkonium chloride (1.0 $\mu\text{g}/\text{ml}$) on 5-hydroxy[^3H]tryptamine release from rat mast cells*
For experimental details see the text. Net percentage release and percentage inhibition are expressed as means $\pm$ s.e.m. for three experiments. Abbreviation: [^3H]5-HT, 5-hydroxy[^3H]tryptamine.

		Net [^3H]5-HT release (%)	Net [^3H]5-HT release with 1.0 μg of benzalkonium chloride/ml (%)	Inhibition (%)
Polyarginine	0.02 μM	4.7 $\pm$ 1.8	1.6 $\pm$ 0.4	61.8 $\pm$ 6.1
	0.05 μM	13.2 $\pm$ 4.3	9.2 $\pm$ 3.9	34.3 $\pm$ 6.3
IgE decapeptide	1 μM	4.1 $\pm$ 1.8	0.3 $\pm$ 0.3	94.6 $\pm$ 3.2
	3 μM	33.5 $\pm$ 4.2	17.4 $\pm$ 4.0	49.6 $\pm$ 6.5
Sheep anti-(rat IgE) serum	1:100	3.8 $\pm$ 0.6	5.0 $\pm$ 0.6	-27.2 $\pm$ 7.0
	1:40	34.3 $\pm$ 9.1	37.9 $\pm$ 9.3	-11.5 $\pm$ 2.2
Ovalbumin	1.0 $\mu\text{g}/\text{ml}$	10.6 $\pm$ 5.3	13.8 $\pm$ 6.7	-44.2 $\pm$ 17.9

Fig. 4. *Additive effect of a fixed concentration of antigen on the concentration-response to polyarginine*

For experimental details see the text. O, Polyarginine alone; $\square$, polyarginine + 1.0 μg of ovalbumin/ml. Results are means $\pm$ s.e.m. for five experiments.

Similar interactive concentration-response characteristics were obtained with combinations of polylysine plus polylysine and of polylysine plus polyarginine (results not shown).

Antigen interacted with polypeptide in a way that differed distinctly from the interactions observed between polypeptides. Antigen was used in preference to sheep anti-(rat IgE) serum in these experiments, since normal sheep serum was found to interfere with the polypeptide-induced secretory response in control experiments. A fixed concentration of antigen induced 5-hydroxy[^3H]tryptamine release that was additive to all concentrations along the polyarginine concentration-response curve (Fig. 4). In five experiments 1 μg of ovalbumin/ml produced a parallel displacement of the polyarginine concentration-response curve amounting to a mean

additional 4.3% release over that induced by polyarginine alone ($P < 0.001$; paired Student's t test).

Effect of benzalkonium chloride

Benzalkonium chloride over the concentration range 0.3–10 $\mu\text{g}/\text{ml}$ inhibited polypeptide-induced 5-hydroxy[^3H]tryptamine release. An optimal concentration of 1.0 $\mu\text{g}/\text{ml}$ was selected for use in subsequent experiments, since concentrations above 10 $\mu\text{g}/\text{ml}$ were found to be cytolytic. Benzalkonium chloride at 1.0 $\mu\text{g}/\text{ml}$ inhibited the release induced by IgE decapeptide and polyarginine and enhanced the release induced by antigen and sheep anti-(rat IgE) serum (Table 1).

Discussion

IgE decapeptide, corticotropin-(1–24)-peptide, polyarginine and polylysine were found to stimulate 5-hydroxytryptamine release from rat peritoneal mast cells. The greater potency of polyarginine (300 basic residues) and polylysine (180 basic residues) compared with IgE decapeptide (2 basic residues) and corticotropin-(1–24)-peptide (7 basic residues) is consistent with the finding that rat mast cells (Jasani *et al.*, 1979) and human basophil leucocytes (Foreman & Lichtenstein, 1980) are more readily activated as the number of basic residues in the polypeptide chain is increased. On a 'per residue' basis, the equipotency of polyarginine and polylysine in mediating secretion suggests that polylysine is more potent than polyarginine. Arginine (pK_a 12.5) is more readily protonated than lysine (pK_a 10.5), and thus net charge is not the only factor contributing to secretory activity. Similarly, IgE decapeptide is considerably more potent than is corticotropin-(1–24)-peptide in relation to the number of basic residues, and again this suggests that chemical features in addition to net charge are important in determining the releasing potency of basic polypeptides. Potency has been shown to be influenced by several features that may act by

facilitating interaction with cell membrane receptors. Hydrophobic residues in the vicinity of the basic region enhance activity, whereas acidic residues diminish activity (Jasani *et al.*, 1973, 1979). In addition, optical isomerism has been found to be critical to the activity of some small basic peptides (Roy *et al.*, 1980).

Previous studies have shown that basic peptides (Jasani *et al.*, 1979; Stanworth *et al.*, 1979), including polylysine (Ennis *et al.*, 1980), stimulate mast cells by a non-cytolytic mechanism. In the present study the close similarity in kinetics and concentration-response characteristics of 5-hydroxytryptamine release induced by small and large polypeptides (Figs. 1 and 2) suggests that they may act via a similar mechanism. This conclusion is supported by the finding that both large and small polypeptides were non-additive in their effects on secretion when the combined concentrations were high. Under these conditions the two polypeptides compete for, and mutually exclude each other from, mechanisms initiating mast-cell secretion, indicating a saturable receptor-like component involved in peptide-induced secretion. Thus the similarity in the releasing characteristics of both large and small polypeptides might be explained by the cross-linking of several receptors by large peptides. In contrast with the interactions observed between large and small polypeptides, 5-hydroxytryptamine release induced by antigen was additive to the whole length of the concentration-response curve to polyarginine (Fig. 4). Thus, when the polycation-stimulated release mechanism is saturated by polycation, antigen is still capable of inducing further release, suggesting that the two stimuli act independently. Some caution in interpreting the results of these additive experiments is necessary, however, since peptides induced mediator release appreciably more rapidly than does antigen, i.e. in 5–10 s compared with 45–60 s (Schwartz *et al.*, 1979). In our experiments sufficient time (5 min) was allowed for both releasing stimuli to complete their effect.

Benzalkonium chloride, a mixture of quaternary benzylmethylalkylammonium chlorides, has been reported to inhibit selectively polyamine-induced histamine release from rat mast cells, but not that induced by antigen and cytolytic agents such as detergents (Read & Kiefer, 1979). The inhibitory activity was shown by the same authors to be due to the membrane-active properties of the benzalkonium chloride molecules, rather than to any effect in the fluid phase. The present study clearly demonstrates the capacity of benzalkonium chloride to inhibit mediator release from mast cells induced by IgE decapeptide and polyarginine, though having an enhancing effect on IgE-dependent release.

In summary, the present observations suggest that IgE-decapeptide stimulation of mediator release

from mast cells closely resembles stimulation by other basic peptides, but in some ways appears to differ from immunological stimulation. This might be taken as preliminary evidence of a fundamental difference between IgE-decapeptide- and antibody-mediated release of mediators from mast cells. This problem deserves further investigation, as it appears to be contrary to the theory of Stanworth (1974) that the immunological triggering of mast cells is dependent on the stimulation of an activation site on the cell membrane by an effector site within the Fc region of the IgE molecule, of which the synthetic decapeptide is representative. In this connection, it should be pointed out that artificial stimulation of mast cells can be affected by a process that obviously does not involve an Fc-region effector site; namely by the cross-linking of mast-cell IgE receptors by an anti-receptor antibody of the immunoglobulin G class (Ishizaka & Ishizaka, 1978b). On the other hand, there are several other ways in which polypeptide-induced mediator release does resemble that brought about by the natural IgE antibody-antigen interaction, including dependence of temperature, energy and Ca^{2+} ions, and inhibition by sodium cromoglycate and phosphodiesterase inhibitors (Stanworth *et al.*, 1979; Ennis *et al.*, 1980).

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