Release of Histamine from Mast Cells by Vasoactive Peptides[1] (37219)

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A number of peptides stimulate smooth muscles and alter blood pressure. Among these, bradykinin (1), eledoisin (1) and substance P (2, 3) are potent hypotensive agents. Although the fall in blood pressure presumably results from a direct action of the peptides on vascular smooth muscle, it might be enhanced by histamine. High concentrations of bradykinin release histamine from mast cells *in vitro* (4). Since both histamine (5) and kinins (6) have been implicated in acute allergic reactions, it was important to determine whether vasoactive peptides liberate histamine. Such an effect may potentiate the activity of the peptides. We tested synthetic kinins and several other vasoactive peptides, including eledoisin, substance P and *Polistes* kinin (7, 8), for histamine releasing action on rat mast cells *in vitro*. The relative potencies of the peptides as histamine releasers were compared with that of compound 48/80.

Methods. Cell suspensions. Serous fluid cells were collected by lavage[2] of peritoneal and pleural cavities of large male Sprague–Dawley rats (9). Cells from 3–6 rats were pooled for each experiment. These preparations contained 6–10% mast cells as determined by the mast cell counting technique of Bray and Van Arsdale (10).

Histamine release. Aliquots of cell suspension (0.9 ml) were warmed to 37° in a metabolic shaker. Peptides (1–1000 μg/ml), or compound 48/80 (10 μg/ml) were added in a volume of 0.1 ml and incubation was continued for 10 min. The cells were separated from the suspending medium by centrifugation at 500*g*. The cell pellets were treated with 2 ml of 0.2 *N* HCl to release residual cell histamine in order to calculate the percentage of total histamine released by various agents. The amounts of histamine in both cell and supernatant fractions was determined by bioassay on strips of guinea pig ileum.[3] Because most of the peptides tested contract guinea pig ileum, each assay sample was adjusted to pH 7.8 and incubated with 100 μg/ml of chymotrypsin (Calbiochem) for 30 min. The tubes were then boiled for 10 min to inactivate chymotrypsin and centrifuged at 500*g* prior to assay for histamine. This procedure effectively eliminated all smooth muscle stimulating activity of the peptides. The technique used for the inactivation of chymotrypsin had no effect on histamine. This was shown by subjecting standard solutions of histamine to the same treatment.

Lactic dehydrogenase release. In experiments where release of lactic dehydrogenase was determined, mast cells were isolated and concentrated by centrifugation through solutions (35%) of Ficoll (Pharmacia) in buffered medium (9). Isolated mast cells were washed 3 times in fresh medium to remove Ficoll and 0.9 ml aliquots of cell suspension were treated with 0.1 ml of peptide (bradykinin, kallidin or methionyl-lysyl bradyki-

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[2] The standard buffered medium (pH 7.0) used for collection and incubation of cells contained 150 m*M* NaCl, 2.7 m*M* KCl, 0.9 m*M* $CaCl_2$, 4 m*M* Na_2HPO_4, 2.5 m*M* KH_2PO_4, 5.6 m*M* dextrose and 0.1% human serum albumin (Fraction V, Nutritional Biochemicals).

[3] The histamine assay medium (pH 7.4) contained 138 m*M* NaCl, 2.7 m*M* KCl, 1.8 m*M* $CaCl_2$, 1.0 m*M* Na_2HPO_4, 1.2 m*M* $NaHCO_3$, 5.6 m*M* dextrose and 1 μg/ml of atropine sulfate.

nin), 48/80, or with Triton X-100 to yield the following final concentrations per milliliter: 50 mmole, 1 μg and 0.5 mg, respectively. The cell suspensions were then incubated for 10 min at 37°. The samples were separated by centrifugation at 500*g* for 10 min, the cell pellets were resuspended in 2.0 ml of 0.2 *M* Tris (pH 8.5) and 1 ml of Tris was added to the supernatant medium. All samples were sonified with a Branson probe sonifier for 10 sec and the extracts were assayed for lactic dehydrogenase activity by a modification of the fluorometric method of Burch *et al.* (11). Aliquots (0.05–0.2 ml) of each cell or supernatant extract were added to 1 ml of 0.2 *M* Tris (pH 8.5) containing 1 mmole of NAD. The production of NADH during 10 min at room temperature (27°) was measured in a Farrand fluorometer before and after addition of lactate as substrate (5 m*M*). The remainder of the sonified extracts was acidified and stored frozen until assayed for histamine.

Trypan blue staining. Suspensions of isolated mast cells were incubated with peptides, 48/80 or Triton X-100 as indicated above. One drop of cell suspension for each sample was placed on a clean glass coverslip and mixed with 1 drop of trypan blue dye solution (2.5% in isotonic saline). The coverslips were placed on glass slides and examined under the light microscope. At least 25 cells in each of 4 different fields were examined at a magnification of 450× and the percentage of cells stained with trypan blue was estimated.

Peptides and drugs. Bradykinin, kallidin, methionyl-lysyl-bradykinin, angiotensin I and II were purchased from Schwarz/Mann (Orangeburg, NY), and crystalline glucagon was purchased from Eli Lilly Co., Inc. (Indianapolis, IN). Human fibrinopeptide A was purchased from Spectrum Medical Industries, Inc. (Los Angeles, CA). Other synthetic peptides were generously donated by the following individuals or companies: Substance P by Dr. S. Leeman (Brandeis University), eledoisin by Farmitalia (Milan), *Polistes* kinin by Dr. J. M. Stewart (University of Colorado), and bradykinin tetrapeptide by Dr. M. O. Ondetti (Squibb Inst, Princeton, NJ). Compound 48/80 was obtained from Burroughs-Wellcome, Inc. (Research Triangle Park, NC). Inactivated bradykinin was prepared by incubating 500 μg of synthetic peptide with either 5 μl of 11 mg/ml carboxypeptidase B (Worthington Biochemicals, Freehold, NJ) or with 0.2 ml of guinea pig serum in 1 ml of buffered medium[3] (pH 7.8) at 37° for 15 min. Inactivation of the peptides was established by testing them on guinea pig ileum.

Results. Release of histamine. Histamine was released from suspensions of rat mast cells by several of the vasoactive peptides tested. In order of ascending potency, bradykinin, kallidin, methionyl-lysyl-bradykinin, substance P and *Polistes* kinin released more than 50% of the total histamine. Bradykinin tetrapeptide was only slightly active at a concentration of 2×10^{-3} *M*. The structure of these peptides is given in Fig. 1. The most potent of the peptides, *Polistes* kinin, was only slightly less active than compound 48/80. When the releasing agents were compared on the basis of ED_{50} (conc that releases 50% of the total mast cell histamine) the value for *Polistes* kinin was 3×10^{-6} *M* as compared with 8.5×10^{-7} *M* for 48/80 (Fig. 2).

Eledoisin (8×10^{-5} *M*), angiotensin I (9×10^{-5} *M*) and glucagon (3×10^{-5} *M*) did not release histamine. Angiotensin II (10^{-4} *M*), fibrinopeptide A (7×10^{-5} *M*) and bradykinin tetrapeptide (2×10^{-4} *M*) were only slightly active and liberated less than 12% of the total histamine. The data in Table I indicate that the release of

Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Kallidin (Lys-bradykinin)	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Met-Lys-bradykinin	Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Polistes kinin	Pyr-Thr-Asn-Lys-Lys-Lys-Leu-Arg-Gly-bradykinin

FIG. 1. Structure of histamine releasing peptides; amino acid sequence of peptides that release histamine.

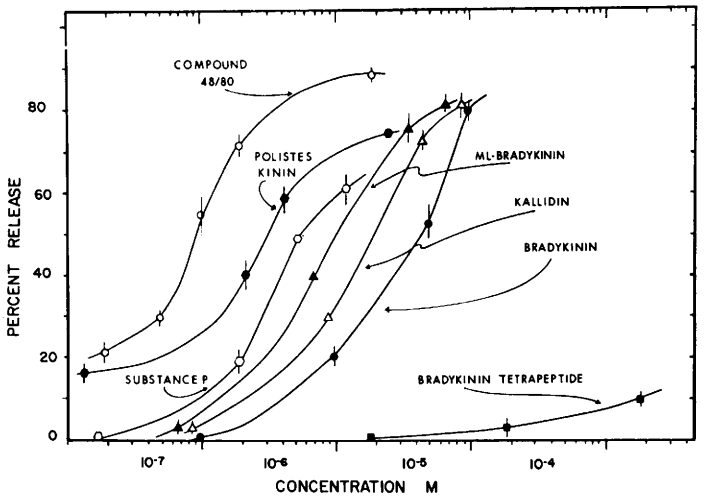

FIG. 2. Release of histamine from rat serous fluid mast cells by peptides and by compound 48/80. Abscissa: M concn of agent; ordinate: percentage histamine released. Values are means ± SE of 3–6 separate determinations. 0 on the abscissa = 1×10^{-7} M concn.

histamine by peptides does not depend on the structure of the N- or C-terminal amino acids but on the number of basic groups in the peptides. We estimate that in incubation medium at pH 7.0, the most potent peptide, *Polistes* kinin has 5 positively charged groups, kallidin, methionyl-lysyl bradykinin and substance P each have 3, and bradykinin has only 2. Removal of even one basic amino acid from bradykinin by the action of carboxypeptidase B (12) results in complete loss of histamine releasing activity. Peptides containing only negatively charged groups were inactive or only slightly active.

Release of lactic dehydrogenase. Mast cells incubated with 48/80 or with any of the three bradykinin derivatives released histamine but not lactic dehydrogenase. In con-

TABLE I. Effect of Biologically Active Peptides on Mast Cells.

Peptide	Concn ($M \times 10^{-5}$)	Amino acid		Release of histamine (%)	No. of expts
		Basic[a]	Acidic[b]		
1. Control (buffer)	—	0	0	3.8 ± 0.3	20
2. Bradykinin	5	2		62.2 ± 4.7[f]	8
3. Kallidin	4	3		72.9 ± 3.7[f]	4
4. Met-Lys-bradykinin	4	3		78.2 ± 3.0[f]	6
5. C-term. tetrapeptide of bk.	18	1		9.5 ± 2.4[e]	3
6. Bradykinin-inactiv.[c]	6	1		3.2 ± 0.9	4
7. Bradykinin-inactiv.[d]	7	1		2.6 ± 0.6	5
8. *Polistes* kinin	0.5	6		61.0 ± 2.8[f]	6
9. Substance P	1	2		65.8 ± 3.9[f]	4
10. Eledoisin	8	1	1	6.6 ± 2.3	4
11. Angiotensin I	9	1	1	8.4 ± 2.0	3
12. Angiotensin II	10	1	1	9.6 ± 1.5[e]	3
13. Glucagon	3	3	3	6.8 ± 1.2	4
14. Fibrinopeptide A	7	1	4	11.3 ± 2.5[f]	4

[a] No. of unsubstituted diamino or guanido amino acids in the peptide chain.
[b] No. of dicarboxylic amino acids.
[c] Bradykinin inactivated by carboxypeptidase B.
[d] Bradykinin inactivated by guinea pig serum.
[e] $p < .01$.
[f] $p < .001$.

TABLE II. Effect of Histamine Releasers on Mast Cells.

Agents	Release of		Cells stained with trypan blue (%)
	Histamine (%)	Lactic dehydrogenase (%)	
Buffer	2.3 ± 1.4	6.9 ± 3.0	7.1 ± 2.0
Compound 48/80 (10^{-6} *M*)	61.8 ± 3.3	5.4 ± 1.0	4.4 ± 1.0
Bradykinin (5×10^{-5} *M*)	62.1 ± 8.0	3.0 ± 0.2	8.3 ± 1.3
Kallidin (4×10^{-5} *M*)	72.9 ± 3.7	3.4 ± 1.1	6.7 ± 1.0
Met-Lys-bradykinin (3×10^{-5})	75.9 ± 3.0	5.7 ± 2.3	4.3 ± 0.8
Triton X-100 (0.05%)	89.1 ± 0.7	96.2 ± 2.1	98.0 ± 0.1

trast, cells treated with Triton X-100 released both histamine and lactic dehydrogenase into the suspending medium (Table II).

Staining with trypan blue. Although the appearance of mast cells incubated with active peptides or 48/80 was altered in comparison with untreated cells, these releasing agents did not increase the number of cells which stained with trypan blue. In samples where histamine was liberated, mast cells appeared slightly swollen and the cell edges were rugged in contrast to the smooth outline of control cells. These changes in morphology are consistent with "degranulation" induced by histamine releasing agents such as 48/80 (13) and antigen (14) and suggest that the peptides affect mast cells in a similar manner. Mast cells which were treated with Triton X-100 were visibly disrupted and more than 90% were stained by trypan blue.

Discussion. These experiments indicate that several biologically active peptides release histamine from rat serous fluid mast cells. Kinins and other hypotensive peptides such as substance P and *Polistes* kinin were effective but the hypertensive angiotensin I and angiotensin II were relatively inactive in this test system.

The releasing activity of the kinins increased with the length of the peptide chain. The most potent agent was *Polistes* kinin. One mole of this peptide liberated up to 47 moles of histamine. For comparison, 100 to 200 moles of histamine were liberated by 1 mole of 48/80.[4]

Release of histamine from mast cells can not be correlated directly with hypotensive actions of the peptides, because eledoisin, one of the most potent vasodepressor substances (15) was inactive. In contrast, the structurally related substance P (16) liberated as much as 60% of the total mast cell histamine. The difference may be due to the presence of basic amino acids in the first and third positions in the peptide chain of substance P while eledoisin contains only a single basic amino acid (lysine) in the third and an acidic one (aspartic acid) as well in the fourth position. Apparently the carboxyl end of the peptide need not contain a free carboxyl group, since substance P terminates in methionylamide. In general, peptides that were active had two or more basic groups (Table I). Where the net charge was lowered by removal of a basic amino acid (peptides 5–7) or by the presence of positively charged groups (peptide 13) no histamine was released.

Other peptides which liberated histamine have been extracted from leukocytes of several species (17–19) and from serum (20, 21). The activity of these agents probably depends also upon a net basic charge.

Although we can not precisely define the mechanisms of histamine release by basic peptides our observations suggest, that, just as 48/80 or antigen, peptides stimulate exocytosis of mast cell granules. This statement is based on the following considerations. First, kinins released histamine but not lactic dehydrogenase from mast cells. Second, even though the cells appeared "degranulated" after exposure to kinins they did not stain with trypan blue. These criteria have been used for the definition of a selective release mechanism by 48/80, antigen (22) and several basic drugs (23). Thus, exclusion of trypan blue and retention of lactic dehydrogenase following release of histamine indicate that the basic peptides stimulate

[4] The molecular weight of 48/80 as trimer (520) was used for this calculation.

exocytosis rather than disrupt cell membranes.

Release of histamine alone can not account for the hypotensive actions of these peptides, but it may add to some of the activities of *Polistes* kinin or substance P. Under our conditions *in vitro* 1 or 3 nmoles of the two peptides already liberated appreciable quantities of histamine. For example, *Polistes* kinin is a constituent of a wasp venom (7, 8). Several histamine liberators such as phospholipase A, mellitin and a "mast cell disrupting" peptide have been extracted from venoms (24), but the activity of *Polistes* kinin has not been completely characterized. Here histamine could contribute to the actions of wasp venom on smooth muscle and blood vessels.

Substance P was synthesized only recently (16) although it has been known that the natural peptide prepared from either brain or intestine has potent hypotensive and smooth muscle stimulating properties (25). Its physiological importance remains unknown. Because substance P occurs in brain and in nerve endings there has been considerable interest in its possible role in the nervous system (26). Our studies do not indicate a specific function for substance P, but if this peptide can release histamine from storage sites in mast cells it may also release amines in brain and other tissues.

Summary. The effect of a variety of synthetic peptides on rat mast cells was investigated. Peptides with two or more basic groups were active histamine releasers, while eledoisin, angiotensin I and II, glucagon, and fibrinopeptide A had slight or no activity. The activity of bradykinin analogues increased with the chain length. Des-arginine derivatives of bradykinin did not release histamine. The most potent histamine liberators were substance P and *Polistes* kinin; the activity of the latter one approached that of 48/80. Because histamine could be released by the kinin peptides without disruption of the mast cell membrane, these agents like 48/80, probably stimulate exocytosis of mast cell granules.

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1. Erdös, E. G., *in* "Advances in Pharmacology" (S. Garattini and P. A. Shore, eds.), Vol. 4, p. 1. Academic Press, New York (1966).

2. V. Euler, U. S., Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. **181**, 181 (1936).

3. Pernow, B., Acta Physiol. Scand. **29**, Suppl. 105 (1953).

4. Goth, A., *in* "Advances in Pharmacology" (S. Garattini and P. A. Shore, eds.), Vol. 5, p. 47. Academic Press, New York (1967).

5. Mongar, J. L., and Schild, H. O., Physiol. Rev. **42**, 226 (1962).

6. Spragg, J., Talamo, R. C., and Austen, K. F., *in* "Bradykinin, Kallidin and Kallikrein; Handbook of Experimental Pharmacology" (E. G. Erdös, ed.), Vol. 25, p. 531. Springer, Heidelberg (1970).

7. Pisano, J. J., in Ref. (6), p. 581.

8. Stewart, J. M., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **27**, 534 (1968).

9. Johnson, A. R., and Moran, N. C., Amer. J. Physiol. **216**, 453 (1969).

10. Bray, R. E., and Van Arsdale, P. P., Proc. Soc. Exp. Biol. Med. **106**, 255 (1971).

11. Burch, H. B., Lowry, O. H., Kuhlman, A. M., Skerjance, J., Diamant, E. J., Lowry, S. R., and Von Dippe, P., J. Biol. Chem. **238**, 2267 (1963).

12. Erdös, E. G., and Yang, H. Y. T., in Ref. (6), p. 289.

13. Uvnäs, B., and Thon, I., Exp. Cell Res. **18**, 512 (1959).

14. Mota, I., and Da Silva, W., Nature (London) **186**, 245 (1960).

15. Erspamer, V., and Anastasi, A., Experientia **18**, 58 (1962).

16. Chang, M. M., Leeman, S. E., and Niall, H. D., Nature (London) New Biol. **232**, 86 (1971).

17. Janoff, A., and Schaeffer, S., Nature (London) **213**, 144 (1967).

18. Ranadine, N. S., and Cochrane, C. G., J. Immunol. **106**, 506 (1971).

19. Frimmer, M., Merk-Jansen, M., Schischke, B., and Waldvogel, G., Pharmacology **1**, 165 (1968).

20. Dias da Silva, W., and Lepow, I. H., J. Immunol. **95**, 1080 (1966).

21. Cochrane, C. G., and Muller-Eberhard, H. J., J. Exp. Med. **127**, 371 (1968).

22. Johnson, A. R., and Moran, N. C., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 1716 (1969).

23. Ellis, H. V., Johnson, A. R., and Moran, N. C., J. Pharmacol. Exp. Ther. **175**, 627 (1970).

24. Habermann, E., Science **177**, 314 (1972).

25. V. Euler, U. S., Ann. N.Y. Acad. Sci. **104**, 449 (1963).

26. Stern, P., *in* "Hypotensive Peptides" (E. G. Erdös, N. Back, and F. Sicuteri, eds.), p. 633. Springer, New York (1966).

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