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INDUCTION OF HISTAMINE RELEASE FROM HUMAN SKIN MAST CELLS BY BRADYKININ ANALOGS*

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Abstract—Kinins are potent proinflammatory peptides that induce histamine release from rodent mast cells. We examined the ability of bradykinin, lysylbradykinin and a series of kinin analogs to cause histamine release from human basophils, human lung mast cells and human skin mast cells. At concentrations ranging from 0.1 μM to 1 mM, bradykinin failed to cause histamine release from any of the human histamine-containing cells studied. Lysylbradykinin was also without effect on basophils and lung mast cells, but was a weak secretagogue for human skin mast cells, inducing 5.5 ± 3% (mean ± SD) of total cellular histamine release at a concentration of 10^{-5} M. Similarly, when sixteen recently developed bradykinin antagonists were examined, these compounds had no effect on basophils or lung mast cells but all sixteen induced dose-dependent histamine release from skin mast cells. The release process was temperature dependent and, at a concentration of 10^{-5} M, the antagonists induced 8–27% histamine release. Although preincubation of cells with 10^{-3} M bradykinin or des(Arg^9) bradykinin significantly inhibited antagonist-induced histamine release, the requirement for such high concentrations of these peptides to cause inhibition suggested that histamine release is not mediated by either B_1 or B_2 kinin receptors. To understand further the mechanism of histamine release, we examined a series of bradykinin analogs with single amino acid substitutions in the bradykinin sequence. Replacement of proline^7 in the bradykinin sequence with D-phenylalanine is the essential change used to convert kinin analogs into antagonists, and 10^{-5} M [DPhe^7]-bradykinin induced 8–10% histamine release. Other analogs, devoid of antagonist activity, however, such as [DPhe^6]-bradykinin and [LPhe^7]-bradykinin were able to induce equivalent levels of histamine release. The ability to induce histamine release appears to be related, at least in part, to aromaticity, since [DTrp^6]-bradykinin and [DTrp^7]-bradykinin induced greater amounts of histamine release than equivalent [DPhe]-analogs, causing approximately 20% histamine release at 10^{-5} M. By contrast, [DAla^7]-bradykinin was an ineffective stimulus. In summary, a single amino acid substitution can convert bradykinin into a secretagogue for human skin mast cells. The ability of kinin analogs to induce histamine release from skin mast cells, but not lung mast cells or basophils, emphasizes the heterogeneity of human histamine-containing cells.

In recent years evidence has begun to accumulate supporting the hypothesis that kinins may be among the important mediators of human allergic disease. Thus, it has been shown that high molecular weight kininogen is consumed during anaphylactic reactions in humans [1]. More recently, it has been demonstrated that the potent vasoactive peptides, bradykinin and lysylbradykinin, are generated in nasal secretions during both the immediate and late responses to allergen challenge [2, 3]. Immunoreactive levels in bronchoalveolar lavage fluids from symptomatic asthmatics, or from asthmatics responding to allergen challenge, are also elevated significantly compared to levels in lavage fluids from normals [4].

The known pharmacologic abilities of kinins to increase vascular permeability, to cause vasodilatation and pain, and to stimulate arachidonic acid metabolism [5] appear to make them ideal mediators of allergic inflammation. Support for this premise is provided by the observations that provocation of the lower airways of asthmatics with bradykinin results in bronchoconstriction [6–8], whereas bradykinin administered to the nasal mucosa induces symptoms of rhinitis, regardless of atopic status [9]. In addition to their actions on vasculature and smooth muscle, however, it has also been shown that kinins can induce mediator release from rodent peritoneal mast cells [10, 11]. If human mast cells or basophils are similarly responsive to kinins, then these peptides could also contribute to the pathogenesis of the allergic response by causing mediator release from

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histamine-containing cells. To examine this possibility we have studied the ability of kinins to induce histamine release from human basophils, human lung mast cells and human skin mast cells. As part of these studies, the abilities of a recently developed series of bradykinin receptor antagonists [12] and of various kinin analogs to act as secretagogues were also examined.

MATERIALS AND METHODS

Materials

The following were purchased: piperazine-*N,N'*-bis[2-ethanesulfonic acid] (PIPES), ethylenediamine tetracetic acid (EDTA), glucose, dextran, chymopapain, elastase type I, penicillin and hyaluronidase (Sigma Chemical Co., St. Louis, MO); Pronase and DNase (Calbiochem, San Diego, CA); calcium/magnesium free Hanks' balanced salt solution (CMF-HBSS), gentamycin, collagenase, fetal; calf serum and RPMI 1640 medium with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (Gibco, Grand Island, NY); synthetic bradykinin, des(Arg^9) bradykinin and lysylbradykinin (Peninsula Laboratories, Belmont, CA); Percoll (Pharmacia, Piscataway, NJ); and human serum albumin (Miles Laboratories, Elkhart, IN). Goat anti-human IgE was provided by Dr. Kimishige Ishizaka, Johns Hopkins University School of Medicine.

Buffers. PAG consists of 25 mM PIPES/110 mM NaCl/5 mM KCl/0.1% dextrose/0.003% human serum albumin, pH 7.4. Histamine release experiments were carried out in PAG supplemented with 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PAGCM).

Methods

Preparation of basophils. Mixed leukocytes containing approximately 0.5% basophils were obtained by dextran sedimentation of venous blood as previously described [13]. Mononuclear cell suspensions containing 1–5% basophils were prepared from whole blood. Twenty milliliters of blood was anticoagulated with 0.8 ml of 0.4 M EDTA, diluted with 50 ml of normal saline, and layered over 10 ml of isotonic Percoll (specific gravity 1.079 g/cm^3). The tubes were centrifuged at 250 *g* for 15 min, and the mononuclear cells were recovered from the plasma–Percoll interface. The cells were washed once in EDTA–saline, twice in PAG, and resuspended in the appropriate volume of PAGCM for challenge experiments. Highly purified basophils were prepared by affinity chromatography according to the method of MacGlashan and Lichtenstein [14].

Human lung mast cells. Human lung tissue was obtained from patients undergoing thoracotomy and lung resection, usually for carcinoma of the bronchus or other neoplasms. Macroscopically normal lung tissue was dissected free of pleura, bronchi and blood vessels, minced into 5-mg fragments, and enzymatically dispersed into single cell suspensions. Mast cells were purified further by countercurrent centrifugation elutriation as previously described [15].

Human skin mast cells. Adult human skin was obtained from either mastectomies for breast cancer or from elective cosmetic surgery procedures. Tissue was placed in CMF-HBSS and used within 1 hr. The skin was separated from subcutaneous fat by blunt dissection and was chopped with surgical scissors to a size of 1 mm. Tissue was washed once in CMF-HBSS and centrifuged at 4° and 200 *g* for 8 min to remove residual fat which can be skimmed from the top of the supernatant. The tissue fragments were then washed twice in CMF-HBSS at 23° and incubated for 3 hr at 37° with constant stirring in CMF-HBSS containing 20 mg collagenase and 5 mg hyaluronidase per g wet weight of tissue and 1000 units/ml of DNase. The incubation volume was approximately 8 ml/g wet weight. The cells were separated from tissue fragments by filtration through Nitex cloth (150 μm pore size) and washed once in CMF-HBSS at 4° and then twice at 23°.

Cells obtained from the above digestion were resuspended in RPMI 1640 medium containing 25 mM HEPES/2 mM L-glutamine/1% penicillin/1% gentamycin/10% fetal calf serum. The suspension (2×10^5 mast cells/ml) was incubated overnight in 5-ml aliquots in 25 cm^2 tissue culture flasks at 37° in 95% air/5% CO_2. After overnight culture, mast cells were harvested, washed twice in PAG at 23°, and resuspended at the appropriate concentration in PAGCM for release experiments.

Cell counts. Mast cells and basophils were enumerated using an alcian blue stain [16].

Bradykinin antagonists and analogs. Peptides were synthesized by the solid phase method of Merrifield [17] using standard procedures [18] on a Beckman 990 automatic synthesizer (Beckman Instruments, Palo Alto, CA). Protected (*t*-butyloxycarbonyl) amino acids used in the synthesis were commercially available (Bachem, Torrance, CA; Peninsula Laboratories, Belmont, CA; Colorado Biotechnology Associates, Boulder, CO), or were obtained from the Southwest Research Institute, San Antonio, TX. The β-(2-thienyl)-alanine was a gift from Floyd Dunn. Single dicyclohexylcarbodiimide-mediated coupling reactions in dichloromethane were used, except that the proline residue at sequence position 2 was routinely recoupled in dimethylformamide. Peptides were cleaved from the resin with anhydrous liquid HF containing 10% anisole, and purified by countercurrent distribution (100 upper phase transfers in either *n*-BuOH:HOAc:H_2O [4:1:5] or *n*-BuOH:1% TFA [1:1]). Peptide homogeneity was determined by thin-layer chromatography (Merck silica gel coated glass plates) in two different solvent systems (*n*-BuOH:HOAc:H_2O [8:3:4] and pyridine:EtOAc:HOAc:H_2O [5:5:1:3]) and by paper electrophoresis (10 V/cm on Whatman No. 1 paper) in 1 N HOAc, pH 2.8. Peptides were visualized by the chlorine–tolidine identification spray for peptides [18]. Peptide identity was confirmed by quantitative amino acid analysis (Beckman 120C analyzer) after hydrolysis in 6 N HCl in sealed glass tubes under N_2.

Histamine release assay. Mast cells and basophils were resuspended in PAGCM at appropriate concentrations and challenged in duplicate with the desired stimulus in a final volume of 0.1 ml. For skin mast cells and lung mast cells, suspensions were selected to provide a final cell number of 2×10^4 mast cells in the 0.1 ml reaction volume, while basophils were suspended at a final concentration of 2×10^4 to 5×10^4 basophils per experimental tube.

Release reactions were performed for 30 min at a temperature of 37° in the case of lung cells and basophils and at 30° for skin mast cells. Supernatant fractions were assayed for histamine by the automated fluorometric technique of Siraganian [19]. Results were expressed as the percentage of total cellular histamine, determined by lysis of cells in 2% perchloric acid, and corrected for spontaneous histamine release as determined from buffer control tubes. Hydrolysis of bradykinin by cell populations was evaluated by measuring kinin levels in aliquots of cell supernatant fractions using a specific radioimmunoassay [2]. Levels were compared to those detected in buffer after 30 min at the appropriate temperature.

Studies to evaluate the ability of bradykinin or des(Arg^9) bradykinin to inhibit analog-induced release from skin mast cells were performed by preincubating cells with des(Arg^9) bradykinin or with bradykinin (10^{-3} M to 10^{-5} M) for 10 min at 30° and then challenging with the desired compound (10^{-5} M) for an additional 20 min at 30°. Histamine release was compared to that from control tubes containing buffer, rather than bradykinin, and percent inhibition was calculated.

RESULTS

Exposure of mixed leukocytes, containing basophils, to bradykinin, even at concentrations up to 10^{-3} M, failed to result in significant histamine release. These mixed leukocyte preparations, however, contain large numbers of neutrophils that are rich in kininase activity [20], so the failure of bradykinin to induce histamine release could be a consequence of its rapid metabolism. To evaluate this possibility, mixed leukocytes and mononuclear cell preparations of varying basophil purities were incubated with 2×10^{-5} M bradykinin for 30 min at 37°, and at the end of that time both the histamine and residual bradykinin content of the cellular supernatant were determined (Table 1). As can be seen, greater than 97% of the added bradykinin was destroyed during a 30-min incubation with mixed leukocytes, and no histamine release was observed. In two other experiments with mixed leukocytes, kinin destruction was 95 and 98% respectively. Again, no significant histamine release was observed in either case. Significant histamine release also failed to occur, however, in mononuclear cell preparations containing from 3 to 60% basophils, even though bradykinin recoveries from these preparations ranged up to 96%. That the cells were capable of responding to a stimulus is indicated by their ability to release histamine upon exposure to anti-IgE. Thus, bradykinin does not induce histamine release from human basophils even under conditions where little or no kinin metabolism occurs.

Similar studies with human lung mast cells and human skin mast cells suggested that these cells were also non-responsive to bradykinin at concentrations up to 10^{-3} M. Again, while significant metabolism of bradykinin by crude preparations of lung mast cells may, at first glance, appear to be a possible explanation of their lack of responsiveness, histamine release also failed to occur in more purified preparations in which 89% of the bradykinin added could be recovered (Table 2). In a second such experiment, recovery of added kinin from a crude lung mast cell preparation was 4%, while recovery of added kinin from the same cells after purification to 25% purity was 85%. No detectable histamine release was observed from either cell preparation. Even with relatively crude preparations of skin mast cells, metabolism of added bradykinin was less than 50% and was not sufficient to explain the lack of histamine release upon exposure to this peptide. Interestingly, although bradykinin failed to induce histamine release from any of the human histamine-containing cells studied, lysylbradykinin, while showing no effect on lung mast cells or basophils, was capable of inducing modest histamine release (5.5 ± 3%; mean ± SD) from skin mast cells at a concentration of 10^{-5} M. Des(Arg^9) bradykinin was inactive in all cell systems.

When a series of competitive bradykinin receptor antagonists were studied, none of these analogs caused histamine release from basophils or lung mast cells at concentrations up to 10^{-4} M. In marked contrast, all sixteen compounds studied were capable of inducing histamine release from human skin mast cells (Table 3). Histamine release ranged from 7 to 30% at analog concentrations of 2×10^{-5} M, with most compounds releasing greater than 10% of total cellular histamine. Of interest is the observation

Table 1. Interaction of bradykinin with human basophils

	Mixed leukocytes		Mononuclear cell fractions							
			Basophil purity							
	(~0.5% basophils)		3%		4%		48%		60%	
Stimulus	%HR	% Kinin recovered	%HR	% Kinin recovered	%HR	% Kinin recovered	%HR	% Kinin recovered	%HR	% Kinin recovered
anti-IgE (0.1 μg/ml)	32		25		19		20		27	
Bradykinin (2×10^{-5} M)	0	2.7	0	63	0	74	0	84	0.2	96

Mixed leukocytes and mononuclear cell preparations of varying basophil purities were incubated with bradykinin for 30 min at 37%; HR = histamine release.

Table 2. Interaction of bradykinin with human mast cells

	Lung mast cells				Skin mast cells			
	4% Purity		20% purity		10% Purity		12% Purity	
Stimulus	%HR	% Kinin recovered	%HR	% Kinin recovered	%HR	% Kinin recovered	%HR	% Kinin recovered
anti-IgE	18		24		15		21	
Bradykinin (2×10^{-5} M)	0.5	17.5	0	89	0	65	0	49

that elongation at the N-terminus of peptides with [DArg^0] appeared to enhance their histamine releasing ability. To further study the abilities of these compounds to act as secretagogues for human skin mast cells we selected a representative compound: [DArg^0-Hyp^3-DPhe^7]-bradykinin. In addition, since all sixteen compounds contain D-phenylalanine in place of proline^7 of the bradykinin sequence and since this is the essential change required to confer antagonist activity, the ability of [DPhe^7]-bradykinin to cause histamine release was also examined. As shown in Fig. 1, the peptide [DArg^0-Hyp^3-DPhe^7]-

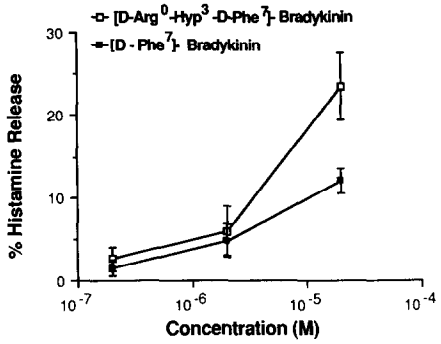

Fig. 1. Dose-dependent histamine release induced from human skin mast cells by kinin analogs. Results are the means ± SE from three experiments.

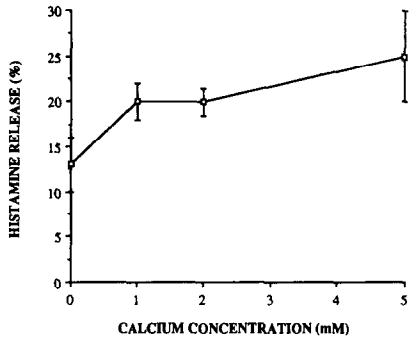

Fig. 2. Calcium dependence of histamine release induced from human skin mast cells by the kinin analog [DArg^0-Hyp^3-DPhe^7]-bradykinin. Results are the means ± SE from four experiments.

Table 3. Induction of histamine release from human skin mast cells by bradykinin analogs

Stimulus*	% Histamine release†
Anti-IgE	21.4 ± 2.5
[‡Thi^{5,8}-DPhe^7]-BK	9.5 ± 2.5
[§Hyp^3-Thi^{5,8}-DPhe^7]-BK	7.5 ± 2.3
[DArg^0-Hyp^3-Thi^{5,8}-DPhe^7]-BK	30.0 ± 5.4
Lys-Lys[Hyp^2-Thi^{5,8}-DPhe^7]-BK	13.0 ± 4.5
Lys-Lys[Hyp^{2,3}-Thi^{5,8}-DPhe^7]-BK	11.8 ± 4.0
Lys-Lys[Hyp^3-Thi^{5,8}-DPhe^7]-BK	16.0 ± 5.0
[DArg^0,Hyp^2-DPhe^7]-BK	20.7 ± 6.0
[DArg^0,Hyp^3-DPhe^7]-BK	25.3 ± 4.0
[Gly^6-Leu^{5,8}-DPhe^7]-BK	7.6 ± 2.5
[Gly^6-DPhe^7]-BK	13.0 ± 5.0
[Hyp^3-DPhe^7]-BK	13.5 ± 4.0
[DArg^0-Hyp^{2,3}-Thi^{5,8}-DPhe^7]-BK	11.0 ± 7.0
[‖EAC-Thi^{5,8}-DPhe^7]-BK	10.3 ± 5.0
Lys-Lys[Hyp^2-DPhe^7]-BK	10.4 ± 6.0
Lys-Lys[Hyp^3-DPhe^7]-BK	9.6 ± 6.2
Lys-Lys[Thi^{5,8}-DPhe^7]-BK	7.3 ± 4.0

* All analogs were used at a concentration of 2×10^{-5} M BK = bradykinin.
† Results are expressed as mean ± SE from three experiments.
‡ Thi = beta(2-thienyl)-alanine.
§ Hyp = 4-hydroxyproline.
‖ EAC = ε-aminocaproic acid.

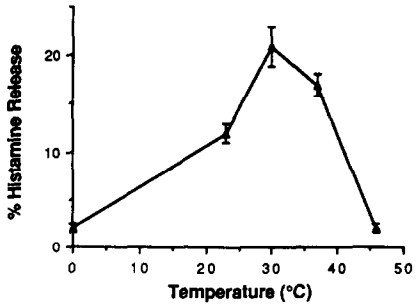

Fig. 3. Temperature dependence of histamine release induced from human skin mast cells by the kinin analog [DArg^0-Hyp^3-DPhe^7]-bradykinin. Results are the means ± SE from three experiments.

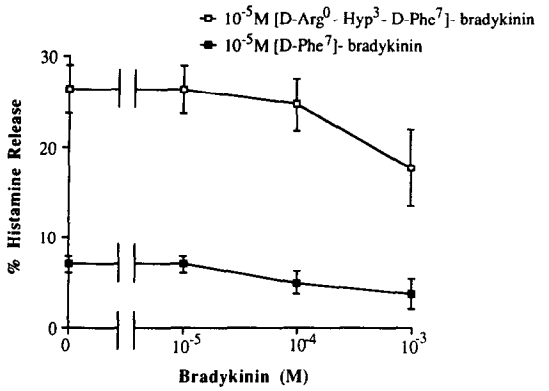

Fig. 5. Inhibition of kinin analog-induced histamine release from human skin mast cells by preincubation with bradykinin. Results are the means ± SE from three experiments.

bradykinin caused histamine release in a dose-dependent fashion at concentrations from 2×10^{-7} M to 2×10^{-5} M. Interestingly, [DPhe^7]-bradykinin also caused at least some histamine release (2.5 to 10%) over the same concentration range. Thus, substitution of a single amino acid in the bradykinin molecule is sufficient to confer secretagogue activity.

Histamine release induced by analogs such as [DArg^0-Hyp^3-DPhe^7]-bradykinin occurs in the absence of calcium (Fig. 2) but with a slight increase in histamine release over a calcium concentration range of 1 to 5 mM. Support for the concept that histamine release is an active process comes from temperature dependence studies (Fig. 3). Little or no release occurred at 0° or 46°, while optimal release occurred at 30°. The ability of human skin mast cells to respond maximally at 30° has been observed previously with other stimuli [21]. The kinetics of histamine release in response to [DArg^0-Hyp^3-DPhe^7]-bradykinin were relatively typical of those for human skin mast cells with other stimuli, such as substance P or morphine, with maximal release occurring in 1 min (Fig. 4).

If these peptides induce histamine release from human skin mast cells via a bradykinin receptor-mediated mechanism, then it should be possible to inhibit release by pretreatment of cells with bradykinin, which has a 10- to 100-fold higher affinity for the B_2 receptor than the antagonists. As can be seen in Fig. 5, however, it was not possible to inhibit release significantly until a 100-fold molar excess of bradykinin was used. The B_1 receptor agonist, des(Arg^9) bradykinin, showed a similar weak inhibitory effect (not shown). Clearly, these data are not consistent with the hypothesis that these peptides induce histamine release via a kinin receptor-mediated mechanism. This being the case, it seemed likely that the ability of these peptides to induce histamine release was unrelated to their bradykinin antagonistic activity. To test this hypothesis, and to further understand the structural requirements for secretagogues, a series of analogs in which single amino acid substitutions had been made in the bradykinin sequence was tested for their abilities to induce histamine release (Table 4). Two major points can be made from these experiments. First, secretagogue activity can, indeed, be dissociated from antagonist activity. Thus, compounds such as [LPhe^7]-bradykinin and [DPhe^6]-bradykinin, that are completely devoid of antagonistic activity, were as potent at causing histamine release as [DPhe^7]-bradykinin. Second, aromaticity would appear to be a major parameter in conferring secretagogue activity.

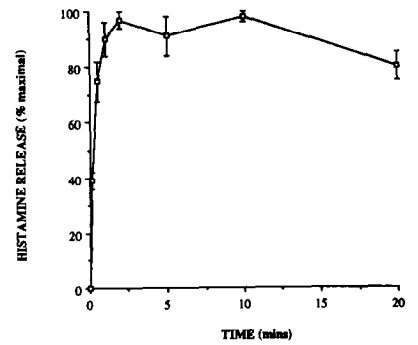

Fig. 4. Kinetics of histamine release induced from human skin mast cells by the kinin analog [DArg^0-Hyp^3-DPhe^7]-bradykinin. Results are the means ± SE from three experiments.

Table 4. Induction of histamine release from human skin mast cells by kinin analogs with single amino acid substitutions

Stimulus*	% Histamine release†
anti-IgE	35.0 ± 11.0
[DPhe^7]-Bradykinin	8.0 ± 2.7
[LPhe^7]-Bradykinin	12.7 ± 2.2
[DPhe^6]-Bradykinin	9.1 ± 2.6
[DPhe^8]-Bradykinin	5.8 ± 2.4
[DTrp^6]-Bradykinin	20.0 ± 4.0
[DTrp^7]-Bradykinin	23.3 ± 2.2
[DAla^7]-Bradykinin	1.1 ± 1.0
[DTyr^7]-Bradykinin	8.0 ± 2.5

* All peptides were used at a final concentration of 2×10^{-5} M.

† Results are means ± SE from three experiments.

Replacement of proline[7] with the nonaromatic alanine resulted in an analog with little, if any, histamine releasing ability, while substitution with aromatic phenylalanine or tyrosine residues resulted in secretagogues. Increased histamine release was observed when even more highly aromatic tryptophan residues were placed at either position 6 or 7. The role of amino acid stereochemistry remains unclear. While substitution of D-phenylalanine for L-phenylalanine at position 8 of bradykinin led to weak secretagogue activity, the placement of L-phenylalanine at position 7 was at least as effective as D-phenylalanine.

DISCUSSION

The ability of bradykinin to induce mediator release from rodent mast cells is well documented [10, 11]. Until the present study, however, no comprehensive attempt had been made to study the effects of kinins on human histamine-containing cells. Since basophils show clear differences compared to mast cells in terms of their sensitivity to anti-IgE and in their response to pharmacologic agents [22] and since more recent evidence suggests a functional heterogeneity of skin and lung mast cells [21, 23, 24], we decided to compare the effects of kinins on all three of these cell types.

In contrast to its effects on rodent mast cells, bradykinin was unable to induce histamine release from any human histamine-containing cell even at supraphysiologic concentrations up to 10^{-3} M. This inactivity was not due to kinin metabolism and is supported by *in vivo* nasal provocation studies in which administration of bradykinin to the nasal mucosa in doses up to 1 mg failed to result in any significant histamine release [9]. It has been reported that the ability of kinins to induce histamine release from rat peritoneal mast cells is dependent upon peptide length and basicity, with lysylbradykinin being a more effective stimulus than bradykinin while des(Arg^9) bradykinin was inactive [10]. In the human systems, des(Arg^9) bradykinin was inactive on all cell types, as may be expected in light of bradykinin's ineffectiveness. Lysylbradykinin was without effect on human basophils or human lung mast cells but was a weak secretagogue for skin mast cells at a concentration of 10^{-5} M. The need for such a high concentration of lysylbradykinin to affect skin mast cells, however, raises clear doubts regarding the physiologic relevance of this activity.

The selectivity of human skin mast cells to respond to kinins was also clear in studies using bradykinin analogs with receptor antagonist activities (Table 3). All sixteen compounds examined were without effect on basophils and lung mast cells but all induced significant levels of histamine release from skin mast cells. Release induced by these compounds is a calcium-independent process that occurs optimally at 30°. The ability of skin mast cells to respond maximally at 30° is observed with other stimuli, including anti-IgE [21], and presumably reflects the lower ambient temperatures normally experienced by these cells compared to lung mast cells or circulating basophils.

There is clear precedent for the ability of human skin mast cells to respond to stimuli which are ineffective on basophils or lung mast cells. In addition to kinin antagonists and analogs, morphine sulfate [21, 24] and substance P [21, 25] are also selective in their abilities to stimulate skin mast cells. The exact mechanism by which such compounds induce release, however, is unclear. Substance P must be used at concentrations considerably greater than would be expected for a normal receptor interaction unless one assumes rapid peptide metabolism or limited accessibility to the receptor. In the case of kinin antagonists, our results clearly suggest that histamine release occurs via a mechanism which is independent of bradykinin receptors, since a large excess of bradykinin is necessary to inhibit release significantly, despite the considerably higher affinity of bradykinin for such receptors. It is also clear that secretagogue activity can be dissociated from receptor antagonistic activity. The temperature dependence of analog-induced histamine release would tend to suggest that release is an active process. The possibility that release occurs via a lytic process, however, cannot be definitively ruled out.

In rodent mast cells, the ability of peptides to cause histamine release has been related frequently to the presence of clusters of basic amino acids, usually lysine and arginine, near the N-terminus [10, 26, 27], and it has been suggested that the presence of hydrophobic amino acids may play a role. Comparisons of release induced from human skin mast cells by substituted bradykinin analogs with antagonist activity (Table 3) and kinin analogs with single amino acid substitutions (Table 4) show some interesting trends. The presence of basic amino acids extending the N-terminus appears to yield mixed results. Obviously, lysylbradykinin is a secretagogue whereas bradykinin is not, and extension of [Hyp^3-Thi^{5,8}-DPhe^7]-bradykinin with two lysines at the N-terminus caused a modest increase in histamine release, while extension with D-arginine quadrupled histamine release. Similarly, addition of [DArg^0] to [Hyp^3-DPhe^7]-bradykinin doubled histamine release. Indeed, the three secretagogues among the antagonists examined that caused the greatest levels of histamine release all had a [DArg^0] addition. On the other hand, addition of two lysines to the N-terminus of either [Thi^{5,8}-DPhe^7]-bradykinin or [Hyp^3-DPhe^7]-bradykinin had no positive effect on histamine release. A more consistent feature that can be associated with histamine-releasing activity is increasing the aromaticity of these peptides by substitutions with aromatic residues such as phenylalanine and, in particular, tryptophan. The ability of added tryptophan residues to enhance secretagogue activity has also been observed on the induction of histamine release from rat peritoneal mast cells by substance P analogs. Foreman [26] reported that [DTrp^{7,9}]-substance P is 12-fold more potent as a secretagogue than the parent peptide, whereas Devillier and coworkers [28] demonstrated that [DTrp^{7,8,10}]-substance P is almost 100-fold more potent than substance P in causing histamine release. It has generally been stated that it is the increased hydrophobicity imparted by increasing the number of tryptophan molecules that leads to enhanced

release. Obviously it is difficult to distinguish between increasing hydrophobicity and aromaticity, but our data suggest that it may, in fact, be the latter parameter which is the important characteristic. For example, replacement of proline[7] with the relatively hydrophilic, but aromatic, tyrosine residue resulted in a secretagogue which was as effective as [DPhe[7]]-bradykinin, whereas substitution with the non-aromatic but weakly hydrophobic [DAla[7]] essentially abolished release (Table 4). By the same token, replacement of the aromatic phenylalanine residues at positions 5 and 8 with nonaromatic, hydrophobic leucine residues in [Gly[6]-Leu[5,8]DPhe[7]]-bradykinin resulted in decreased histamine release compared to [Gly[6]-DPhe[7]]-bradykinin (Table 3).

In summary, we have demonstrated that bradykinin has no effect on any human histamine-containing cell. A single amino acid substitution, however, can convert bradykinin into a secretagogue for human skin mast cells but not mast cells or basophils, providing additional support for the concept of human mast cell heterogeneity. The mechanism by which kinin analogs induce release from skin mast cells requires further study but is obviously independent of bradykinin receptors and depends, at least in part, on the aromaticity and basicity of the peptide.

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