Anaphylactic Release of a Basophil Kallikrein-like Activity

II. A MEDIATOR OF IMMEDIATE HYPERSENSITIVITY REACTIONS

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ABSTRACT This report describes the immune release of a new mediator from human peripheral leukocytes, a basophil kallikrein-like activity (BK-A). The release process is initiated by the interaction of antigen or anti-IgE with cell-bound IgE, and appears to be similar in mechanism to the release of histamine and other mediators of the immediate hypersensitivity reaction. The dose-response relationships and kinetics of histamine and BK-A release from antigen-challenged peripheral leukocytes are similar. The release of the BK-A is calcium and temperature dependent, requires metabolic energy, and is controlled by hormone-receptor interactions that influence the cellular level of cyclic AMP, as has been described for other mediators of immediate hypersensitivity reactions. The data indicate that the interaction of BK-A with human plasma kininogen, generates immunoreactive kinin. We conclude that the antigen-IgE interaction leads to the release from human basophils of a new mediator, a basophil kallikrein-like activity which may well be a link between reactions of immediate hypersensitivity and the plasma and/or tissue kinin-generating systems.

INTRODUCTION

Human leukocyte preparations from allergic donors challenged with appropriate antigens release several chemical mediators including histamine, slow-reacting substance of anaphylaxis (SRS-A)[1] and eosinophil chemotactic factor of anaphylaxis (ECF-A) (1–3).

The release process of some mediators of inflammatory or allergic reactions—histamine, SRS-A, ECF-A—has been extensively described (2). These agents are actively secreted after antigen challenge of cells (basophils and mast cells) sensitized with IgE antibody (3–6). The release process is calcium and temperature dependent, requires metabolic energy, and is controlled by hormone-receptor interactions which influence the intracellular level of cyclic nucleotides (3, 7–10). We have now demonstrated the immune release of another mediator from human basophils; a basophil kallikrein-like activity (BK-A). We earlier demonstrated that a protease released by antigen-challenged leukocytes which co-chromatographs with the BK-A, generates a kinin from human plasma kininogen (11). The purpose of the experiments described here is to characterize the mechanism of BK-A release and to compare that mechanism to the release of other mediators, particularly histamine. The data suggest some similarities in the release process of BK-A and histamine. More importantly are the differences in the release process of BK-A and histamine which, in different individuals, may prove to be of some importance in their response to inflammatory stimuli.

METHODS

Materials. The *p*-toluenesulfonyl-L-arginine [^{3}H]methyl ester ([^{3}H]TAMe) (210 mCi/mmol) was purchased from Biochemical and Nuclear Corp. Burbank, Calif. Tris buffers used in the release of histamine and the arginine esterase from basophils were made of 0.025 M preset Tris, pH 7.35 at 37°C (Sigma Chemical Co., St. Louis, Mo.), 0.12 M sodium chloride, 5 mM potassium chloride, and 0.03% human serum albumin (Behring-Werke, Marburg/Lahn, West Germany). The above constitutes Tris-A; Tris-ACM contains, in addition, calcium 0.6 mM and magnesium 1.0 mM (3). The following were purchased from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.: TAMe and Tris from which the buffer used in the [^{3}H]TAMe basophil arginine esterase assay was made (0.06 M), and adjusted with HCl to pH 8.0 at 25°C. Theophylline, histamine, isoproterenol, and colchicine were purchased from the Sigma Chemical Co.; diatrizoate sodium (Hypaque) from Winthrop Laboratories, New York; Ficoll from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.; 2-deoxyglucose from Aldrich Chemical Co., Inc., Milwaukee, Wisc.; deuterium oxide (D_2O) from Bio-Rad Laboratories, Richmond, Calif.;

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[1] *Abbreviations used in this paper*: AgE, ragweed antigen E; BK-A, basophil kallikrein-like activity; D_2O, deuterium oxide; ECF-A, eosinophil chemotactic factor of anaphylaxis; SRS-A, slow-reacting substance of anaphylaxis; TAMe, *p*-toluenesulfonyl-L-arginine-methyl ester.

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prostaglandin E1 was kindly provided by Dr. J. Pike of The Upjohn Company, Kalamazoo, Mich.; Cholera enterotoxin by Dr. R. A. Finkelstein, Dallas, Tex. Antigen E was kindly provided by Dr. T. P. King of The Rockefeller University, New York, anti-IgE by Dr. K. Ishizaka, and the ionophore A23187 by Dr. R. Hamill, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

Leukocyte preparations. Human Leukocytes from donors allergic to ragweed or grass and from normal volunteers were separated from the other formed elements of blood by sedimentation for 60–90 min in a mixture of dextran-EDTA and dextrose. The cells were washed twice in Tris-A buffer, then resuspended in a serum-free Tris-ACM buffer at a concentration of $\cong 10^7$ cells/ml, as previously described (3). The immunologic reaction was initiated by the addition of antigen or anti-IgE to the cell preparations and the reaction allowed to proceed for 30–45 min in the dose-response studies, or for increasing periods up to 45 min in the kinetic studies. At the completion of the reaction, the cells were centrifuged and the quantity of histamine released into the supernate as well as that present in an aliquot of untreated cells were determined by a modification of the fluorometric method of Shore et al. (3, 12, 13). The BK-A was assayed as described below. Experimental tubes were run in duplicate except in studies where standard deviations were obtained where quadruplicate determinations were made. Duplicates did not differ by >5–10%. Each study included a control to which no antigen was added and this value was subtracted from all experimental tubes when measuring the quantity released of either histamine or the arginine esterase. We have previously shown that spontaneous histamine release represents <2% of the total cellular histamine (3). The spontaneous esterase release is at a similar level.

Arginine esterase activity (BK-A). Arginine esterase activity of the supernate was determined by a radiochemical technique employing [^{3}H]TAMe which was devised by Beaven et al. (14) for the measurement of human urinary kallikrein and modified for the determination of prekallikrein (15) and arginine esterase activity in supernate (16). The experimental tubes with leukocytes were run in duplicate, and the determinations of arginine esterase activity were run in quadruplicate. The total cellular arginine esterase activity was determined using sonicated aliquots of untreated cells. Sonication studies with 1-ml aliquots of leukocyte preparations showed that, when cells were sonicated for 30-s periods, maximal arginine esterase activity was released with 2½ min of sonication (Branson Sonic Power Co., Danbury, Conn.).

A convenient chemical assay of a kallikrein is the measurement of its esterolytic activity on a synthetic amino acid ester substrate such as TAMe. The chemical assay utilizes the measurement of methanol liberated upon hydrolysis of the ester (17). The [^{3}H]TAMe assay measures methanol as [^{3}H]-methanol, after the hydrolysis of [^{3}H]TAMe. The [^{3}H]TAMe radiochemical assay gives values for arginine esterase activity comparable to those obtained by the chemical assay and is 100- to 200-fold more sensitive (14). For the measurement of arginine esterase activity of the supernate, a 40-μl aliquot of supernatant fluid was added to a 0.06 M Tris buffer, pH 8.0 (20 μl) in a polypropylene microtube (Eppendorf microtube 3810) and the reaction initiated by the addition of [^{3}H]TAMe (0.035 μCi, 10 μl). The microtube containing the reaction solution was floated in a closed counting vial containing 10 ml of toluene-liquiflor (Econofluor, New England Nuclear, Boston, Mass.) and 50 μl stop solution (1 vol glacial acetic acid added to 10 vol 0.02 M TAMe), during the 30-min incubation period at room temperature. The reaction was terminated by shaking the vial for 10 s and the vial counted by a liquid scintillation spectrometer (Isocap 300, Searle Diagnostics Inc., subsidiary of G. D. Searle & Co., Des Plaines, Ill.).

The hydrolyzed [^{3}H]methanol is soluble in toluene and hence counted, whereas the unhydrolyzed [^{3}H]TAMe is insoluble in toluene, remaining in the water phase, and does not contribute a significant number of counts. A standard curve relating [^{3}H]methanol formation from [^{3}H]TAMe as a function of time, by anti-IgE-generated basophil arginine esterase (Fig. 1A) shows a linear release of [^{3}H]methanol from 0 to 90 min (0.035 μCi, or 30,000 cpm is added to each microtube). This linear range encompasses our experimental conditions.

RESULTS

Dose response. The dose-response relationships of histamine and arginine esterase release have been studied with leukocyte preparations from 25 allergic and 23 normal individuals challenged with either the purified protein antigens from ragweed (AgE) (18) or grass (19) or with highly specific anti-IgE (20). An arginine esterase dose-response curve is shown in Fig. 1B. The precision of the arginine esterase assay for quadruplicate determinations is indicated by the standard deviations (Fig. 1B). In general, the pattern of the dose-response curves for histamine and arginine esterase release are similar, whether the release is initiated by antigen (Fig. 2A) or anti-IgE (Fig. 2B), but the maximal percentage of histamine or arginine esterase released by a leukocyte preparation often differ (Fig. 3A). The data in Fig. 3B show the average dose-response relationships of histamine and the arginine esterase released from basophils by anti-IgE in 23 nonallergic subjects. The maximal percent histamine released averaged 38% whereas the arginine esterase released averaged 23%. For both histamine and the arginine esterase, however, the range may vary from 0 to 100%. The differential release of amounts of histamine and BK-A (Fig. 3) is paralled by the reports of others showing differential release of enzymes from azurophilic and specific granules of polymorphonuclear leukocytes (21), and differential release of histamine and SRS-A (22).

Kinetic studies. The rates of histamine and esterase release are compared in Fig. 4. There is no significant difference. Both release processes are temperature dependent (Fig. 5). Incubation of cells at 15° and 25°C allows little release of either mediator, whereas the percent released increases at 32° and is maximal at 37°C. Note the kinetic and temperature parallelism in the release of the two mediators, even though, in this experiment, there is a maximum of $\cong$90% histamine release and only 20% esterase release.

Not only is the initiation of BK-A release temperature sensitive, but decreasing the temperature at any time during the release process promptly stops the reaction. In the experiment illustrated in Fig. 6, the following procedures were used: A suspension of

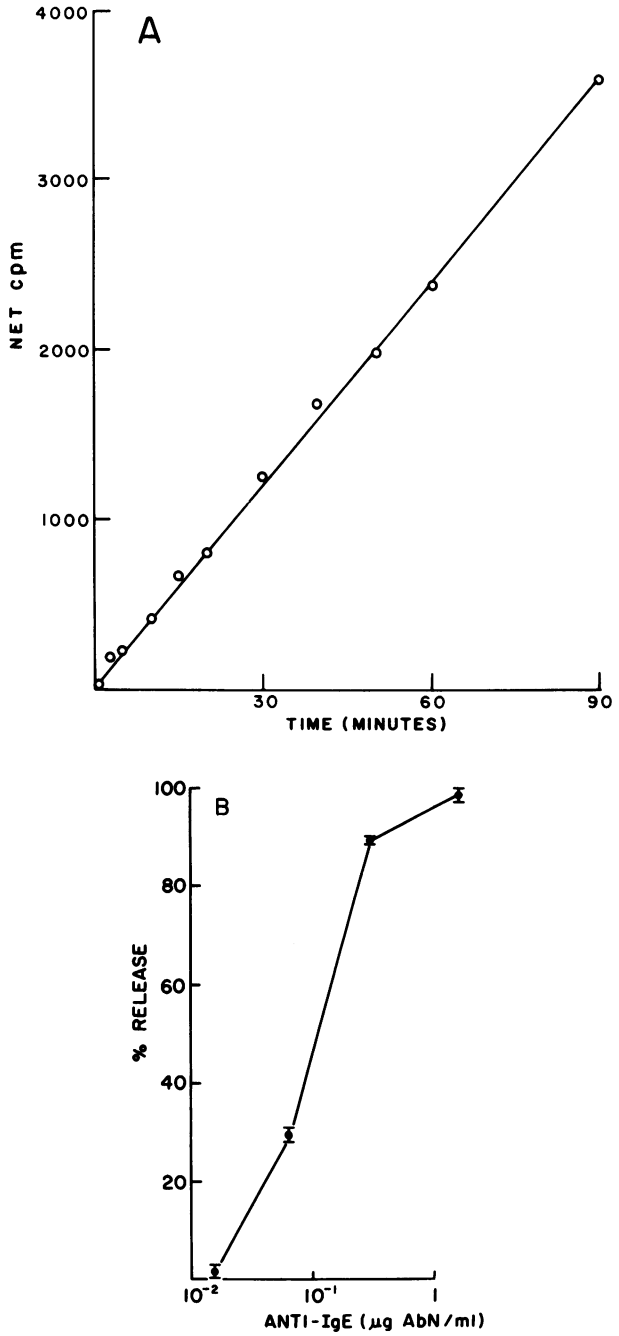

FIGURE 1 (A) [^{3}H]Methanol formation from [^{3}H]TAMe by anti-IgE generated BK-A as a function of time. There is a linear release of [^{3}H]methanol between 0 and 90 min. (B) Dose response of anti-IgE-induced release of BK-A. The precision of the esterase assay is indicated by the SD.

leukocytes was brought to 37°C and the reaction initiated in a "parent" flask by the addition of ragweed antigen (AgE). At various time intervals after the addition of antigen, leukocytes were withdrawn from the parent flask and treated in one of three ways: (*a*) Duplicate aliquots of 1 ml of cells were added to tubes containing 0.2 ml of buffer and centrifuged for 1 min. This established the basic kinetics of release by rapidly centrifuging the cell preparations to separate the cells from the supernate. (*b*) Duplicate 1-ml aliquots of cells, at the time intervals indicated by arrows in Fig. 6, were also transferred to tubes containing 0.2 ml of buffer and placed immediately in a 4°C bath, and allowed to incubate until the end of the experiment. It is clear that the release process stopped when the temperature was reduced. (*c*) Duplicate 1-ml aliquots of cells, at the time intervals indicated by arrows in Fig. 6, were also transferred to tubes containing 0.2 ml of EDTA (3 mM final concentration) and allowed to incubate at 37°C until the end of the experiment. Fig. 6 illustrates the divalent cation (e.g. calcium) dependence of the esterase release process. The addition of EDTA at any time during the reaction led to prompt cessation of the esterase release. These findings with respect to the esterase release are similar to those described for basophil histamine release (3).

Effect of cell number. We have reported that, when the concentration of antigen in the reaction mixture is sufficient for maximal response, the quantity of histamine released is directly proportional to the cell number (3). The effects of cell number on esterase release were studied in the following experiments. Leukocytes were obtained from a nonallergic donor, and serially diluted by a factor of 2 (highest concentration $\cong 10^7$ cells/ml) after which the reaction was initiated by adding a constant amount of anti-IgE, earlier determined to be sufficient for maximal esterase release. The release of the arginine esterase (Fig. 7A) is directly proportional to the cell number. The supernate from an aliquot of anti-IgE challenged cells was likewise serially diluted. The arginine esterase activity was proportional to the serially diluted aliquots (Fig. 7B).

Cell of origin of the arginine esterase. Blood from donors allergic to ragweed or grass, and from normal volunteers was drawn in heparin (10 U/ml) then diluted with 2 vol of 0.9% saline (1:3 dilution), layered on a Hypaque-Ficoll cushion (d = 1.077) and centrifuged at 400 g for 40 min (20). The cells at the interface contain lymphocytes, monocytes, and basophils. When passed over glass bead columns, lymphocyte fractions (>99% lymphocytes) were eluted with plasma and basophil-rich fractions (2–10%) were eluted with EDTA (20, 23). The granulocytes and erythrocytes remaining at the bottom of the Hypaque-Ficoll cushions were resuspended in homologous plasma and the erythrocytes sedimented in a mixture of dextran-EDTA and dextrose. The granulocyte-rich fraction was obtained from the suspension after 60–90 min of sedimentation, while the erythrocyte-rich fraction

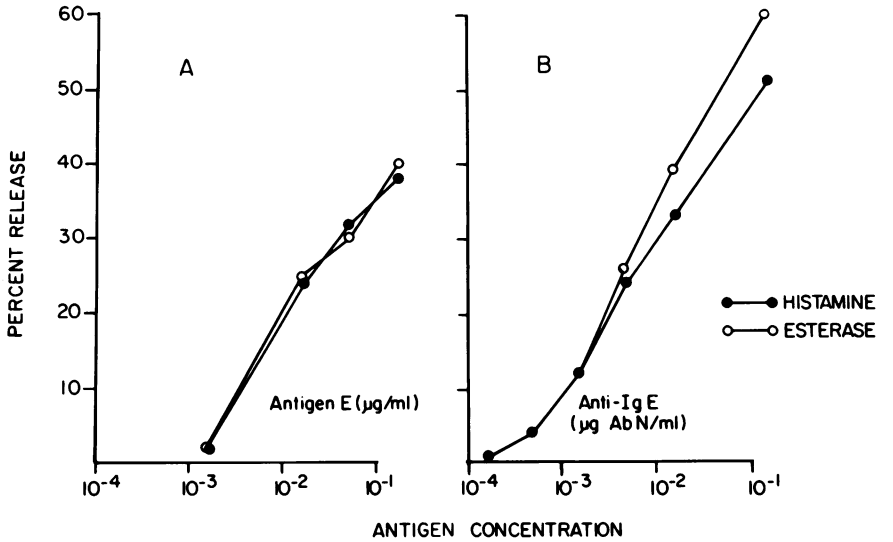

FIGURE 2 Release of histamine and BK-A activity. The pattern of the dose-response curves for histamine and BK-A are similar whether the release is initiated by antigen (A), or by anti-IgE (B).

was at the bottom of the mixture. Eosinophil-rich fractions were obtained by the procedure described by Day and Litt (24, 25). The types of cells in these preparations were determined by counting 500–1,000 cells that were stained with Wright's stain or toluidine blue. The total cell number was determined by counting in a Neubauer chamber. The cell preparations thus obtained were either sonicated to determine the total arginine esterase activity, or dose-response curves were established using anti-IgE, antigen, or the calcium ionophore A23187 (26, 27).

Lymphocytes. Cell preparations of lymphocytes (>99% lymphocytes) from both allergic and nonallergic donors were challenged with both anti-IgE and the calcium ionophore A23187. Since previous work has demonstrated that the basophil is the only human leukocyte type which fixes IgE (20, 28), the anti-IgE challenged lymphocytes, as anticipated, released no esterase activity. The calcium ionophore causes nonspecific, nonimmunologic, and noncytotoxic release of histamine (27) and the arginine esterase from human basophils. Ionophore also releases large quantities of SRS-A from human granulocytes (29). Thus, one would anticipate that, if the lymphocyte contained the arginine esterase, it might be released by ionophore. In six experiments, however, the ionophore-challenged lymphocytes released no BK-A activity. Sonicated lymphocyte preparations had TAMe esterase

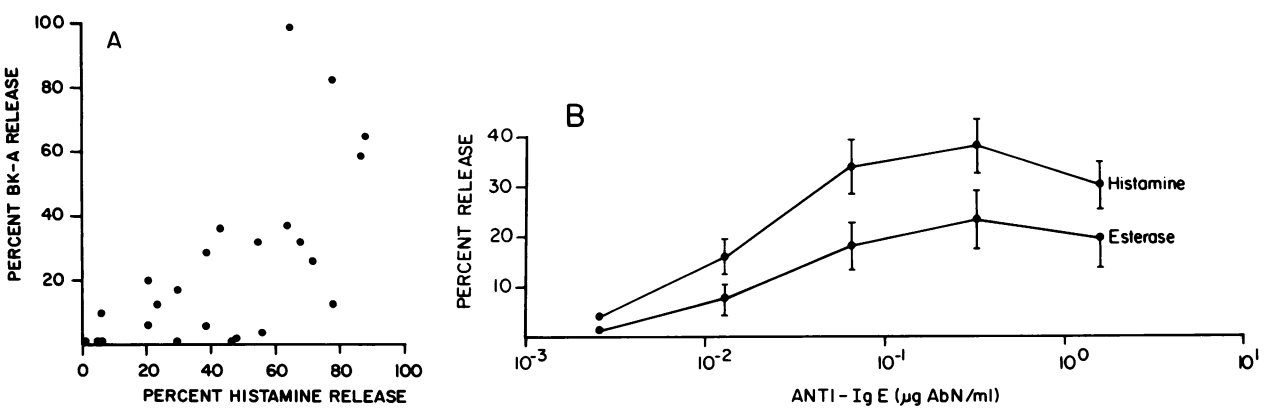

FIGURE 3 (A) Maximal percent release of BK-A and histamine. The maximal release of BK-A and histamine from a leukocyte preparation often differed (25 subjects). (B) Dose-response relationships of histamine and BK-A in 23 nonallergic subjects. At most anti-IgE levels, the percent released was significantly greater for histamine. Vertical bars represent ±1 SEM.

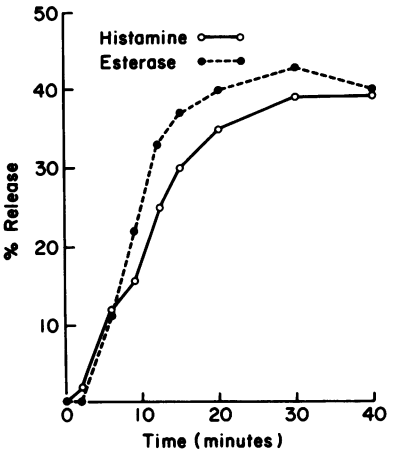

FIGURE 4 Kinetics of AgE-induced (17 ng/ml) histamine and BK-A release from basophils. The time-courses of release for histamine and BK-A are essentially identical.

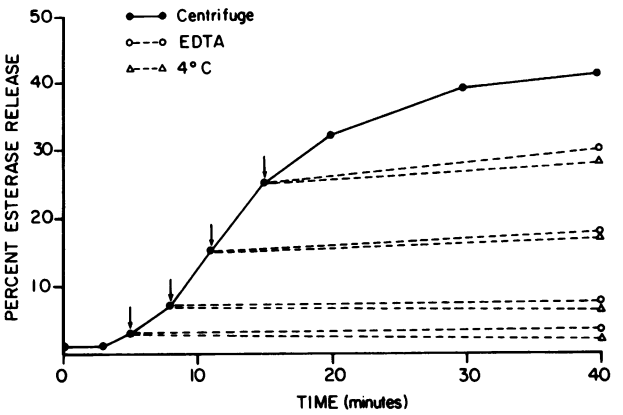

FIGURE 6 Effects of EDTA and temperature on AgE-induced BK-A release. Decreasing the temperature to 4°C or the addition of EDTA (3 mM final concentration) at any time during the release process (indicated by arrows) stops the reaction.

activity which was 0.1–1% that of the basophils (per 10^6 cells) as determined by their relative abilities to hydrolyze [^{3}H]TAMe. The 15,000-*g* supernate from sonicated lymphocyte preparations contained no arginine esterase activity; all of the activity was in the pellet. The 15,000-*g* supernate from sonicated basophil-lymphocyte preparations contained, on the other hand, most of the arginine esterase activity, with only minor activity in the pellet. Thus, the basophil and lymphocyte enzymes appear to be different.

Other blood cells. Polymorphonuclear leukocytes (95–98% pure) and eosinophils (90–98% pure) were challenged with both ionophore (0.01–5 μg/ml) (27) and anti-IgE (10^{-4} to 10^{-1} μg AbN/ml), as were the lymphocytes. In five experiments, neither ionophore nor anti-IgE caused release of the arginine esterase.

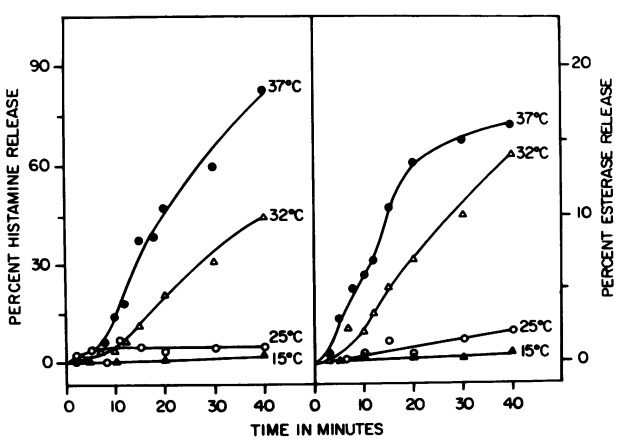

FIGURE 5 Effect of different temperatures on AgE-induced histamine and BK-A release from basophils. The release process of both mediators is temperature dependent.

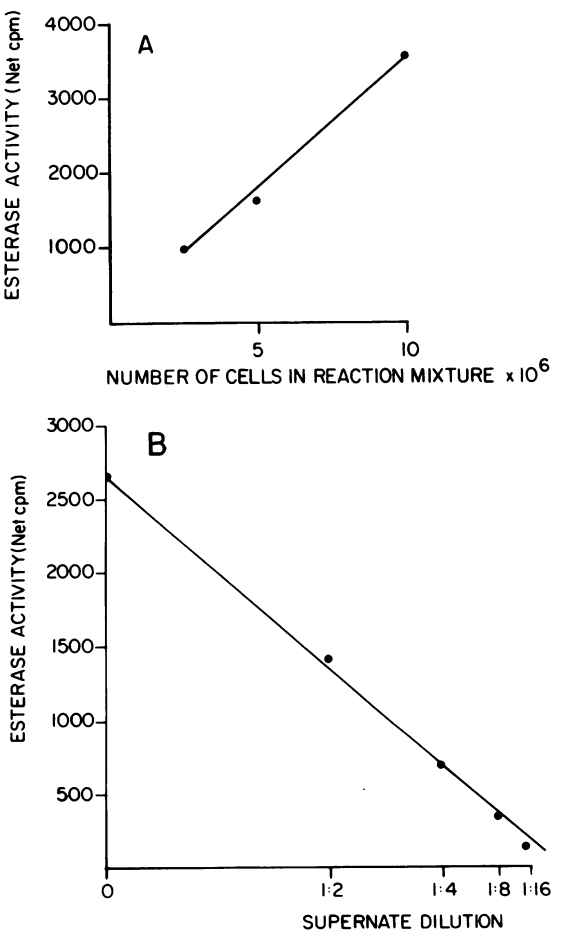

FIGURE 7 Anti-IgE-induced BK-A release as a function of cell number (A), and BK-A activity as a function of serial dilution (B). Both BK-A release and activity are proportional to cell number and serial dilution, respectively.

Sonicates of pure platelets ($>10^8$/ml) obtained by light centrifugation of leukocyte preparations likewise failed to demonstrate arginine esterase activity. We were unable to obtain monocytes in high concentrations. However, sonicates of fractions from the glass bead columns, with varying percentages of basophils (0–10%), lymphocytes (up to 99.8%), and monocytes (0.2–20%) suggest that if the lymphocytes in the monocyte and lymphocyte-rich fractions are similar, then monocytes also contain minor arginine esterase activity, similar to the lymphocytes. Sonicates of erythrocytes had no arginine esterase activity.

Effect of cell number on esterase release (Is there an esterase releasing factor?). Studies were designed to attempt to determine the cell of origin of the TAMe esterolytic activity. Human peripheral leukocytes from nonatopic donors were aliquoted from a parent flask then studied in three ways. In "A" (Table I), leukocytes were passively sensitized with antiserum from a ragweed-sensitive donor, then challenged with AgE. In "B", leukocytes were "sham" passively sensitized with antiserum from a nonragweed-sensitive donor, then challenged with AgE. In "C", aliquots of leukocytes from "A" and "B" with equal numbers of cells were mixed, then challenged with AgE. As shown in study I, challenge of the sensitized leukocytes led to 50% histamine and 16% esterase release. Challenge of the sham-sensitized cells led to 0% release of both histamine and the esterase. Challenge of the mixed cells (equal numbers of cells from "A" and "B") led to 25% histamine and 8% esterase release, suggesting that in "C", like histamine, the esterase was released as a result of IgE-AgE interaction (from the 50% of the basophils that were sensitized), and that the presence of excess numbers of other cell types, including the presence of excess numbers of nonsensitized basophils, did not influence the quantity or percent of esterase that was released. This data suggest that, as with histamine, the esterase is derived from basophils as a direct result of IgE-AgE interaction, and that the esterase is not released as a result of a basophil-derived releasing factor, which might affect other cell populations, or nonsensitized basophils.

TABLE I
Effect of Cell Number on Esterase Release

	Histamine			Esterase activity		
	A	B	C	A	B	C
	% Release			% Release		
Study I	50	0	25	16	0	8
Study II	57	0	26	29.1	0	15.6

(A) Peripheral leukocytes passively sensitized then challenged with AgE; (B) Peripheral leukocytes sham passively sensitized then challenged with AgE; (C) Mixture of equal number of leukocytes from A and B challenged with AgE.

Modulation of BK-A release. Earlier studies have shown that cyclic AMP itself and agents which increase the cyclic AMP level of target cells of the immediate hypersensitivity reaction inhibit the release of histamine (8, 9). In the following experiments, we studied the modulating effects of certain cyclic AMP-active drugs on the release of BK-A and histamine. The pharmacological effects of the drugs were determined by adding aliquots of a leukocyte suspension to a series of test tubes containing a constant amount of AgE and variable concentrations of the drugs. The test tubes were incubated at 37°C for 30 min, and the percent of BK-A and histamine released into the supernate determined. The percent inhibition of release resulting from each drug was determined from the equation: percent Inhibition = $(C - E)/C \times 100$, where C and E represent the percentage of BK-A or histamine release in the control (C) and experimental (E) tubes, respectively.

Fig. 8A shows the influence of theophylline on the AgE-induced release of BK-A (arginine esterase activity) and histamine. The molar concentrations of theophylline required for 50% inhibition of release are similar for the BK-A and histamine. Fig. 8B shows that the molar concentrations of dibutyryl cyclic AMP required for 50% inhibition of release are likewise similar for the BK-A and histamine. The molar concentrations of prostaglandin E1 (PGE1) (Fig. 9A) and cholera enterotoxin (Fig. 9B) required for 50% inhibition of release are likewise similar for the BK-A and histamine. Similarly, the molar concentrations of other drugs (0.1 μM isoproterenol and 1 μM histamine) required for 50% inhibition of release were essentially identical for the BK-A and histamine.

Recent evidence suggest that cyclic AMP levels might control the state of aggregation of microtubules which, in turn, regulates the secretory process. It has been shown that colchicine which causes disaggregation of microtubules, is a potent inhibitor of histamine release (30), whereas heavy water (D_2O) which favors microtubule aggregation is a potent enhancer of histamine release, and that together the two agents are antagonistic so that their effects cancel (30, 31). Leukocyte suspensions were exposed to either D_2O or colchicine as earlier described (30), then challenged with a constant concentration of AgE or anti-IgE. Table II and Fig. 10 show that colchicine inhibits whereas D_2O enhances the release of BK-A and histamine.

Energy requirement for BK-A release. Mediator release is an active process. It has been demonstrated that, in the secretory process, there is an absolute requirement for metabolic energy and if glycolytic processes are impaired, histamine release ceases (9). We studied the effects of a metabolic inhibitor, 2-deoxy-

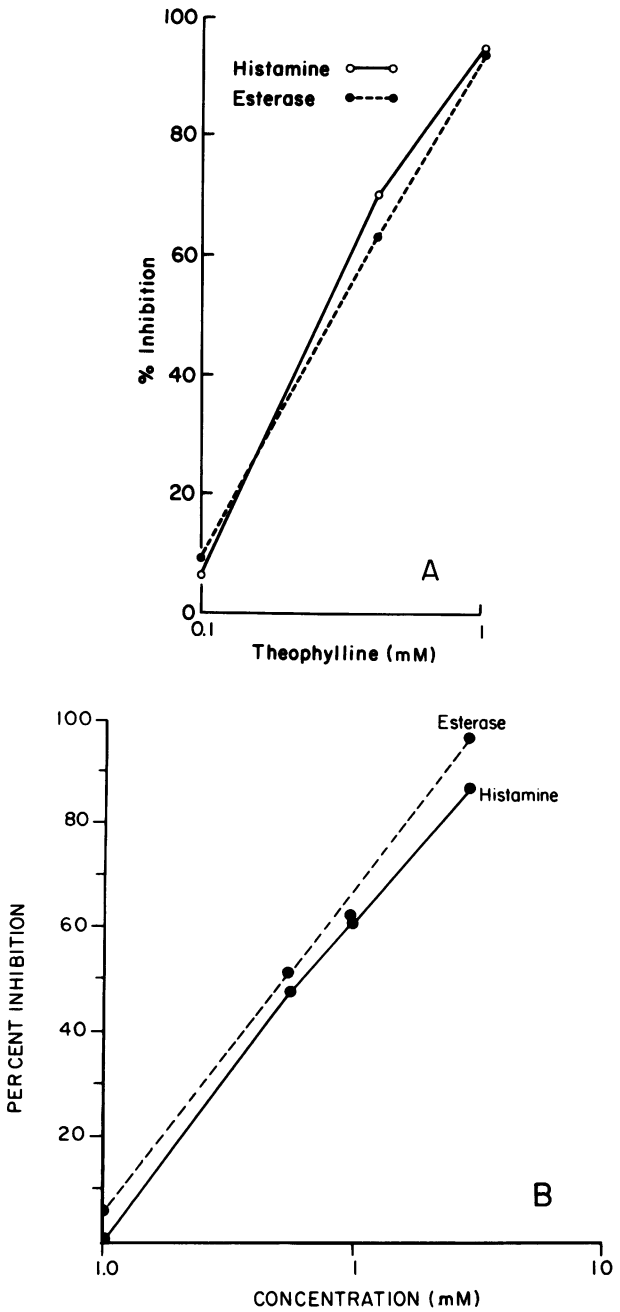

FIGURE 8 Inhibitory effects of theophylline (A) and dibutyryl cyclic AMP (B) on BK-A and histamine release from basophils. For each drug, the molar concentrations required for 50% inhibition of release are similar for histamine and BK-A.

glucose on the release of BK-A and histamine. The pharmacological effects of 2-deoxyglucose were determined in a fashion similar to the above studies on the modulation of BK-A release (9). The release process of BK-A is energy dependent (it is inhibited by 2-deoxyglucose). The molar concentrations of 2-deoxyglucose required for 50% inhibition of release are similar for the BK-A (0.4 mM) and histamine (0.3 mM).

DISCUSSION

A variety of mediators of potential pathogenetic importance in reactions of immediate hypersensitivity have been described, each with different pharmacological properties. We have now described an additional mediator (BK-A) which is released after antigen-IgE antibody interaction (Fig. 1) and which has the important property of generating kinins from plasma kininogen (11, 32, 33). This is the first mediator from basophils or mast cells which is able to interact with the serum cascade systems (the coagulation and kallikrein-kinin systems) to generate biologically relevant peptides. In the present manuscript, we have explored the mechanism of the release of the BK-A compared

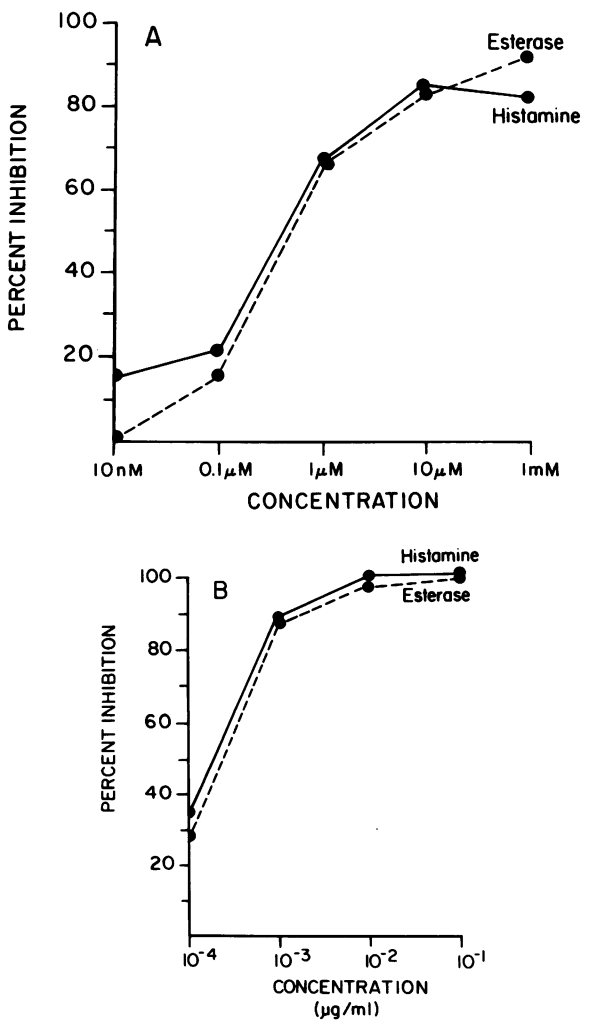

FIGURE 9 Inhibitory effects of prostaglandin E1 (A) and cholera enterotoxin (B) on BK-A and histamine release.

TABLE II
Effect of Colchicine (0.3 mM) on Histamine and Esterase (BK-A) Release

Subject	Histamine release		Esterase release	
	Anti-IgE	Anti-IgE + Colchicine	Anti-IgE	Anti-IgE + Colchicine
	%		%	
1	57.9	40.3 (30)	44.6	7.2 (84)
2	37.2	14.7 (61)	18.1	4.5 (75)

Leukocytes were challenged with anti-IgE 0.33 and 0.07 μg AbN/ml in subjects 1 and 2, respectively. Numbers in parentheses represent percent inhibition of release by colchicine.

to the mechanism of release of the standard mediator, histamine. Both mediators are preformed, i.e., antigen challenge is not required for the generation of the mediator. This is unlike mediators such as SRS-A which is generated only after antigen-antibody interaction (1, 2). It is also unlike ECF-A, which in basophils is not a preformed mediator (1, 2). Another similarity between histamine and BK-A is that qualitatively the dose-response relationship and the kinetics of the two mediators are similar whether release is initiated by antigen or by anti-IgE (Figs. 2–4). However, an analysis of these curves indicates an important difference between the two mediators. That is, there is no quantitative relationship between the amount of histamine and BK-A which is released from the cells of a given individual (Fig. 3A). This observation was unexpected because although this might be anticipated when comparing histamine and SRS-A (22), a preformed and nonpreformed mediator, it might not be expected when studying the release of two preformed mediators. One explanation for this difference is that histamine and BK-A are localized in two different granules. There exists an analogy in the neutrophil which contains two types of granules. In the neutrophil, which shares a common cell of origin with the basophil, the release of β-glucuronidase from the azurophil granule and lysozyme from the specific granule are often quite different (21, 34).

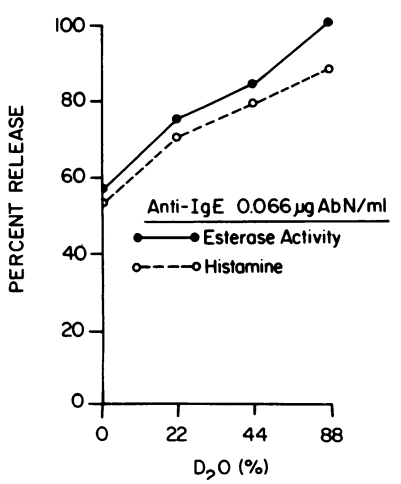

FIGURE 10 Effect of D_2O on histamine and esterase (BK-A) release. D_2O enhanced the release of both histamine and BK-A.

In most other respects, the release mechanisms for BK-A and histamine are similar. Thus, both have an absolute requirement for calcium (Fig. 6). The release process is totally dependent upon calcium throughout its course; the removal of extracellular divalent cations by EDTA promptly stops the release process of BK-A, as has been described for histamine (3). The release processes of both mediators are also highly temperature dependent (Fig. 5), with minimal release at temperatures under 22°C and maximal release at ≅37°C. Not only is the initiation of BK-A release temperature sensitive, but decreasing the reaction temperature at any point during the release process promptly stops the reaction (Fig. 6), as earlier described for histamine (3). Finally, both release processes require metabolic energy as indicated by the inhibition of release by 2-deoxyglucose, an inhibitor of glycolysis. All of these characteristics indicate that BK-A release, like histamine release, is an active, secretory event and not a cytotoxic process.

We, and other investigators, have described data which indicate that the release of both preformed and nonpreformed mediators is controlled by hormone receptor interactions which influence the intracellular level of cyclic AMP (7–9). As anticipated, the release of BK-A is similarly controlled and, thus, agents which act on adenylate cyclase to increase cyclic AMP levels inhibit the release of histamine and BK-A (Figs. 8–9). The phosphodiesterase inhibitor, theophylline, has a similar effect on the release of both mediators and dibutyryl cyclic AMP itself also similarly inhibits the release of both mediators. Although there may be some quantitative differences in the effects of these inhibitors of release, qualitatively the results are similar, suggesting that BK-A release is also controlled by the intracellular level of basophil cyclic AMP. Finally,

the release of both preformed and nonpreformed mediators have been shown to be influenced by agonists which putatively act on the state of aggregation of microtubules. In each instance, colchicine inhibits and D_2O enhances the release of these mediators (30, 31). Similar results were obtained with respect to the modulation of BK-A release (Fig. 10, Table II), although the magnitude of the effects of D_2O and colchicine on the release process of histamine and BK-A differed.

Studies to establish the cell of origin of BK-A indicate that no arginine esterase activity was derived from antigen, anti-IgE, or ionophore challenge of purified preparations of lymphocytes, neutrophils, eosinophils, erythrocytes, or platelets. The data suggest that BK-A is derived solely from basophils. There is, however, in sonicates of lymphocytes, some arginine esterase activity. The lymphocyte TAMe esterase activity is only $\cong$1% that of basophils (per 10^6 cells), has a different profile of substrate specificity for the synthetic amino acid esters (11), and is associated with the 15,000-*g* fraction of lymphocyte sonicates. In all of these respects, the lymphocyte esterase differs from the basophil arginine esterase activity. Nonetheless, in some of the experiments, the lymphocyte esterase may have influenced the determination of the percentage of arginine esterase release. Although this may have accounted to some degree for the difference between the percent of histamine and BK-A release (Fig. 3B), a major part of this difference cannot be accounted for in this way, supporting the hypothesis that the two mediators may be derived from different granules, or may be differentially secreted from the same granule, as occurs with polymorphonuclear leukocytes (21).

It has been previously pointed out that certain chemical mediators, such as histamine, participate not only as mediators of immediate hypersensitivity reactions but also function in important aspects of the entire inflammatory response. Whereas the role of BK-A in immediate hypersensitivity reactions and in other inflammatory processes is not yet clear, it does appear to represent an important interface between cell systems and the serum cascade systems. BK-A, in addition to generating kinins from human plasma kininogen (11), has also been shown to activate Hageman factor (33) and, by this route, may influence inflammation by generating other biologically active compounds. Through the activation of Hageman factor, BK-A is able to influence the coagulation sequence which is felt to participate in both subacute and chronic cell-mediated, inflammatory lesions (35). Thus, BK-A may be an important link between several types of inflammatory processes and, although its pharmacologic control is similar to that of histamine, differential release of the two mediators in different individuals may eventually prove to be of some importance in the pathogenesis of inflammation.

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