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Research Article

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Anaphylactic Release of a Basophil Kallikrein-like Activity

I. PURIFICATION AND CHARACTERIZATION

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ABSTRACT These studies describe the IgE-mediated release of a basophil kallikrein-like enzyme that is an arginine esterase and is inhibited by plasma, diisopropylphosphofluoridate, and Trasylol. The substrate specificity for the synthetic amino acid ester substrates *p*-toluenesulfonyl-L-arginine methyl ester, benzoyl-arginine methyl ester, and acetyl-tyrosine methyl ester is similar for the basophil enzyme and plasma kallikrein. The interaction of arginine esterase-active fractions from ion-exchange (DEAE-Sephacel) and gel filtration (Sepharose 6B) chromatography, with human plasma kininogen, generates immunoreactive kinin. The basophil arginine esterase and kinin-generating activities co-chromatograph on Sepharose 6B and the quantity of kinin generated is, in general, proportional to the arginine esterase activity of the column fractions, suggesting that these two activities are subserved by the same protease. The ability of this protease to generate kinin equally well from heat- and acid-treated plasma, as from fresh human plasma, suggests that this protease has kallikrein-like activity. These data suggest that kallikrein-like activity can be generated from human basophils as a direct result of a primary IgE-mediated immune reaction, thus providing a potential link between reactions of immediate hypersensitivity and the plasma and(or) tissue kinin-generating systems.

INTRODUCTION

Kallikrein is a generic term used for the designation of serine proteases capable of generating peptides called kinins from protein substrates (1). These proteases have been found in plasma (plasma kallikrein [2]), urine (urinary kallikrein [3]), as well as glandular tissues (glandular kallikrein [3, 4]). Engleman and Greenbaum (5) and Greenbaum et al. (6) have reported kallikrein-like activity from the rabbit polymorphonuclear leukocyte and human lymphocytes. In contrast to the former neutral proteases, the kinin-generating enzyme reported by Engleman and Greenbaum (5) is an acid protease. The neutral proteases (plasma, urinary, and glandular kallikreins) are variably inhibited by plasma, soybean trypsin inhibitor (SBTI),[1] diisopropylphosphofluoridate (DFP), and Trasylol (7–9).

Kinins have been implicated as mediators of inflammatory processes (10). Even though the cascade of reactions which generate bradykinin in plasma is well defined (10), little or no information exists regarding a mechanism by which kinins can be generated by immune processes. There have been reports of the immune release of a kallikrein-like kinin-generating factor from perfused, sensitized guinea pig lung (11, 12). This release process, however, was not calcium dependent, and more recent studies on this system suggest that the kinin-generating factor was not produced by the primary immune reaction, but, rather, as the result of a secondary event (13).

The present studies describe the IgE-mediated release of a basophil enzyme which is an arginine esterase (hydrolyses *p*-toluenesulfonyl-L-arginine methyl ester (TAMe), and is inhibited by plasma, DFP, and Trasylol. A protease which is generated by IgE-mediated mechanisms and co-chromatographs with the above arginine esterase activity, generates a kinin from human plasma kininogen (a basophil kallikrein-like activity). The data suggest that this kinin-generating protease and the basophil TAMe esterase activity are

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[1] *Abbreviations used in this paper*: BK-A, basophil kallikrein-like activity; DFP, diisopropylphosphofluoridate; HSA, human serum albumin; SBTI, soybean trypsin inhibitor; SDS, sodium dodecyl sulfate; TAMe, *p*-toluenesulfonyl-L-arginine methyl ester.

subserved by the same enzyme. Thus, kallikrein-like activity can be generated from human basophils as a result of a primary IgE-mediated immune reaction, providing a potential link between reactions of immediate hypersensitivity and the kinin-generating systems.

METHODS

Materials. The [^{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 (HSA) (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 (14). The following were purchased from Schwarz Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.: acetyl-tyrosine methyl ester; TAMe; benzoyl-arginine methyl ester; and Tris from which the buffer used in the [^{3}H]TAMe basophil arginine esterase assay was made (0.06 M), and adjusted to pH 8.0 at 25°C. SBTI, acetyl-lysine methyl ester, and dithioerythritol were purchased from the Sigma Chemical Co.; diatrizoate sodium (Hypaque) from Winthrop Laboratories, New York; L-alanyl-L-alanyl-L alanine methyl ester from Vega-Fox Biochemicals Div., Newbery Energy Corp., Tucson, Ariz.; and Sepharose 6B, preswollen DEAE-Sephacel, Sephadex G-200, and Ficoll from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J. Antigen E was kindly provided by Dr. T. P. King of The Rockefeller University, New York; anti-IgE by Dr. K. Ishizaka, The Johns Hopkins University, and the ionophore A23187 by Dr. R. Hamill, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.; and Trasylol (10,000 Kunitz inhibitor units/ml) by Dr. M. E. Webster, National Heart and Lung Institute of National Institutes of Health, Bethesda, Md. (purchased from Farbenfabricken Bayer AG, Leverkusen, Germany).

Leukocyte preparations. Human leukocytes from donors allergic to ragweed or grass and from 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 (14). 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 or 45 min in the dose-response studies, or for 90-min periods when generating large quantities of the basophil enzyme. At the completion of the reaction, the cells were centrifuged and the esterase released into the supernate as well as that present in an aliquot of untreated cells was determined by the radiochemical technique of Beaven et al. (15), as previously described (16). Experimental tubes were run in duplicate except in studies where standard deviations were obtained where quadruplicate determinations were made. Duplicates usually differed by <5%. 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. We have previously shown that spontaneous histamine release represents <2% of the total cellular histamine (14), and the spontaneous esterase release is at a similar level.

Arginine esterase activity. Arginine esterase activity of the supernate was determined by a radiochemical technique employing [^{3}H]TAMe (16) which was devised by Beaven et al. (15) for the measurement of human urinary kallikrein and modified for the determination of prekallikrein (17) and arginine esterase activity in supernate (18). The experimental tubes with leukocytes were run in duplicate, whereas 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.).

Definition of TAMe unit. A TAMe unit is defined as that quantity of the basophil enzyme which hydrolyses 1 pmol of the substrate [^{3}H]TAMe/min. The activity of a sample is expressed as units per milliliter of the sample, or picomoles per minute per milliliter of sample. The specific activity of the [^{3}H]TAMe used in these studies is such that cpm/10 μl of sample reported in this manuscript may be converted to units per milliliter by simply dividing counts per minute by 10,000.

Kinin-forming activity. The ability of basophil kallikrein-like activity (BK-A) purified by chromatography to generate immunoreactive kinin was tested using kininogen substrate prepared from normal human plasma by the method of Diniz and Carvalho (19). The plasma was prepared by drawing venous blood from normal donors into plastic tubes containing sodium citrate (1 vol of 3.8% sodium citrate/9 vol of blood), and centrifuging at 4°C for 20 min. 100-μl aliquots of Sepharose 6B or DEAE-Sephacel eluates, buffer, or trypsin (100 μg, Worthington Biochemical Corp., Freehold, N. J.) were incubated in duplicate for 20 min at 25°C in 200 μl of kininogen substrate in the presence of 3 mM 1,10-phenanthrolene, and the reaction was terminated by addition of 20 μl of 20% trichloroacetic acid. After addition of 1 ml of 0.1 N acetic acid, the entire reaction mixture was applied to 3-cm columns (in siliconized transfer pipets) of CG-50 gel (100–200 mesh) in 0.1 N acetic acid. After washing with 10 ml of 0.1 N acetic acid, the kinin bound to the columns was eluted with 5 ml of 50% acetic acid, concentrated by flash evaporation, and then quantitated by a kinin radioimmunoassay, as previously described (20).

Chromatography. Samples were applied to the various columns and the pattern developed with a peristaltic pump (model 2120, LKB Instruments, Inc., Rockville, Md.). The eluate was continuously monitored with an in line Uvicord III spectrophotometer (model 2089, LKB Instruments, Inc.) with absorbances at 206 and 280 nm. At the completion of the chromatographic studies, absorbance was again determined using a spectrophotometer (model 26, Beckman Instruments, Fullerton, Calif.).

RESULTS

Dose response. Dose-response curves of 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 (21) or grass (22) or with highly specific anti-IgE (23). A typical arginine esterase dose-response curve is shown in Fig. 1. The precision of the arginine esterase assay for quadruplicate determinations is ≅3% as is indicated by the standard deviations (Fig. 1).

Purification of the basophil arginine esterase. To generate large quantities of the basophil arginine esterase, leukocytes from 200 to 400 ml of blood were

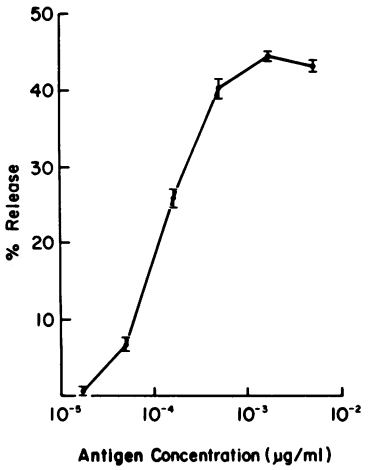

FIGURE 1 Dose-response of Rye group I antigen-induced release of BK-A. The precision of the [^{3}H]TAMe arginine esterase assay is indicated by the standard deviations.

challenged with either anti-IgE or ragweed antigen E. The supernates from these preparations were concentrated 10- to 20-fold by vacuum dialysis at 4°C using collodion bags no. 100 and stored at −70°C until used for chromatographic studies. The basophil enzyme obtained by challenge of leukocytes with anti-IgE was sequentially chromatographed on Sephadex G-200, DEAE-Sephacel, and Sepharose 6B on 12 occasions utilizing cells from seven donors. The recovery after chromatography on the three columns was variable, but averaged ≅20% (Table I).

Sephadex G-200. A 2.6 × 40-cm column was packed with Sephadex G-200 to a height of 35 cm, and equilibrated with 0.02 M PO_4 buffer, pH 6.8, at 4°C. 10–15 ml of concentrated supernate was applied to the column and the pattern developed by upward flow of 10 ml/h using a peristaltic pump. 80 3-ml fractions were collected and we determined arginine esterase activity and absorbance (280 nm).

Only one major esterase-active area was observed when concentrated supernate was applied to Sephadex G-200 (Fig. 2). This esterase-active area eluted with the first OD peak (the void volume). The esterase-active fractions from Sephadex G-200 were further purified by ion-exchange chromatography (DEAE-Sephacel).

DEAE-Sephacel chromatography. A 1.6 × 20-cm column was packed with Sephacel to a height of 10 cm, equilibrated with 0.02 M PO_4 buffer, pH 6.8, at 4°C. 40–60 ml of esterase-active eluate of the Sephadex G-200 was applied to the Sephacel column and the pattern developed by downward flow of 30 ml/h using a peristaltic pump. The column was washed with 200 ml of the equilibrating buffer and eluted with a linear salt gradient of 30 ml of equilibrating buffer and 30 ml of 0.02 M PO_4 buffer, pH 6.8, containing 0.50 M NaCl. A second-step salt gradient was developed with 30 ml of equilibrating buffer containing 0.50 M NaCl and 30 ml of 0.02 M PO_4, pH 6.8, containing 1 M NaCl. 30 2-ml fractions were collected from each NaCl gradient and we determined arginine esterase activity, conductivity (model 31, Yellow Springs Instrument Co., Yellow Springs, Ohio), and absorbance (280 nm). Only one esterase-active area was observed when Sephadex G-200 esterase-active fractions were eluted from DEAE-Sephacel (Fig. 3). This esterase-active area eluted with the first NaCl gradient, and coincided with an OD peak. A second OD peak eluted with the second gradient, however, it had no esterase activity. The esterase-active fractions eluted with a conductivity of 10 mmho, corresponding to a salt concentration of 0.1 M NaCl. The esterase-active fractions from DEAE-Sephacel were further purified by molecular sieve chromatography (Sepharose 6B).

Sepharose 6B. A 1.6 × 100-cm column of Sepharose 6B was equilibrated with 0.02 M PO_4 buffer, pH 6.8, at 4°C and calibrated with blue dextran, thyroglobulin, ferritin, catalase, and HSA. 6–8 ml of concentrated

TABLE I
*Partial Purification of a Leukocytic Protease**

	Protein	Esterase activity	sp act
	μg/ml	*cpm*	
Crude	3,600	31,595	8.8
Sephadex G-200	310	3,111	10
DEAE-Sephacel	810	5,423‡	6.7‡
Sepharose 6B	15	1,259	84

* 17% recovery after three columns.
‡ The TAMe esterase assay is influenced by high NaCl, hence values are actually higher.

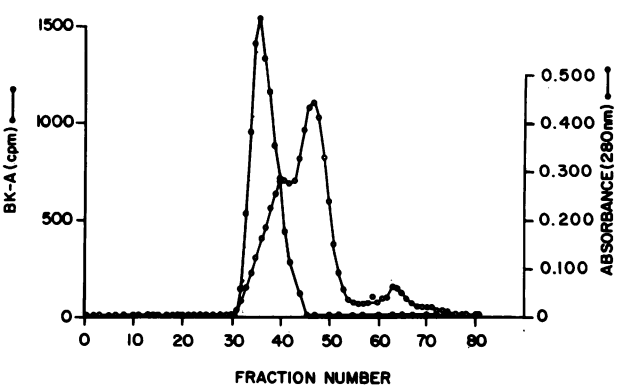

FIGURE 2 Sephadex G-200 chromatography of anti-IgE generated BK-A. The major arginine esterase-active peak eluted in the void volume and is mostly contained in the first OD peak.

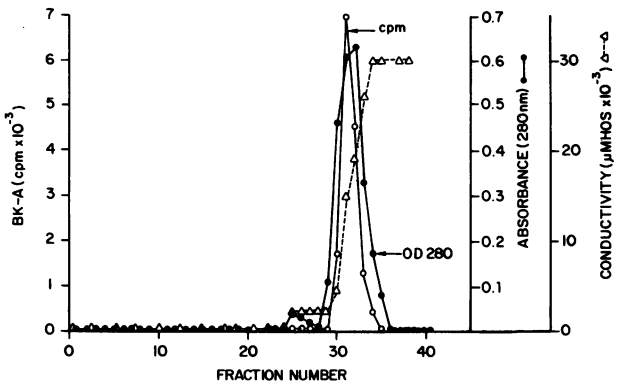

FIGURE 3 DEAE-Sephacel chromatography of the arginine esterase-active fractions from the void volume of Sephadex G-200 (Fig. 2). The arginine esterase-active peak coincides with the first OD peak after initiation of the NaCl gradient (0.5 M NaCl in limiting buffer). A second-step NaCl gradient (1.0 M NaCl in limiting buffer) eluted more protein which had no esterase activity.

esterase-active fractions from DEAE-Sephacel was applied to the column and the pattern developed by upward flow of 10 ml/h using a peristaltic pump. 100 3-ml fractions were collected and we determined arginine esterase activity and absorbance (280 nm).

One major esterase-active area was observed when concentrated samples from DEAE-Sephacel were applied to Sepharose 6B (Fig. 4). This esterase-active area coincided with the first OD peak and eluted with an estimated 1.2,000,000 mol wt. A second arginine esterase-active area is usually present, and is quantitatively of smaller magnitude and of smaller molecular weight (≅400,000). A third arginine esterase-active area is variably present, and is quantitatively of smaller magnitude than the 1.2,000,000 or the 400,000 forms. This third form also has the smallest molecular weight (<100,000). The use of dithioerythritol (24) in the supernates (1%) and in the eluting buffer (0.5%) did not affect the molecular weight of the BK-A. The esterase active fractions were used for further studies, including kinin generation and sodium dodecyl sulphate (SDS)-polyacrylamide disc gel electrophoresis.

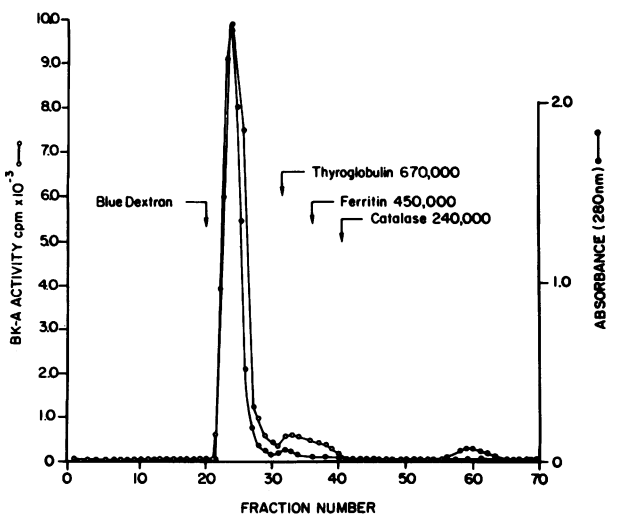

FIGURE 4 Sepharose 6B chromatography of the esterase-active peak from DEAE-Sephacel. Fraction volume is 3 ml.

Linear gradient polyacrylamide gel electrophoresis. Basophil enzyme from Sepharose 6B eluates with an estimated 1.2,000,000 mol wt were inactivated with DFP (10 mM final concentration) and subjected to a 2.5–27% linear gradient polyacrylamide gel (rods from Isolab, Inc., Akron, Ohio) electrophoresis using Tris-borate-EDTA buffer, pH 8.4, stained with 0.25% Coomassie Blue and destained by diffusion (25–27). Bovine serum albumin (68,000), HSA (69,000), bovine thyroglobulin (670,000), and human IgM (920,000 [N. L. Cappel Laboratories Inc., Cochransville, Pa.]) were included as molecular weight markers. Fig. 5A compares the electrophoretic pattern of the enzyme (gel 2 containing 50 μg in 50 μl) with human IgM (gel 1) and HSA (gel 3). The molecular weight of the enzyme was estimated to be 950,000±70,000 (mean±SD) in four runs. Analyses of overloaded gels containing up to 200 μg of the enzyme protein (in 200 μl) failed to reveal low molecular weight proteins.

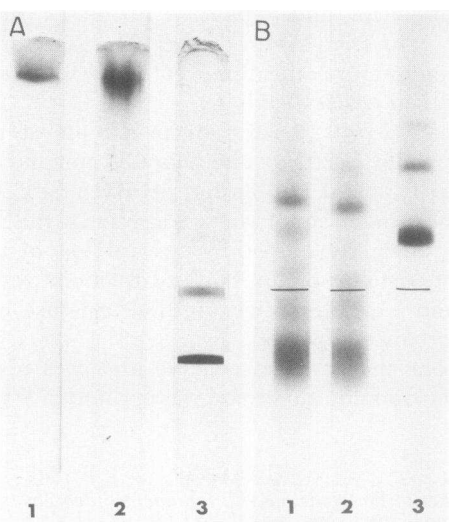

FIGURE 5 (A) 2.5–27% linear gradient polyacrylamide gel electrophoresis. Gel 1 contains human IgM; gel 2 contains 50 μg (in 50 μl) of BK-A inactivated with DFP; and gel 3 shows the monomer (lower band), and probably aggregated (upper band) HSA. Migration is downward toward the anode. (B) 3.3% polyacrylamide disc gel electrophoresis containing SDS. Gel 1 contains DFP-inactivated BK-A, reduced; gel 2 contains DFP inactivated BK-A, unreduced; gel 3 shows the monomer (lower band), and probably aggregated (upper bands) HSA, reduced. All samples were denatured at 100°C × 1 min. Migration is downward toward the anode. A wire marks the position of the tracking dye.

SDS-polyacrylamide disc gel electrophoresis. SDS-polyacrylamide (3.3%) disc gel electrophoresis was performed according to the method of Weber et al. (25). Gels were cast in 5.5-mm (ID) × 12.5-cm siliconized tubes to a height of 10 cm. Urea was not added to samples. Basophil enzyme from Sepharose 6B eluates with an estimated 1.2,000,000 mol wt was inactivated with DFP (10 mM final concentration), and denatured with 2% SDS and 2% dithiotheitol (if reduced) at 100°C × 1 min or 37°C × 2 h. Gels were stained with Coomassie Blue, destained by diffusion, and visually inspected. Each run contained the following standards: RNA polymerase (39,000; 155,000; 165,000), bovine serum albumin (68,000), bovine thyroglobulin (670,000), human IgM (920,000), as well as a concentrated buffer containing HSA (69,000). As shown in Fig. 5B, both reduced (gel 1) and unreduced (gel 2) samples of DFP inactivated basophil enzyme subjected to 100° and 37°C denaturing conditions yielded bands with the following molecular weight (mean±SD, estimated relative intensity): 16,000±0, dark; 30,500±700, faint; 46,000 ±1,400, faint; 90,000±700, dark. In addition to these bands, unreduced samples of BK-A (gel 2 [and possibly reduced samples, gel 1]) exhibited a faint band with a 135,000 mol wt which moved to a 66,000 mol wt upon reduction (gel 1). The reduced, concentrated buffer (in which the leukocytes were challenged) containing HSA is shown in gel 3. Analysis of basophil enzyme samples which had not been inactivated with DFP usually resulted in a diffuse staining of the bottom two-thirds of the gel (low molecular weight proteins).

Kinin generation by the basophil arginine esterase. The interaction of the basophil arginine esterase with the plasma kinin-generating system was studied. In these experiments, the esterase-active chromatographic fractions from antigen or anti-IgE challenged cells, or buffer, were incubated with a kininogen preparation in duplicate and the kinin generated was assayed in quadruplicate by radioimmunoassay (20). Fig. 6 shows that esterase-active fractions from Sepharose 6B generated kinin, and that the quantity of kinin generated is in general proportional to the esterase activity of the column fractions. Because the kinin radioimmunoassay is extremely time consuming, not all fractions were assayed; rather, we assayed those fractions with TAMe esterase activity. The data in Table II suggest that, for equivalent arginine esterase activity, the basophil enzyme is as active as human plasma kallikrein in the proteolytic cleavage of human plasma kininogen. Purified human plasma kallikrein (kindly provided by Dr. John Griffin and Dr. Charles Cochrane, Scripps Research Foundation, La Jolla, Calif.) and basophil enzyme (sequentially purified on Sephadex G-200, DEAE-Sephacel, and Sepharose 6B) were incubated with human plasma kininogen. For equivalent arginine esterase activity (0.4 TAMe units) plasma kallikrein generated 110 whereas the basophil enzyme generated 213 ng of kinin/ml of substrate. 100 μg of trypsin was used as a positive control, and it generated 250 ng of kinin. The basophil enzyme is also active in the proteolytic cleavage of Hageman factor (28) where, for equivalent arginine esterase activity, the basophil enzyme is 16-fold more active than plasma kallikrein in cleaving Hageman factor.[2] Studies to determine the kinetics of kinin generation with purified high and low molecular weight kininogens (29) are currently ongoing.

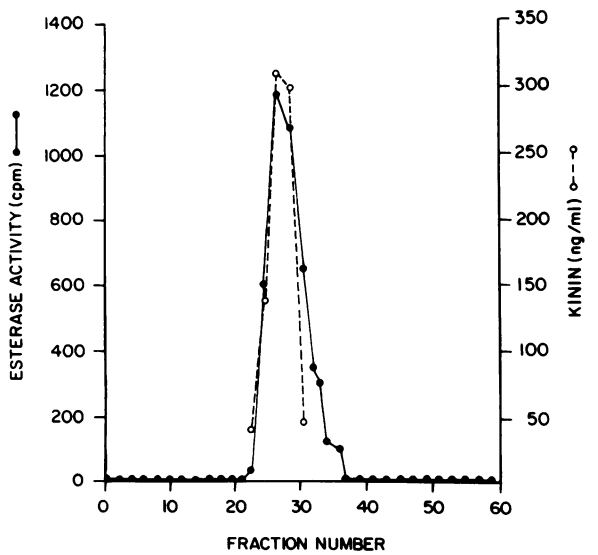

FIGURE 6 Arginine esterase activity and kinin generation by anti-IgE generated supernate chromatographed on Sepharose 6B (cpm × 10^{-1}).

TABLE II

Kinin Generation by Purified Human Plasma Kallikrein (0.4 TAMe units), BK-A (0.4 TAMe units), and Trypsin

Protease	Kinin generated
	ng/ml substrate
Plasma kallikrein	110
BK-A	213
Trypsin (100 μg)	250

Inhibitors of the arginine esterase. In the following experiments, we studied the inhibitory capacity of certain agents on the arginine esterase activity of the basophil enzyme. The pharmacological effects of the agents were determined by adding aliquots of a constant amount of basophil enzyme to a series of test tubes containing variable concentrations of the agents. The percent inhibition resulting from each agent was determined from the equation: percent inhibition = (C-E)/

[2] Newball, H. H., S. Revak, and C. Cochrane. Unpublished observations.

C × 100, where C and E represent the esterase activity of the basophil enzyme in the control (C) and experimental (E) tubes, respectively. The arginine esterase activity of the basophil enzyme was minimally inhibited by SBTI (11% at 0.1 mM). Unlike its inhibition of plasma kallikrein (8, 30), SBTI only poorly inhibits the basophil enzyme. With respect to its inhibition by SBTI, the basophil esterase is unlike the kallikreins of sweat glands, intestinal wall, brain, and carcinoid tumors; but similar to the kallikrein of urine which is unaffected by SBTI (8). In three studies, plasma consistently inhibited the arginine esterase activity of the basophil enzyme (Table III). Trasylol and DFP, likewise, inhibit the basophil arginine esterase activity (Figs. 7 and 8). As with other kallikrein, Trasylol does not completely inhibit the basophil esterase activity even at concentrations of 1,000 Kunitz inhibitor units/ml. This may be explained by the relatively high dissociation constant of the kallikrein-inhibitor complex (8).

Substrate specificity. The studies portrayed in Fig. 9 show that the pattern of substrate specificity of the basophil esterase for synthetic amino acid ester substrates is generally similar to that reported for plasma kallikrein (30). In our studies, we looked at the competitive inhibition of "cold" TAMe, benzoyl-arginine methyl ester, acetyl-lysine methyl ester, acetyl-tyrosine methyl ester, and L-alanyl-L-alanyl-L-alanine methyl ester on the hydrolysis of [^{3}H]TAMe by the basophil arginine esterase. The inhibition pattern was similar for TAMe, benzoyl-arginine methyl ester, and acetyl-tyrosine methyl ester (Fig. 9).

Temperature inactivation. Fig. 10 shows that the basophil enzyme is totally inactivated when incubated at 61°C for 30 min. Incubation of the enzyme for 30 min at 4° and 25°C led to no loss of arginine esterase activity, whereas incubation for 30 min at 56° and 61°C led to 85 and 98% inactivation, respectively.

pH optimum. Arginine esterase activity, reflecting the hydrolysis of [^{3}H]TAMe by the basophil enzyme was optimum at pH 8.5 (Fig. 11). Nonenzymatic hydrolysis of the substrate [^{3}H]TAMe was increased above pH 8.0. The signal to noise ratio, that is, the enzymatic to nonenzymatic hydrolysis ratio, was optimal at pH 8.5. Hence, pH 8.5 is now being used for all assay procedures.

TABLE III
Effect of Human Plasma on Esterolytic Activity of BK-A

	Esterolytic activity
	net cpm
BK-A (diluted one-half)	1,538
Plasma (diluted one-half)	415
BK-A plus plasma	431

When equal volumes of BK-A and plasma were mixed (equivalent to a one-half dilution of each), the counts per minute were equivalent to plasma diluted one-half.

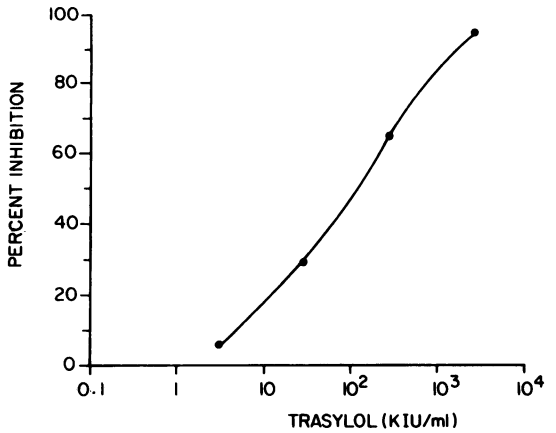

FIGURE 7 Inhibition of arginine esterase activity of BK-A by Trasylol. 50% inhibition was obtained with 100 Kunitz inhibitor units (KIU)/ml.

DISCUSSION

We earlier reported the IgE-mediated release of a TAMe esterase from anti-IgE stimulated peripheral human leukocytes. The supernates from anti-IgE stimulated peripheral leukocytes also had kinin-generating activity (31). The purpose of these studies was to attempt to purify and characterize the basophil TAMe esterase and kinin-generating activities, and determine whether the two activities were subserved by the same protease. It is reasonable to assume that the kinin-generating protease and the basophil arginine esterase activity might be subserved by the same protease, since, like all known kallikreins (7, 8), the basophil enzyme activity is an arginine esterase, and, like many kallikreins, it is inhibited by plasma, DFP, and Trasylol

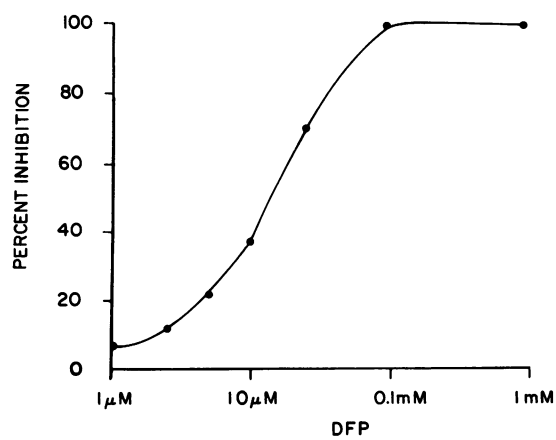

FIGURE 8 Inhibition of arginine esterase activity of BK-A by DFP. 50% inhibition was obtained with 40 μM.

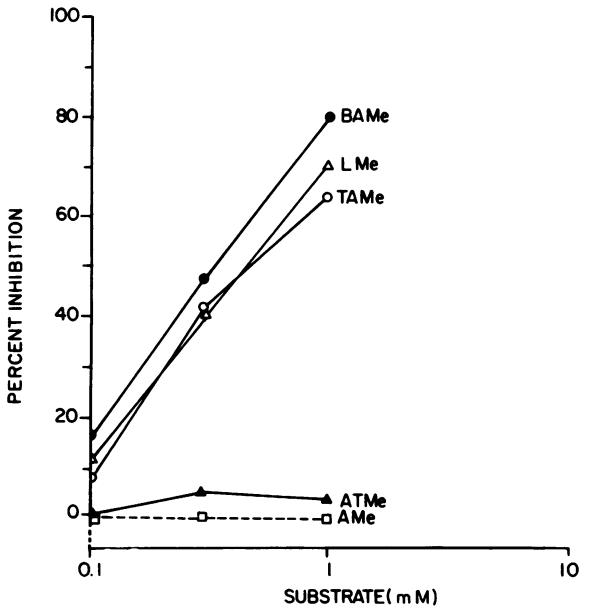

FIGURE 9 Substrate specificity of the BK-A for synthetic amino acid esters. TAMe, benzoyl-arginine methyl ester (BAMe), and acetyl-lysine methyl ester (LMe) were good substrates, whereas acetyl-tyrosine methyl ester (ATMe) and L-alanyl-L-alanyl-L-alanine methyl ester (AMe) were poor substrates for BK-A.

(Figs. 7 and 8, Table III). Moreover, the IgE-generated basophil TAMe esterase and kinin-generating activities co-chromatograph on Sepharose 6B (Fig. 6). Furthermore, the TAMe esterase-active fractions from Sepharose 6B and DEAE-Sephacel generate kinin and the quantity of kinin generated is proportional to the TAMe esterase activity of the column fractions. The data support the hypothesis that the basophil-derived arginine esterase activity and the basophil-derived kinin-generating activity are subserved by the same protease, however, further work will be required to establish their identity.

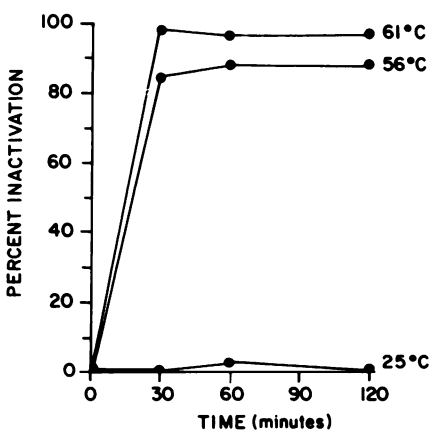

FIGURE 10 Effect of time and temperature on the inactivation of BK-A. At 61°C, BK-A it totally inactivated in 30 min. After a 30-min incubation at 25°C, BK-A loses no arginine esterase activity. After a 30-min incubation at 56°C or 61°C, BK-A loses 85 and 98% esterase activity, respectively.

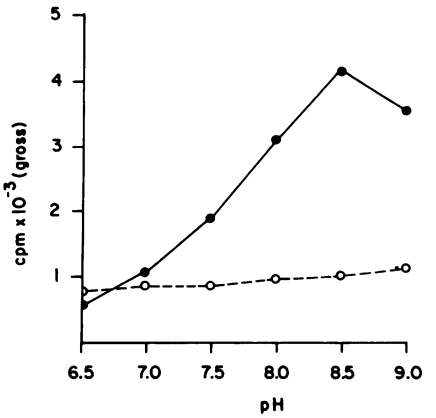

FIGURE 11 Determination of pH optimum for the hydrolysis of [^{3}H]TAMe by BK-A. Values obtained without enzyme are indicated by the dashed line. The solid line represents the net hydrolysis at each point (enzymatic minus nonenzymatic activity).

To purify the basophil enzyme, leukocytes from peripheral blood were challenged with anti-IgE and the supernate was sequentially chromatographed on Sephadex G-200, DEAE-Sephacel, and Sepharose 6B. During attempts to bind the basophil enzyme to concanavalin A-Sepharose, the esterolytic activity consistently flushed through, although some nonesterase glycoproteins were bound to the column. Fig. 2 illustrates the elution profile of the basophil enzyme on Sephadex G-200. A major arginine esterase-active area is observed, which elutes in the void volume. Chromatography on Sephadex G-200 accomplished two purposes: (*a*) the basophil enzyme was desalted for subsequent chromatography on DEAE-Sephacel, and (*b*) the enzyme was minimally purified—it eluted in the void volume, thus allowing separation from smaller molecular weight proteins. The arginine esterase-active fractions from the void volume of Sephadex G-200 were further purified on DEAE-Sephacel. As shown in Fig. 3, only one arginine esterase-active area is eluted from DEAE-Sephacel with the first NaCl gradient. The second-step NaCl gradient eluted more protein which had no esterase activity. The esterase-active fractions from DEAE-Sephacel were chromatographed on Sepharose 6B. The major esterase activity eluted with an estimated 1.2,000,000 mol wt (Fig. 4). A second esterase-active area was present, but quantitatively of smaller magnitude and molecular weight (≅400,000). A third esterase-active area was variably present, and had a molecular weight of <100,000. The relationship of one form of esterase activity to the other is not clearly understood. The data suggest that either dif-

ferent enzymes elute from the Sepharose 6B in the regions of 1.2,000,000, 400,000, and 80,000 or, alternatively, there may be three forms of a single enzyme.

The basophil enzyme generates a kinin(s) from human plasma kininogen. Plasma kininogen may not, however, be the natural substrate of the basophil enzyme. There are more than one type of kininogen substrate, i.e., low and high molecular weight kininogens (29). There may be another kininogen, i.e. a tissue kininogen that may be a better substrate for the basophil enzyme. The data in Table II suggest that, for equivalent arginine esterase activity, the basophil enzyme is at least as active as plasma kallikrein in the proteolytic cleavage of human plasma kininogen. The continuous elaboration of the basophil enzyme over a prolonged IgE-mediated event could conceivably lead to the generation of sufficient quantities of kinins that would be important in the pathogenesis of inflammation. Studies are in progress to determine the nature of the kinin being generated. The antibody used in the kinin radioimmunoassay is able to detect all kinins containing the bradykinin nonapeptide sequence, including Lys-bradykinin, Met-Lys-bradykinin and the pachykinin Gly-Arg-Met-Lys-bradykinin (32).

Engleman and Greenbaum (5) and Greenbaum et al. (6) have reported that kallikrein-like enzymes are found in leukocytes. These enzymes are acid proteases and cleave leukokinins from a protein substrate known as leukokininogen. Leukokinins are polypeptides having 20–25 amino acids, and are distinct from the nonapeptide bradykinin. Moreover, leukokinins do not contain the bradykinin sequence in their molecule and would not be detected by our radioimmunoassay. The substrate from which leukokinins are cleaved (leukokininogen) is not a normal constituent of plasma (6).

The kinin-forming activity of human lymphocytes has been studied by Engleman and Greenbaum (5). After incubation of whole cell lysates or subcellular fractions for 3 or 15 h at pH 4 with purified human kininogen, a kinin-like material was found in the whole cell lysates of lymphocytes, measured by bioassay on the rat uterus. It was found that this kinin had a relatively greater potency in the bioassay, compared to native bradykinin. Half of the enzymatic activity was found in the heaviest cell fraction (250 g), whereas 25% of the total activity was found in the 15,000 g pellet and the 15,000 g supernate. Inhibition of this enzymatic activity could be achieved only with STI, and not with a variety of other agents, including Trasylol, which is an inhibitor of the basophil enzyme (Fig. 7). The results suggest that, at low pH, lymphocytes may release kinin-forming activity. It is clear, however, that the lymphocyte enzyme is different from the basophil enzyme herein reported.

Movat et al. (33) have described a neutral protease released by polymorphonuclear leukocytes after their interaction with antigen-antibody complexes or IgG-coated latex particles. This protease has alanine esterase activity in contrast to the basophil protease (Fig. 9) and recent evidence suggests that the polymorphonuclear leukocyte protease is an elastase.[3] Wintroub et al. (34) have described a neutrophil-dependent pathway for the generation of a neutral peptide which is cleaved from a plasma protein substrate by an α-1-antitrypsin-inhibitable serine protease. This peptide is distinguished from the kinin peptides by a neutral isoelectric point, susceptibility to inactivation by trypsin, as well as chymotrypsin, and activity on the isolated, atropinized, and antihistamine-treated guinea pig ileum with relatively little action on the estrous rat uterus.

This report indicates that kallikrein-like activity can be generated from human basophils as a result of a primary immune reaction. The basophil enzyme may generate kinins directly by the proteolytic cleavage of human plasma kininogen. The basophil enzyme may also generate kinins indirectly through the activation of human Hageman factor (28). Thus, this kinin-generating activity which we have described appears to be unique, in that it is the first recognized mechanism whereby IgE-mediated immune reactions may directly activate the kallikrein-kinin system. Kinins are believed to mediate a variety of allergic and inflammatory conditions. The demonstration of the IgE-mediated release of a basophil kallikrein-like enzyme allows us to begin the study of the role of this system in the pathogenesis of allergic and inflammatory processes.

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