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Hereditary angioneurotic oedema: characterization of plasma kinin and vascular permeability-enhancing activities

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SUMMARY

The mediator(s) responsible for localized enhanced vascular permeability that characterizes an exacerbation of hereditary angioneurotic oedema (HAE) is thought to be a product of either contact or complement system activation. In contrast to normal individuals, plasma from these patients generates both kinin and vascular permeability-enhancing activity following incubation at 37°C. Depletion of C1 inhibitor in both normal and C2-deficient plasma, but not in contact factor-deficient plasmas, resulted in generation of these activities. The kinin activity from incubated HAE plasma was susceptible to kininase inactivation and was blocked by a Bk_2 receptor antagonist. Furthermore, this activity was isolated from HAE plasma; amino acid sequence analysis proved it to be bradykinin. Similarly, the vasopermeability-enhancing activity from ethanol-fractionated or boiled HAE plasma, collected during either attack or remission, co-eluted with bradykinin on reverse-phase high performance liquid chromatography (HPLC). These studies conclusively demonstrate that bradykinin is the major kinin and mediator of enhanced vascular permeability generated during incubation of HAE plasma. The role of other bioactive products, such as the C2 kinin, at local sites of oedema formation remains to be further defined.

Keywords bradykinin angioedema C1 inhibitor classical complement pathway kallikrein-kinin system

INTRODUCTION

The biochemical process responsible for the potentially life-threatening episodes of painless, circumscribed oedema that occur in patients with either acquired or hereditary angioneurotic oedema (HAE) is incompletely understood. HAE is inherited as an autosomal dominant trait resulting from heterozygous deficiency in the activity of C1 inhibitor (C1 INH), the only plasma protease inhibitor known to regulate C1, and a major inhibitor of the proteases involved in contact system activation. In addition, C1 INH may have a minor role in the regulation of the plasma coagulation and fibrinolytic systems.

The dual regulatory roles of C1 INH for both the contact and the classical complement systems suggested that mediation of the oedema is probably due to an activation product of either or both of these proteolytic cascades. Indeed, both vasopermeability and kinin (oestrus rat uterus contracting) activities may result from activation of either system. Evidence for contact system involvement was suggested by studies that demonstrated its activation in plasma during attacks of angioedema [1–4] and in the fluid of suction blisters induced in HAE patients [5]. *In vitro* experimental evidence that suggested bradykinin generation during incubation (at 37°C) of remission HAE plasma supports this conclusion [6–9].

The observation that intradermal injection of active C1s induced a vasopermeability response provided evidence that the classical complement pathway might be important in the mediation of angioedema [10]. Homozygous C2-deficient individuals demonstrated a reduced permeability response to intradermal injection of purified proteolytically active C1s, while C3-deficient individuals revealed a normal response [11,12]. Guinea pigs with hereditary deficiency of C2 or C4 also revealed a reduced permeability response to intradermal C1s [13–15] which, in the C2-deficient animals, could be reconstituted by infusion of purified C2 to a concentration approximately 36% of normal [15]. These data further supported a role for the classical complement pathway, and suggested a direct requirement for C2 in generation of the vascular permeability response.

In 1969, Donaldson *et al.* [16] partially characterized a permeability-enhancing activity in incubated remission HAE plasma. This material behaved as a kinin, yet was biochemically distinct from bradykinin. Subsequent experiments demon-

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strated a requirement for C1s, C2, and plasmin for *in vitro* generation of this activity [13]. Plasmin digestion of C1s-cleaved C2 was shown to occur largely at the carboxy-terminus of C2b [17]. Synthetic peptides corresponding to those derived from potential plasmin cleavage sites in this region were found to have both kinin and vascular permeability-enhancing activity in both guinea pig and human skin [16]. Others, however, have been unable to generate kinin activity by incubation of purified C2 with C1s and either plasmin, kallikrein, or trypsin [9].

We report the results of experiments designed to identify individually the kinin and vasopermeability-inducing substances generated during incubation of HAE plasma. These studies implicate bradykinin as the sole mediator of kinin activity in HAE plasma incubated *in vitro*. In addition, bradykinin is the primary vascular permeability-enhancing mediator generated in plasma *in vitro* when measured using the guinea pig skin vasopermeability model.

MATERIALS AND METHODS

Reagents and proteins

Reagents were purchased from the following sources: bradykinin and D-Arg-(Hyp^3, Thi^{5,8}, D-Phe^7)-Bk from Calbiochem (La Jolla, CA); porcine pancreas carboxypeptidase B (EC 3.4.15.1), rabbit lung angiotensin converting enzyme (ACE) (EC 3.4.17.2), trifluoroacetic acid, and Evans blue dye from Sigma Chemical Co. (St Louis, MO); acetonitrile from Fisher Scientific (Pittsburgh, PA); triprolidine HCl from Borroughs-Wellcome and Co. (Research Triangle Park, NC); diethylstilbestrol from Miles Pharmaceuticals (West Haven, CT); and IgG from polyclonal goat anti-human-C1 INH, C3, albumin, and IgM, IgG and IgA from Incstar (Stillwater, MN). C1s, purified as previously described [18], was a gift from Dr David Bing, Centre for Blood Research (Boston, MA). C1 INH was isolated as described [19].

Plasma preparations

Platelet-poor plasmas in 10 mM Na EDTA were obtained from HAE patients during remission and from normal controls. Plasma deficient in prekallikrein, both high and low molecular weight kininogen (HK and LK), and Hageman factor (factor XII) were purchased from George King Bio-Medical, Inc. (Overland Park, KS). Each had normal C2 concentrations as determined by radial immunodiffusion. Na EDTA was added to these citrated plasmas to obtain a final concentration of 10 mM EDTA. C2-deficient EDTA plasma was obtained from two different individuals with homozygous C2-deficiency, one of which was a gift from Dr Chester Alper (Centre for Blood Research, Boston, MA). The absence of C2 antigen was confirmed by radial immunodiffusion. Plasmas were kept frozen at −70°C in polypropylene tubes and thawed immediately before use.

Immunodepletion and C1s consumption of C1 INH

IgG from polyclonal goat antiserum against human C1 INH, C3, albumin, or immunoglobulin was added to normal, C2-deficient, and contact system-deficient plasma, or to combinations of contact system-deficient plasmas (equal volumes of each deficient plasma). The antibodies to C3, albumin and IgG were used as controls to show that no kinin or vascular permeability-enhancing factor was generated by the immunodepletion itself. Saline was added to yield an antibody:plasma:saline ratio of 2:3:2. The resulting mixture was incubated at 4°C for 4 h followed by 37°C for 1 h. Each sample was promptly tested for kinin activity.

Purified C1s (14 μg in 5 μl) or saline (5 μl) was added to 100 μl of normal, C2-deficient or contact system-deficient plasmas, as well as mixtures (50:50 v/v) of the contact system-deficient plasmas before incubation at 37°C for 60–180 min. Afterward, the reaction was terminated by addition of purified C1 INH (50 μg in 5 μl) to each sample. Samples were tested for kinin activity, and they were diluted from 1:20 to 1:40 with PBS before intradermal injection for measurement of vasopermeability-enhancing activity.

Purification of kinin and vasopermeability-enhancing activities

Plasma obtained from HAE patients in remission was first incubated at 37°C for 60 min or 180 min. The plasma was cooled and ethanol was added to a final concentration of 67%. Following centrifugation, the supernatant was removed, lyophilized, and fractionated by reverse-phase high performance liquid chromatography (HPLC). The lyophilized supernatant was dissolved in water and applied to a C18 column (Waters Delta Pak C18-300A) in 0·1% trifluoroacetic acid, and was developed with a linear acetonitrile gradient from 4% to 80%. Collected fractions were lyophilized and re-dissolved in 0·02 M Na/K phosphate, 0·15 M NaCl, pH 7·0, before testing for kinin and vasopermeability-enhancing activities.

Induction of oestrus rat uterus contraction

Kinin activity was measured using an assay originally developed for the assay of bradykinin. The elapsed time was determined following injection of the test substance before contraction of a uterine horn suspended in an organ bath in aerated de Jalon's solution [20] (9·0 g NaCl, 0·4 g KCl, 0·03 g $CaCl_2$, 0·15 g $NaHCO_3$ and 1·0 g glucose in 1·0 *l* distilled water) at 31°C. The uterine horn was resected from a 200–250 g virgin Sprague–Dawley rat with oestrus induced by i.p. injection of 10 μg/100 g body weight of diethylstilbestrol approximately 48 h earlier. Muscle contractions were recorded on a kymograph by suspending the free end of the uterine horn to a lever, without opposing tension, that was connected to a differential transformer. The dose of each test substance, before dilution in the organ bath, was recorded. In experiments involving the Bk_2 antagonist, this compound was injected 30 s before the test substance. A standard curve for bradykinin was determined several times during each experiment with each uterine horn, and each muscle was sensitive to a bradykinin concentration of 0·2 nM or less.

Unless otherwise stated, all preparations were incubated at 37°C for 1 h in polypropylene tubes before testing for kinin activity. De Jalon's solution was added during incubation of either ACE or carboxypeptidase B with the plasmas or with bradykinin, since these enzymes require divalent cations for activity. Coagulation was not observed in these plasma samples during the experiments.

Sequence identification of kinin activity

The lyophilized ethanol supernatant from approximately 12 ml of incubated, remission HAE plasma was subjected to reverse-phase HPLC separation. Preliminary experiments had established that kinin activity eluted during a single 2-min period

from approximately 25 min to 27 min. The peaks within this interval were collected individually, lyophilized, and a fraction of each peak was tested for kinin activity. The active peak, which eluted at a reproducible time, was lyophilized, and subjected to amino terminal amino acid sequence analysis (performed by R. A. Harrison, MRC Centre, Cambridge, UK).

Vasopermeability of guinea pig skin

Female albino guinea pigs, weighing 300–450 g, were shaved over their sides and back, initially with electric clippers followed by an electric rotary-blade face razor. This was preceded by an i.p. injection of a sedative cocktail (ketamine 5 mg, xylazine 0·5 mg, acepromazine 0·1 mg per 100 g body weight) and was followed by an i.p. injection of 0·45% saline, 1 ml per 100 g body weight, to ensure adequate hydration. Approximately 3 h later the effect of the sedative was no longer present, and each animal received 0·25 ml per 100 g body weight of a sterile 3% solution of Evan's blue dye in 0·45% saline via subclavian venipuncture. After 15 min, 0·1 ml of each test sample was injected intradermally over the back and sides of each animal. In preliminary experiments, triprolidine was added to the Evan's blue solution to a concentration of 0·4 mg/ml, to provide a dose of 0·1 mg per 100 g body weight to each animal. No difference in vasopermeability response was observed in its presence or absence. The animals were killed approximately 30 min following the last i.d. injection by intracardiac injection of a lethal dose of barbiturate. The vasopermeability activity of each test substance was immediately determined by measuring the largest diameter of the corresponding blued skin lesion and its perpendicular, determining the mean of these values, and calculating the area of dye extravasation.

The vasopermeability response was normalized for each guinea pig by dividing the mean area of blueing from each C1s-incubated mixture by the mean area of blueing from the C1s-incubated mixture of factor XII-deficient and prekallikrein-deficient plasmas in that same animal. Before calculation of these ratios, the area of blueing resulting from each control sample incubated in the absence of C1s was subtracted from the area of blueing induced by test samples. This plasma mixture was used as the positive control in order to standardize the amount of citrate and EDTA chelation among the samples tested, and because it provided the most consistent quantity of kininogen among the plasma mixtures. Each sample was tested in at least four guinea pigs.

RESULTS

Kinin activity of normal, HAE, and ethanol fractionated plasmas

Kinin activity was consistently present in incubated HAE plasma and absent in incubated normal plasma (Fig 1a). Unincubated HAE plasma occasionally revealed trace amounts of activity. Repeated freezing and thawing of the plasmas sometimes generated minute amounts of kinin activity, and hence was avoided. Following fractionation of plasma by ethanol precipitation, most of the kinin activity remained in the supernatant (Fig. 1b). Ethanol precipitation itself did not generate activity in normal plasma. This procedure also removed plasma kininase activity, since the lyophilized ethanol supernatant dissolved in solutions containing divalent cations did not lose activity (see below).

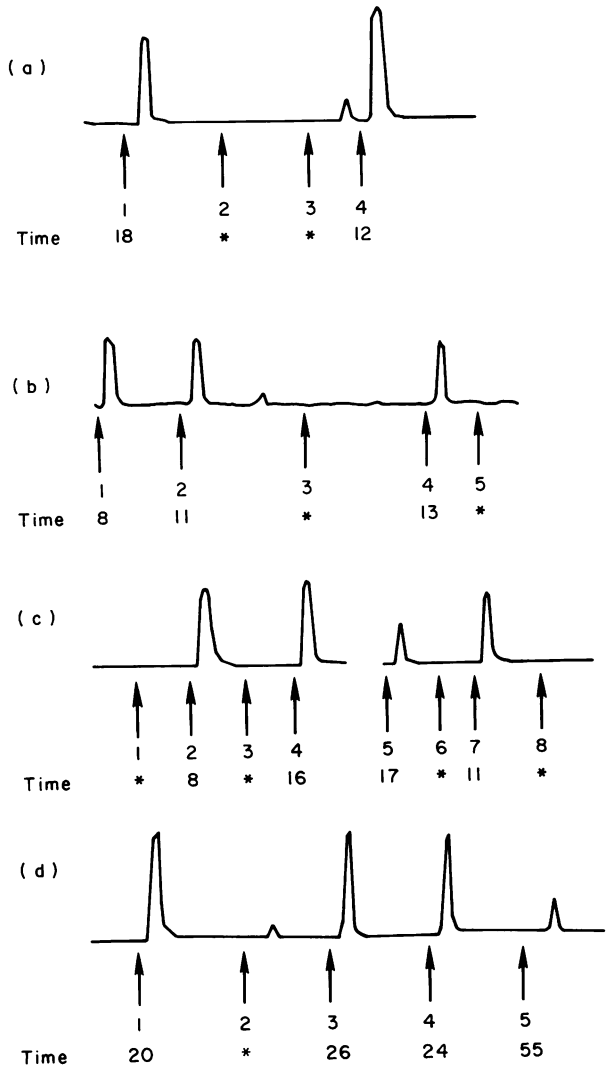

Fig. 1. Rat uterine contraction in response to hereditary angioneurotic oedema (HAE) plasma and ethanol fractionated HAE plasma. The response time (in seconds) is as indicated. *A response time of greater than 60 s, which was interpreted as an absence of kinin activity. The arrows indicate injections of test substances: a, 1, bradykinin (5 ng); 2, normal plasma incubated 60 min at 37°C (30 μl); 3, HAE plasma, unincubated (30 μl); 4, HAE plasma incubated 60 min at 37°C (30 μl); b, 1, bradykinin (5 ng); 2, HAE plasma incubated 60 min at 37°C (10 μl); 3, normal plasma incubated 60 min at 37°C (10 μl); 4, ethanol supernatant of the incubated HAE plasma shown in (2) (40 μl); 5, ethanol supernatant of the incubated normal plasma shown in (3) (40 μl); c, 1, bradykinin (10 ng) incubated with angiotensin converting enzyme (0·002 U) in de Jalon's solution; 2, bradykinin (4 ng); 3, HAE plasma (15 μl) incubated with angiotensin converting enzyme (ACE) (0·002 U) in de Jalon's solution (15 μl); 4, HAE plasma incubated 60 min at 37°C (20 μl); 5, bradykinin (5 ng); 6, bradykinin (5 ng) incubated with carboxypeptidase B (60 μg) in de Jalon's solution (20 μl); 7, HAE plasma (20 μl) incubated 60 min at 37°C; 8, HAE plasma (20 μl) incubated with carboxypeptidase B (60 μg) in de Jalon's solution (20 μl); d, inhibition of kinin activity with the bradykinin antagonist. The antagonist was injected 30 s before the test substance. 1, bradykinin (5 ng); 2, bradykinin (5 ng) and D-Arg-(Hyp^3, Thi^{5,8}, d-Phe^7)-Bk (200 ng); 3, bradykinin (5 ng); 4, HAE plasma (15 μl) incubated 60 min at 37°C; 5, HAE plasma (15 μl) incubated 60 min at 37°C and D-Arg-(Hyp^3, Thi^{5,8},d-Phe^7)-Bk (200 ng).

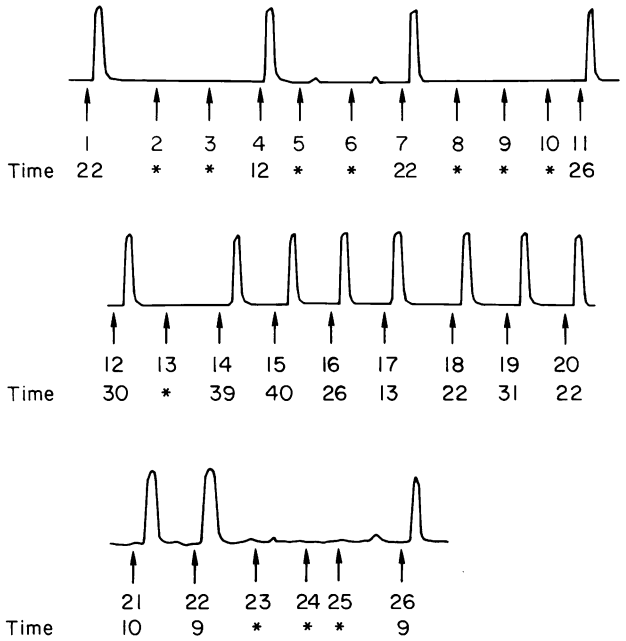

Fig. 2. Generation of kinin activity in plasma depleted of C1 inhibitor (C1 INH). Conditions and symbols are as described in Fig. 1. Each plasma sample was incubated with antibody, C1s or buffer for 60 min at 37°C. 1, normal plasma (15 μl)+anti-C1 INH; 2, normal plasma (15 μl)+anti-IgG, -IgA, -IgM; 3, normal plasma (15 μl)+anti-C3; 4, bradykinin (3 ng); 5, C2-deficient plasma (15 μl)+anti-C3; 6, C2-deficient plasma (15 μl)+anti-IgG, -IgA, -IgM; 7, C2-deficient plasma (15 μl)+anti-C1 INH; 8, high molecular weight kininogen (HK)- and low molecular weight kininogen (LK)-deficient plasma (15 μl)+anti-C1 INH; 9, prekallikrein-deficient plasma (15 μl)+anti-C1 INH; 10, factor XII-deficient plasma (15 μl)+anti-C1 INH; 11, bradykinin (1 ng); 12, bradykinin (1 ng); 13, prekallikrein-deficient plasma (7·5 μl)+HK- and LK-deficient plasma (7·5 μl)+anti-C1 INH; 14, HK- and LK-deficient plasma (7·5 μl)+factor XII-deficient plasma (7·5 μl)+anti-C1 INH; 15, prekallikrein-deficient plasma (7·5 μl)+factor XII-deficient plasma (7·5 μl)+anti-C1 INH; 16, C2-deficient plasma (7·5 μl)+HK- and LK-deficient plasma (7·5 μl)+anti-C1 INH; 17, C2-deficient plasma (7·5 μl)+factor XII-deficient plasma (7·5 μl)+anti-C1 INH; 18, C2-deficient plasma (7·5 μl)+prekallikrein-deficient plasma (7·5 μl)+anti-C1 INH; 19, prekallikrein-deficient plasma (5 μl)+HK- and LK-deficient plasma (5 μl)+factor-XII-deficient plasma (5 μl)+anti-C1 INH; 20, bradykinin (2 ng); 21, normal plasma (25 μl)+C1s; 22, C2-deficient plasma (15 μl)+C1s; 23, HK and LK plasma (15 μl)+C1s; 24, prekallikrein-deficient plasma (15 μl)+C1s; 25, factor XII-deficient plasma (15 μl)+C1s; 26 normal plasma (15 μl)+C1s.

Effect of kininases and the Bk_2 antagonist on kinin activity

Incubation of HAE plasma with de Jalon's solution and either ACE or carboxypeptidase B resulted in rapid depletion of all kinin activity (Fig. 1c). Loss of kinin activity from plasma, but not from the ethanol purified material, was observed during incubation in de Jalon's solution without these enzymes, presumably due to plasma carboxypeptidase N. This loss was less rapid than that observed with added ACE or carboxypeptidase B. Similarly, the kinin activity of the ethanol supernatant from 37°C incubated HAE plasma was lost by incubation of the supernatant with ACE or with carboxypeptidase B.

At a dose sufficient to prevent contraction of the rat uterine horn stimulated with 5 ng of bradykinin, D-Arg-(Hyp^3, Thi^{5,8}, d-Phe^7)-Bk consistently antagonized the kinin activity of incubated HAE plasma (Fig. 1d). Likewise, the activity in the ethanol supernatant from HAE plasma was prevented by exposing the muscle to the Bk_2 antagonist.

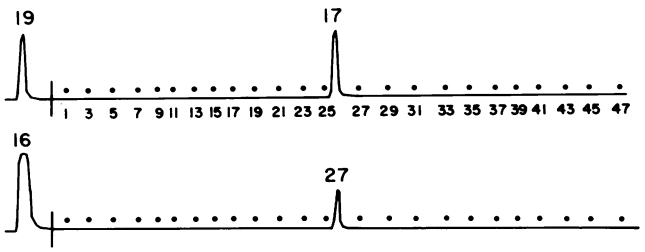

Fig. 3. Kinin activity of fractions obtained from reverse phase high performance liquid chromatography (HPLC). A sample of hereditary angioneurotic oedema (HAE) plasma (top) (30 μl) incubated at 37°C for 60 min and an ethanol supernatant from 100 μl of this plasma (bottom) were fractionated as described in Materials and Methods. The first fraction was collected from 0–1 min, with subsequent fractions collected over 2-min intervals up to 47 min. The response time, in seconds, is displayed over the peak of the contractions. Top, left of vertical bar, kymograph of incubated HAE plasma (10 μl); bottom, left of vertical bar, ethanol supernatant from incubated HAE plasma (equivalent to 50 μl plasma). The dots above the kymograph tracings indicate the points at which fractions were injected. No response was observed with any fractions other than the fraction eluting at 25 min. Segments of the kymograph tracing (none of which showed a response) between 2 min post-injection and the succeeding injection have been removed from the figure to save space.

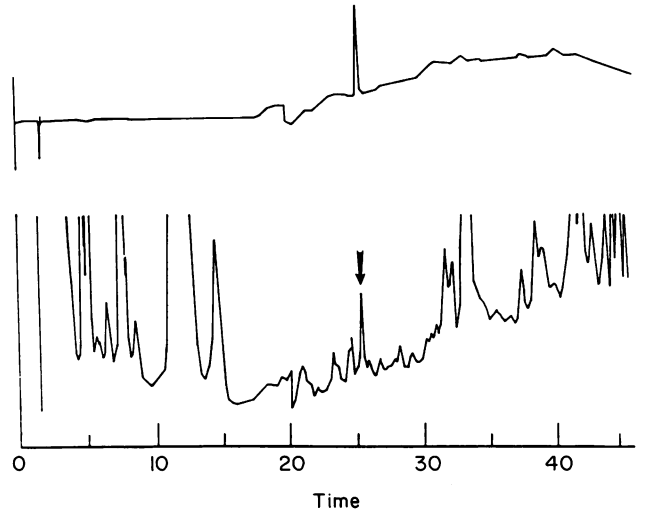

Fig. 4. Reverse phase high performance liquid chromatography (HPLC) analysis of bradykinin and of partially purified kinin from hereditary angioneurotic oedema (HAE) plasma. Samples were fractionated as described in Materials and Methods. Top, bradykinin (500 ng), absorbance units full scale (AUFS)=0·3; bottom, lyophilized, redissolved ethanol supernatant from incubated HAE plasma (2 ml), AUFS=0·25.

Effect of immunodepletion and C1s consumption of C1 INH

Kinin activity was generated in normal and C2-deficient plasma incubated with anti-C1 INH antibody, but not in the same plasmas incubated with antibody to C3, albumin, or immuno-

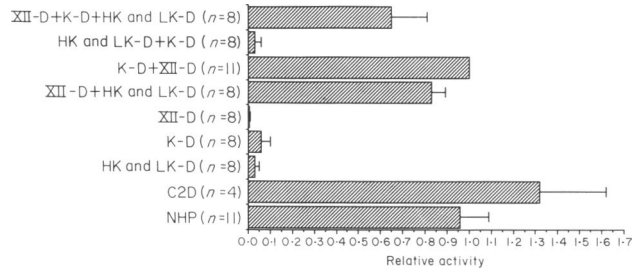

Fig. 5. Generation of vasopermeability-enhancing activity. Vasopermeability was analysed in guinea pig skin as described in Materials and Methods. The relative activities were calculated for each animal by dividing the area of blueing from each injection by the area of blueing from the C1s-incubated mixture of factor XII-deficient and prekallikrein-deficient plasmas in the same animal, as described. Each sample was incubated with C1s followed by C1 inhibitor (C1 INH) as described in Materials and Methods, and 0·1 ml of each was injected. NHP, normal human plasma; C2D, C2-deficient plasma; HK and LK-D, kininogen-deficient plasma; K-D, prekallikrein-deficient plasma; XII-D, factor XII-deficient plasma.

globulin. Plasma from individuals with hereditary deficiency of factor XII, prekallikrein, or HK and LK did not generate kinin activity following C1 INH immunodepletion (Fig. 2). However, when any two of these plasmas were combined before incubation, activity was generated in all instances except the combination of prekallikrein-deficient plasma with HK- and LK-deficient plasma. Presumably, this mixture remains deficient in prekallikrein because the plasma from patients with inherited deficiency of HK and LK (Fitzgerald trait) is also prekallikrein-deficient [21–24].

The addition of excess C1s to normal plasma and to C2-deficient plasma resulted in kinin generation during subsequent incubation. In contrast, when plasma deficient in factor XII, prekallikrein, or HK and LK was similarly incubated with C1s, no kinin activity was generated (Fig. 2). As with immunodepletion, when mixtures of these plasmas were incubated, all combinations developed kinin activity except the mixture of HK- and LK-deficient prekallikrein-deficient plasma (not shown). When an equal volume of saline rather than C1s was added before incubation, kinin generation was consistently absent.

Isolation and identification of kinin activity

Only one 2-min fraction (25 min) collected by reverse-phase HPLC separation of the ethanol supernatant from HAE plasma demonstrated kinin activity (Fig. 3). This activity was contained within a single peak on the chromatogram, which eluted at the same time as bradykinin (Fig. 4). Amino-terminal sequence analysis of this peak revealed a single sequence that was identical to the amino terminal 8 amino acids of bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe).

Guinea pig skin vasopermeability studies

Incubation of normal plasma or of C2-deficient plasma with excess C1s followed by inactivation with added C1 INH resulted in the generation of vasopermeability-enhancing activity (Fig. 5). With each of the contact system deficient plasmas, incubation with C1s resulted in the generation of minimal vasopermeability-enhancing activity. However, equal mixtures of either HK- and LK-deficient or prekallikrein-deficient plasmas with factor XII-deficient plasma, incubated with C1s, generated quantities of vasopermeability-enhancing activity similar to the activity from normal plasma. Little activity was generated from the combination of prekallikrein-deficient and HK- and LK-deficient plasma, similar to the findings in the kinin assay. Minimal responses were observed at the injection sites from all the plasmas and plasma mixtures that had been incubated with saline rather than C1s.

Vasopermeability-enhancing activity was generated in HAE, in C1s-treated normal and in C1s-treated C2-deficient plasmas by incubation at 37°C as described in Materials and Methods. In all these plasmas, this activity fractionated identically to the kinin activity: it was present in the supernatant following ethanol precipitation, and was entirely contained within the HPLC peak, described above, that eluted at the same time as bradykinin (Figs 3 and 4). No activity was present at any other time point through the chromatogram. In addition, one incubated HAE plasma obtained during remission and one obtained during an attack were initially fractionated by boiling to precipitate the bulk of the plasma proteins, as described [16]. The vascular permeability-enhancing activities in these heat-stable fractions also eluted from reverse-phase HPLC as a single peak with a retention time identical to that of bradykinin. Addition of bradykinin to the plasma before fractionation revealed an identical single peak.

DISCUSSION

The activity of vasopermeability-enhancing agents can be measured by quantifying the extravasation of dye–albumin complexes into the intradermal space. The induction of the vasopermeability response has been characterized by analysis of the conditions required for its generation, the effect of protease inhibitors upon its generation, its susceptibility to inactivating agents, and the response to receptor blockade. Furthermore, kinin activity in different smooth muscle bioassays can be quantified [25]. The vasopermeability-enhancing activity of C1s was found to correlate with its esterolytic activity [9,26]. When added to C1s before i.d. injection into guinea pigs, C1s-induced vasopermeability was blocked by C1 INH, but not by soybean trypsin inhibitor (SBTI), which does not inhibit C1 [10]. Similarly, in humans, the response to C1s was not diminished by co-administration of antihistamine or SBTI, but it was prevented by C1 INH [11]. Since SBTI is an effective inhibitor of kallikrein, these early studies suggested that the vasopermeability-enhancing activity generated by injection of C1s, and possibly during attacks in HAE patients, was independent of contact system activation.

The vasopermeable agent(s) responsible for oedema formation in HAE patients has been studied in attack and remission plasma, *in vitro* [13,14,16]. This substance was found to have kinin activity, but was felt to be different from bradykinin due to its inactivation by dansylation and trypsin digestion, and a difference in optical density ratio at 210 and 280 nm [13]. Furthermore, i.d. injection of this substance produced oedema with minimal erythema and pain [13]. The generation of this activity from remission HAE plasma was prevented by coincubation with SBTI, but, once formed, it was not inactivated by SBTI [16]. Thus, the active factor(s) generated *in vitro* appeared to differ from the vasopermeability agent in animals that had received i.d. C1s, at least with respect to susceptibility of its

generation to SBTI. Similarly, Smith & Kerr demonstrated that SBTI prevented the generation of kinin activity in HAE plasma [7,9].

The experiments presented here conclusively demonstrate the presence of bradykinin in incubated HAE plasma, and that its activity is largely, if not entirely, responsible for the kinin and vasopermeability-enhancing activity present in incubated HAE plasma. Removal of C1 INH from normal plasma either by immunodepletion or via complex formation with C1s was associated with generation of kinin and vasopermeability-enhancing activity. Each possessed the characteristics of bradykinin. Furthermore, the only peptide identified by amino acid sequence analysis in the single active peak isolated by reverse phase HPLC, was bradykinin. Similar conclusions were deduced from experiments in which C1 INH and C2 were depleted from plasma with Sepharose-bound antibody, although potential non-specific contact system activation was not excluded [7]. Identification of bradykinin in activated HAE plasma by amino acid sequence has not been accomplished previously, although the co-elution of kinin activity with bradykinin was reported [6], and it has been detected by radioimmunoassay [27]. The requirement for the presence of an intact contact system for the *in vitro* generation of both the kinin and vasopermeability activities was also demonstrated by the generation of activity following reconstitution of the factor XII-, kallikrein- and HK- and LK-deficient plasmas (each of which had normal C2 concentrations). The fact that activity was readily generated in C2-deficient plasma further supports the above findings. In addition, this finding suggests that, at least in whole plasma depleted of C1 INH, a C2-derived peptide does not make up the majority of the vasopermeability-enhancing activity.

The results of these studies do not account for the failure of i.d. injections of C1s to enhance vascular permeability in homozygous C2-deficient individuals and in C4- or C2-deficient guinea pigs. In addition, the results do not explain the reconstitution of the vasopermeability response in the C2-deficient guinea pigs following i.v. infusion of C2. Several explanations could account for the reduced permeability response observed in the early complement component deficient guinea pigs and humans, while still limiting the vasopermeable response generated in plasma to an effect of contact system activation. The contact system may play a role in C1 activation in HAE [25]. Activation of C1, prekallikrein and plasminogen by factor XIIa could result in the release of multiple mediators [25]. It is also possible that bradykinin is synergistic with products of classical complement pathway activation, such as the C2 kinin [17]. Further, it is possible that the C2 kinin (or other potential active peptides) can be efficiently generated only via contact with tissue-associated proteases (such as plasminogen activator). It is possible that, in angioedema *in vivo*, the generation of mediators at local extravascular sites is more important than that which takes place in plasma, and that this may involve mechanisms that cannot be duplicated in plasma *in vitro*. In any case, it seems quite clear from the studies described here that in plasma, in the absence of C1 INH, the vast majority of the kinin and permeability-enhancing activities that result are due to bradykinin. Bradykinin, therefore, probably plays a major role in the induction of angioedema. However, these studies do not allow exclusion of a role for other bioactive molecules, particularly the C2-derived peptide, as important mediators that may be generated extravascularly [11,13–17]. Further analyses *in vivo* will be required to define the relative roles of bradykinin, the C2-kinin and other potential mediators in the induction of angioedema.

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