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Research Article

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Effect of C'1 Esterase on Vascular Permeability in Man: Studies in Normal and Complement-Deficient Individuals and in Patients with Hereditary Angioneurotic Edema

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ABSTRACT When purified human C'1 esterase is injected intradermally in man, increased vascular permeability results. This effect is not blocked by soybean trypsin inhibitor and is not abolished by pretreatment with the antihistamine, pyribenzamine, or by compound 48/80. Thus, the effect is not due to the release of endogenous histamine. The decreased permeability response of individuals with a specific hereditary deficiency of C'2 is evidence for the complement-dependent nature of this reaction. The apparently normal response to intradermal C'1 esterase developed by individuals with an acquired specific deficiency of C'3 suggests that the vasoactive substance may be derived from one of the early reacting complement components. Characteristic attacks of angioedema have been provoked by the intradermal injection of human C'1 esterase in two individuals with hereditary angioneurotic edema. Patients with hereditary angioneurotic edema are unresponsive to intradermal injections of C'1 esterase immediately after attacks.

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INTRODUCTION

The first component (C'1) of the serum complement system normally circulates as a macromolecular complex (1). When this macromolecule interacts with an antigen-antibody complex, C'1 is converted to an enzyme which has esterolytic activity, designated C'1a or C'1 esterase. The second component (C'2) and the fourth component (C'4) of the complement system are the natural substrates of the activated first component (2, 3). C'1 esterase also hydrolyzes several synthetic organic esters (4). Of these, *N*-acetyl-l-tyrosine ethyl ester (ALTEE) is the optimal synthetic substrate for human C'1 esterase (5). The action of C'1 esterase upon its natural and synthetic substrates is inhibited by a naturally occurring serum globulin, C'1 esterase inhibitor (EI) (6).

The serum of individuals with hereditary angioneurotic edema (HANE) lacks the normal inhibition directed against C'1 esterase (7). These individuals have been shown to have measurable C'1 esterase activity in their plasma during attacks of angioneurotic edema (8). They simultaneously have decreased titers of C'2 and C'4 in their serum (8, 9). These observations suggest that C'1 esterase may be involved in the pathogenesis of episodes of angioneurotic edema. Highly purified human C'1 esterase has evoked a measurable in-

crease in vascular permeability when injected i.d. into guinea pigs (10). The present study is concerned with the enhanced vascular permeability in man after the i.d. injection of purified human C'1 esterase.

METHODS

Isolation of C'1 esterase. Blood was drawn from eight normal individuals who have repeatedly served as donors of plasma. Serial serum samples were obtained from the recipients of this plasma at regular intervals for 4 months after the plasma transfusions, and assayed for their content of glutamic oxalacetic acid transaminase and glutamic pyruvic acid transaminase. In all instances the results of the determinations were within normal limits. In addition, there were no instances of clinical hepatitis. Such donors were presumed to be free from hepatitis virus.[1] C'1 esterase was purified by the method of Haines and Lepow (5) by first activating the C'1 in a euglobulin fraction precipitated at pH 5.5, and, subsequently, with chromatography on DEAE-cellulose and TEAE-cellulose as described (5). The C'1 esterase eluted from the TEAE-cellulose was concentrated by ultrafiltration and further purified by electrophoresis on a Pevikon block measuring $20 \times 50 \times 1$ cm, equilibrated in barbital buffer, $\mu = 0.05$, pH 8.6. The block was cut into 1.0 cm sections, and the protein desorbed from the Pevikon was assayed for esterolytic activity. The protein from the region with the highest specific activity was pooled and used in this study. One of the four preparations was labeled with ^{125}I by the method of Reif (11) before electrophoresis on Pevikon. The esterase from the Pevikon segments with highest specific radioactivity was pooled, and an aliquot was added to whole human serum which was then subjected to agarose electrophoresis (12). A radioautograph was made as shown in Fig. 1. The preparation used was free from Hageman factor activity and contained less than 1/1000 of the original fibrinolytic activity of the starting serum and represented a 2400-fold purification with respect to starting serum.

[1] We are grateful to the Protein Foundation, Inc., Jamaica Plain, Mass., for the hepatitis-free blood donors.

Assay of C'1 esterase. C'1 esterase activity was assayed using *N*-acetyl-1-tyrosine ethyl ester (ALTEE)[2] as substrate, as described by Levy and Lepow (13). The assay mixtures were back titrated to pH 7.4 on a Radiometer Titrograph, Radiometer, Copenhagen, Type SBR 2c. The purified C'1 esterase preparations used in these experiments contained 120–180 U/OD Unit. 1 U of C'1 esterase is that amount which hydrolyzes 0.5 μmole of ALTEE in a standard assay mixture in 15 min (13).

Effect of C'1 esterase on vascular permeability. Subjects in whom the C'1 esterase was assayed for vascular permeability activity were pretreated, i.v., with 5 ml of Evan's blue.[3] After 10 min, 0.1 ml of C'1 esterase in doses of 0.01–80 U/ml in phosphate (5) or Veronal (14) buffer were injected i.d. through a 26 gauge needle. Two perpendicular diameters of the resulting wheals were measured at varying intervals up to 20 min after injection. Within this time period all subjects developed maximal responses. At the same time the intensity of the bluing was estimated and scored on a 0–4+ scale. The diameters of erythema were also measured.

Subjects. Six healthy individuals acted as control subjects in this study. Five were males between ages 32 and 42; one was a female 25 yr of age. Two individuals with specific hereditary C'2 deficiency have been described previously (15, 16). Five patients with hereditary angioneurotic edema (17) and two patients with an acquired isolated deficiency of C'3 (β_{1C} globulin) due to progressive glomerulonephritis (18, 19) have already been reported. These individuals were well informed as to the predicted action of C'1 esterase on vascular permeability before they consented to participate in these studies.

RESULTS

When C'1 esterase was injected i.d. into six normal individuals, increased vascular permeability resulted. The size of the wheal and degree of

[2] *N*-acetyl-1-tyrosine ethyl ester was obtained from Mann Research Laboratories, Inc., New York 10006.

[3] Evan's blue injection, USP was obtained from Warner-Chilcott Laboratories, Morris Plains, N. J.

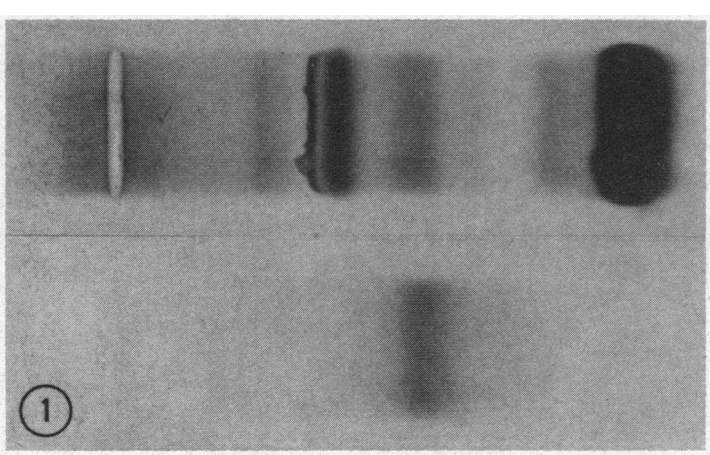

FIGURE 1 Agarose electrophoresis of ^{125}I-labeled C'1 esterase preparation in normal human serum. Top, stained pattern of whole human serum. Bottom, radioautograph of ^{125}I-labeled C'1 esterase in whole human serum. The mobility of the C'1 esterase was slightly faster than the band in the stained preparation formed by the α_2-macroglobulin and types 2–2 and 2–1 haptoglobulin.

bluing varied with the amount of C'1 esterase injected. Maximal reactions were obtained with 1.0 U and larger doses did not result in a further increase in the size or intensity of bluing of the wheals (Fig. 2). In normal individuals detectable reactions could be elicited with doses as small as 0.001 U. The wheals did not have any pseudopods, nor was their development accompanied by itching.

Effects of various agents on the vascular permeability activity of purified human C'1 esterase

Antihistamines. Two normal subjects were pretreated with 100 mg of pyribenzamine orally before i.d. injection of C'1 esterase. Doses of 4, 1, 0.1, and 0.01 U of C' esterase were given before and 1 hr after the ingestion of the antihistamine. No decrease in vascular permeability was observed when C'1 esterase was given after an antihistamine (Table I). The same subjects were also given varying doses of histamine i.d., before and after the oral administration of 100 mg of pyribenzamine. A definite decrease was noted in the degree of bluing elicited by the 1.0 and 0.1 μg doses of histamine after the administration of the antihistamine (Table I).

Compound 48/80 (*P-methoxy phenethyl methylamine*).[4] The failure of pretreatment of human subjects with an antihistamine to diminish the response to human C'1 esterase differed from the results obtained when human C'1 esterase was given i.d. to guinea pigs (10). To define further the requirement for endogenous histamine in the vascular permeability response to C'1 esterase, compound 48/80 was used to deplete the histamine content of an area of skin. Two normal individuals were given 50 and 25 μg of compound 48/80 i.d. in the same skin site at 24-hr intervals on 3 successive days. Each successive injection of compound 48/80 provoked a diminishing response. The final injection did not elicit a wheal. 2 hr after the last injection of compound 48/80, 1.0 U of C'1 esterase was injected into the area of skin depleted of histamine (20). A permeability response was evoked by C'1 esterase in the histamine-depleted skin. This response was somewhat less than a control reaction in the adjacent, untreated area of skin. Two subjects were given 25 μg of compound 48/80 i.d., after the administration of 100 μg of pyribenzamine. A marked decrease was noted in the response to this agent (Table I).

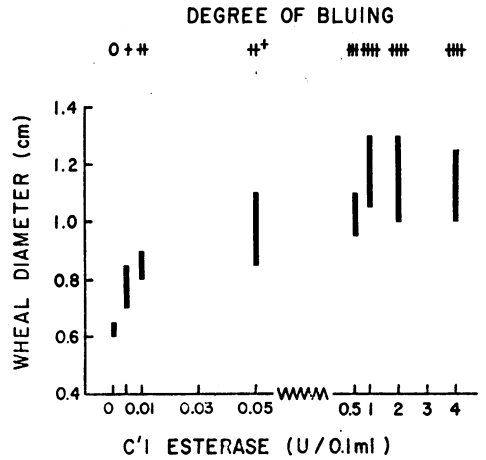

FIGURE 2 The relation of the magnitude of the vascular permeability response to the amount of C'1 esterase injected in six normal individuals.

Soybean trypsin inhibitor.[5] 2 U of C'1 esterase in 0.1 ml of Veronal buffer was mixed and incubated with an equal volume of saline containing 20 μg of soybean trypsin inhibitor for 15 min at 37°C. Two normal individuals each received 0.1 ml of this mixture (1 U of C'1 esterase + 10 μg of soybean trypsin inhibitor) i.d. 1 U of C'1 esterase in 0.1 ml of Veronal buffer was injected simultaneously into an adjacent site. Soybean trypsin inhibitor did not diminish the permeability response to C'1 esterase (Table II).

Human C'1 esterase inhibitor. Purified human C'1 esterase inhibitor was prepared by Dr. Jack Pensky from the serum of individuals judged to be free from hepatitis virus. Aliquots containing 0.2 U (13) of C'1 esterase inhibitor were mixed with 1.0 U of C'1 esterase, so that the final mixture injected contained 0.1 U of inhibitor and 0.5 U of C'1 esterase. This amount of inhibitor will block the action of 1 U of C'1 esterase. The mixture was incubated at 37°C for 15 min and then 0.1 ml was injected i.d. into a normal individual. The permeability response was completely inhibited (Table III). The wheal induced by the injec-

[4] Compound 48/80 was kindly supplied by Dr. M. Winkleman, The Mayo Clinic, Rochester, Minn.

[5] Soybean trypsin inhibitor, crystallized, was obtained from Worthington Biochemical Corporation, Freehold, N. J.

TABLE I

Effect of Pretreatment with an Antihistamine (Pyribenzamine) upon the Response to i.d. C'1 Esterase, Histamine, and Compound 48/80

	Pyribenzamine −		Pyribenzamine +	
	Wheal	Degree of bluing	Wheal	Degree of bluing
	mm		*mm*	
C'1 esterase, *units i.d.*				
1.0	12 × 11	4+	12 × 11	4+
0.1	11 × 11	4+	11 × 11	3–4+
0.01	9 × 8	1–2+	9 × 8	1–2+
Histamine, μg				
10.0	15 × 15	4+	16 × 16	3–4+
1.0	14 × 12	3–4+	11 × 10	2+
0.1	9 × 9	2+	8 × 7	±
Compound 48/80 μg				
25	12 × 12	4+	11 × 10	2+
Saline	10 × 9	0	10 × 9	0

tion of C'1 inhibitor alone was of smaller diameter than the buffer control (Table III).

The permeability response in individuals with specific complement deficiencies. In an attempt to ascertain whether or not the vascular permeability response provoked by C'1 esterase is mediated by the interaction of C'1 esterase with other components of complement, individuals with specific, isolated complement deficiencies were injected with C'1 esterase. Two individuals with specific hereditary deficiency of the second component of complement (15, 16) were found to have markedly diminished responses to C'1 esterase. In both, 4.0 U, which is four times the amount required for a maximal response in normal individuals, was required to evoke a minimal vascular permeability response. The response to 8.0 U of C'1 esterase was only slightly increased compared with the response to 4.0 U of C'1 esterase (Table IV).

To ascertain whether the diminished response to i.d. C'1 esterase was due to a specific inability to generate a permeability response of normal magnitude, one of the C'2-deficient individuals was also given i.d. doses of 10, 1.0, and 0.1 μg of histamine. His response to 25 μg of compound 48/80 was also noted. The response to the doses of histamine were of the same intensity as those in normal individuals, and the wheal elicited by 25 μg of compound 48/80 was greater than that elicited by the same dose in normal individuals.

The serum of two patients with progressive

TABLE II

Effect of Soybean Trypsin Inhibitor (SBTI) on the Response to i.d. C'1 Esterase in Normal Subjects

Material injected	Subject	Wheal	Degree of bluing
		mm	
C'1 esterase, *1.0 U*	A	12 × 10	4+
	B	12 × 10	4+
C'1 esterase, *1.0 U* +	A	14 × 12	4+
SBTI, *10 μg*	B	13 × 10	4+
SBTI, *10 μg*	A	7 × 6	0
	B	7 × 6	0

TABLE III

Effect of C'1 Esterase Inhibitor on the Response to C'1 Esterase in a Normal Subject

	Units	Wheal	Degree of bluing
		mm	
C'1 esterase	0.5	12 × 8	4+
C'1 esterase + C'1 esterase inhibitor	0.5 + 0.1	8 × 8	0
C'1 esterase inhibitor	0.1	2 × 2	0
Veronal buffer	0	7 × 6	0

TABLE IV
Hereditary C'2 Deficiency: Response to i.d. C'l Esterase

	C'1 esterase	Wheal	Degree of bluing
	U	*mm*	
1.	8.0	12 × 8	1–2+
	4.0	12 × 8	1+
	0.4	7 × 6	±
	PO_4 buffer	12 × 10	0
2.	8.0	8 × 8	2+
	4.0	5 × 5	1+
	PO_4 buffer	7 × 6	0

glomerulonephritis (18) contained 9 and 7 mg/100 ml (β_{1C} globulin) C'3 by immunochemical estimation (21) (normal range, 110–175 mg/100 ml), but normal hemolytic titers of C'1, C'4, and C'2. The specific serum deficiency of C'3 has been shown to result from an acquired defect in the biosynthesis of C'3 (22). When these two patients were injected with 0.4–4.0 U of C'1 esterase they developed wheals of the same size and intensity of bluing as did normal individuals given the same doses.

The vascular permeability response in patients with hereditary angioneurotic edema. The serum of individuals with hereditary angioneurotic edema fails to inhibit C'1 esterase. C'1 esterase has been implicated in the pathophysiology of hereditary angioneurotic edema (HANE) by the demonstration that, attacks of edema are uniquely associated with increased amounts of C'1 esterase in the plasma (8). Two patients with hereditary angioneurotic edema who had been asymptomatic for 10 days after the termination of an attack of edema were given 4.0 U of C'1 esterase i.d. in the left forearm. Both had markedly exaggerated responses. In normal individuals the reaction to C'1 esterase disappears within an hour; however, both of these individuals reacted with progressive swelling of the forearm over the ensuing 8–12 hr. The swelling completely subsided within 24 hr of the time of injection. When three patients with hereditary angioneurotic edema were given i.d. C'1 esterase during an attack of edema, they were found to have responses comparable to those observed in normal individuals. Two individuals were given i.d. C'1 esterase within 24 hr of the termination of an attack of angioedema and were found to have markedly decreased responses (Table V).

DISCUSSION

The activated first component of human complement is intimately involved in the development of a complement-mediated enhancement of vascular permeability (10, 23). The markedly diminished response of individuals with a specific hereditary deficiency of the second component of complement is evidence for the complement dependent nature of this response. On the other hand, the apparently normal response to i.d. C'1 esterase of individuals

TABLE V
Response of Individuals with Hereditary Angioneurotic Edema to i.d. C'l esterase

Subject	C'1 esterase	Activity of illness	Wheal	Degree of bluing
	U		*mm*	
1. H.A.S.	4.0	postattack	7 × 6	2+
	1.0	"	6 × 6	1+
	4.0	asymptomatic	12 × 14	4+
	2.0	"	12 × 8	2+
2. W.J.	4.0	"	12 × 12	4+
	1.0	"	14 × 8	4+
3. C.S.	4.0	during attack	13 × 10	4+
	0.5	"	10 × 8	4+
	4.0	postattack	9 × 8	1+
4. L.K.K.	4.0	during attack	13 × 11	4+
5. A.T.	2.0	"	18 × 18	3+

with a specifically acquired deficiency of C'3 suggests, but does not prove, that the vascular permeability factor is not generated from this protein and does not require its participation, since C'3 may be present in the extra vascular space in sufficient quantity to yield a vasoactive product despite the low serum concentration in these patients.

When human serum is exposed to an antigen-antibody complex, complement-dependent factors, termed "anaphylatoxin" (24), are generated from C'3 and C'5 (25, 26) that enhance vascular permeability in guinea pig skin and contract and desensitize smooth muscle of guinea pig ileum. The effects of these factors are mediated through the release of histamine and they can be blocked by antihistamines (27–29). The increase in vascular permeability in man after C'1 esterase injection is not dependent upon the release of endogenous histamine, since the response is unaltered by pretreatment with an antihistamine and is not abolished by histamine depletion by the prior local administration of compound 48/80. Furthermore, the lesions evoked by histamine and C'1 esterase differ in certain significant aspects. No itching is noted in the response to i.d. C'1 esterase. The wheals evoked by C'1 esterase never have pseudopods present. These are consistently noted in the response to histamine. Whereas the i.d. injections of either C'1 esterase or histamine evoke an erythematous response in addition to wheal formation, the erythema induced by C'1 esterase is consistently much less intense than that induced by histamine.

The lack of histamine dependence distinguishes the response to purified human C'1 esterase in man from the response developed in guinea pig skin. Species specificity of certain complement-mediated reactions has also been previously demonstrated in hemolytic systems (30) and in smooth muscle contraction (25). It is likely that the permeability response generated by i.d. C'1 esterase in both man and guinea pig differs in its final chemical mediator.

Since the plasma or serum of normal individuals contains a number of protein systems whose physiological activity results in enhanced vascular permeability (31), the precise definition of these individual systems, while complex, is necessary to an understanding of the inflammatory response. When plasma in contact with glass is diluted with saline, a factor is generated which enhances vascular permeability in guinea pig skin (32). This factor has been designated PF/dil by Miles and Wilhelm (33). Its action is inhibited by soybean trypsin inhibitor, di-isopropylphosphofluridate (DFP), but not by pretreating guinea pigs with antihistamines (34). PF/dil possesses esterolytic activity against TAMe (*p*-toluenesulfonyl-l-arginine methyl ester) but does not hydrolyse ALTEE (35). In vivo PF/dil is thought to exist in an inactive form which is activated through the action of Hageman factor (36).

Another vasoactive globulin present in normal plasma, kallikrein (37, 38), circulates as an inactive precursor which may possibly be activated by PF/dil (39). Kallikrein also possesses esterolytic activity for TAMe, similar to PF/dil, and acts upon an α-globulin substrate to release the nonapeptide, bradykinin (40, 41). Kallikrein, like PF/dil, is inhibited by soybean trypsin inhibitor and DFP. Its vascular permeability effects in the guinea pig are not blocked by pretreatment with antihistamines (42). The enhancement of vascular permeability derived through the action of C'1 esterase in man appears to be different from the systems described above. It is not blocked by soybean trypsin inhibitor.

The serums of individuals who have hereditary angioneurotic edema do not inhibit C'1 esterase. Consequently free C'1 esterase can be detected in the plasma of these individuals, particularly during attacks of edema, and their serum titers of C'4 and C'2, the natural substrates of C'1, are depressed. These observations have provided a basis for the suggestion that, attacks of angioedema are provoked by C'1 esterase. In the present study the i.d. injection of C'1 esterase has provoked characteristic attacks of angioedema in two patients with this disorder. However, these observations do not rule out the possibility that the kallikrein system may also play a role in the pathogenesis of angioedema. Landerman, Webster, Becker, and Ratcliff (43) have demonstrated the lack of serum inhibition to kallikrein in two patients with hereditary angioneurotic edema. Kagen and Becker have shown that a partially purified preparation of C'1 esterase inhibitor also inhibits the permeability effects of preparations PF/dil and kallikrein in guinea pig skin (44). However, a kinin-like material has been generated and identified in the plasma

of patients with hereditary angioneurotic edema (23) which seems to differ from previously identified kinins generated by the kallikrein system.[6] Its source is not known. The results of the present study suggest that the permeability effects of C'1 esterase in man are mediated by the complement system and raise the possibility that a kinin-like substance is derived from one of the early reacting complement components, most likely C'2. It appears that C'3 is not required for this effect. The hemolytic activity and electrophoretic mobility of C'3 were both unaffected by attacks of hereditary angioedema (8). In addition no conversion products of C'3 are detectable in venous plasma–draining areas of edema and the rate of catabolism of β_{1C} globulin is not increased during attacks of angioneurotic edema (45). This is evidence that this complement component does not participate in the enzymatic events that are associated with increased vascular permeability characteristic of this disease.

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Dr. Virginia H. Donaldson is an established Investigator of The American Heart Association; her present address is Shrine Institute for Burn Research and the Department of Medicine, University of Cincinnati, Cincinnati, Ohio. Dr. Fred S. Rosen is a recipient of U. S. Public Health Career Development award 1-K3-AM-19650-04.

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