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ASSAY OF FACTORS OF THE KININ SYSTEM IN PLASMA FROM PATIENTS WITH SPECIFIC, EXOGENOUS ALLERGIES

By

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It is a well-established fact that histamine is the dominant chemical mediator of allergic reactions of the immediate or reaginic type (Type I). In recent years, however, there has been a growing interest in the possibility of other mechanisms being present in addition to the histamine reaction. It has previously been reported that the amounts of kinin released by submaximum concentrations of hog pancreas kallikrein or by acetone-activated human plasma were lower in plasma specimens from patients with allergies than in plasma specimens from healthy subjects. At the same time, the initial rate of kinin release was higher in plasma from the patients than in plasma from the control group (2). It was suggested that the lower degree of kinin release in plasma from the allergic individuals might be due to an increased level of kallikrein inhibitor. The increased rate of kinin release caused by acetone-activated plasma in the same plasma specimens might have been caused by an increased level of prekallikrein. In addition to active kallikrein, acetone-activated plasma also contains prekallikrein activator (22). The fact, however, that hog pancreas kallikrein also induced a rapid release of kinin in plasma specimens from the patients suggested that this ob-

servation must be explained by some other mechanism. Hog pancreas kallikrein is known to act directly on kininogen, but is not supposed to activate prekallikrein.

In the present work the assumptions mentioned above were tested by assaying the following parameters in plasma specimens from patients with specific, exogenous allergies and from healthy subjects: Prekallikrein activator, prekallikrein, kallikrein inhibitors (C_I-inactivator, α -2-macroglobulin, α_1 -antitrypsin). In addition, kininogenase and arginylesterase activity in plasma were determined after incubation with submaximum amounts of hog pancreas kallikrein.

MATERIALS AND METHODS

Human citrated plasma (CPL) was prepared from blood collected into 1/10 vol. of 0.1 M sodium citrate dihydrate solution and centrifuged at $1300 \times g$ for 30 min at 10°. Siliconized equipment was used throughout. The plasma was stored at -20° if not used at once.

Human plasma kininogen (HP-kin) was prepared by incubating citrated plasma with EDTA-2Na (0.04 ml 10 per cent w/v per ml plasma) for 30 min at 37°. 60°-heated plasma kininogen (HP-kin/60°) was prepared by heating for 1 h in a water bath.

Human plasma kallikrein (HP-kall/17 per cent and HP-kall/25 per cent) was prepared by acetone activation (3).

Kaolin-activated CPL or HP-kin was prepared by incubation with the same volume of kaolin 10 mg/ml in saline (5, 6).

Partially purified human plasma-prekallikrein (11) was kindly supplied by A. M. Vennerød.

Bioassay of the kininogenase activity of HP-kall was carried out after incubating HP-kin/60° with HP-kall for 10 min at 37° (3). The activity was calculated on the basis of a standard preparation tested in parallel. The kinin determinations were carried out on the isolated rat uterus as "bracketing assays" with a dose ratio of 3:2. Bradykinin was used as reference standard.

Esterolytic assay of HP-kall was carried out at 25° with benzoyl-arginine ethyl ester (BAEe) as substrate (10, 21).

Esterolytic assay of kaolin-activated CPL or HP-kin was carried out at 25° with BAEe as substrate (5, 6, 11).

Esterolytic assay of prekallikrein activator was carried out by estimating the BAEe-activity obtained upon activation of a partially purified prekallikrein with kaolin-activated CPL (11, 12).

Immunological assay of protease inhibitors. The plasma concentrations of α_2 -macroglobulin, C_I-inactivator and α_1 -antitrypsin were determined by single

TABLE I
Survey of Case Material with Bronchial Asthma.

Case no.	Age	Family disposition	Asthma duration (years)	Other manifest. (years)	Skin test	Eos./mm ³
1	26	(+)	20-21	N, C, S	++++	-
2	27	+	> 1/2	N	++	69
3	20	(-)	1 1/2	N, C	+++	250
4	20	(+)	7-8	N, C	++++	519
5	20	-	4	N 16	++++	225
6	21	(-)	> 3-5	N, C	+++	462
7	24	+	1 1/2	N, C, S, 14-16	++++	50
8	29	+	1/2	N, C 17-22	++	-
9	20	+	8	N, C 8, S 19	+++	175
10	24	+	Several	N, C	++	200
11	18	(-)	10-15		++	-
12	22	-	1/2	N, C 5	+++	313

Symbols: N, nose; C, conjunctiva; S, skin.

Skin reactions: +, mild, but definite; ++, moderate; +++, marked (equals histamine control); +++++, strong to maximal.

radial immunodiffusion in 1 per cent (w/v) agarose in 0.07 M diethylbarbituric acid buffer (13). The wells were circular, with a diameter of 2.6 mm, and with 0.01 ml samples added. The concentrations of antisera in the agarose were: Anti- α_2 -macroglobulin 1.6 per cent, anti-CI-inactivator 3.3 per cent and anti- α_1 -antitrypsin 3.3 per cent.

Chemicals. *Alcohol dehydrogenase.* Alkohol-Dehydrogenase (Kristall suspension), Boehringer, Mannheim, Germany. *α -N-benzoyl-L-arginine ethyl ester (BAEe)*, Sigma Chem. Co., St. Louis, Miss., U.S.A. *Bradykinin*, Sandoz, AG, Basel, Switzerland.

β -diphosphopyridine nucleotide from yeast, grade III, Sigma Chem. Co., St. Louis, Miss., U.S.A. *Ethylenediamine tetraacetic acid disodium salt (EDTA-2Na)*, Titriplex III, Merck, AG, Darmstadt, Germany. *Hog pancreas kallikrein*, Padutin® R, Farbenfabriken Bayer AG, Leverkusen, Germany. *Lima bean trypsin inhibitor (LBTI)*, Worthington Biochemical Corp., Freehold, New Jersey, U.S.A. *Anti- α_1 -antitrypsin serum*, *anti-CI-inactivator serum* and *anti- α_2 -macroglobulin serum*, Behringwerke AG, Mahrburg-Lahn, Germany.

Statistical method. Student's *t* test was used. A probability level of less than 5 per cent was considered significant.

Patient Material

Among the various "allergic" syndromes with asthma as the predominating symptom, patients with "specific, exogenous allergy" of the immediate (or anaphylactoid) reaction type, constitute the best-defined and most homogeneous group. The patient material, all males, was selected to correspond roughly with that of a previous investigation (2). Some of the characteristics of the cases are summarized in Table 1.

Control Material

The donors of blood for plasma control specimens were not specifically screened in advance with regard to possible allergy. They were all male, claiming to be in good health at the time of the investigation. Age distribution was 20 to 29 years (average 24 years) as compared with 18 to 29 years (average 23 years) for the patients. Considering the high incidence of allergic diseases in the general population, a random sample may be expected to include persons with previous allergies, or who might be potentially allergic through an allergic family predisposition. In order to investigate this possibility, each person in the control group was asked to complete a questionnaire in which, with regard to the following conditions: eczema, urticaria, allergic rhinitis, allergic conjunctivitis, asthma, chronic infection in nose or sinuses, or chronic or recurrent bronchitis, they were asked to give information about any such case in the family, relationship specified, and, in the case of previous personal illness, the year(s). As we expected there were some positive responses: Case no. 1 reported chronic infection in nose and sinuses in family (not parents).

TABLE 2

Prekallikrein Activator, Kininogenase and Arginylesterase Activated in Plasma Specimens from Patients with Specific Exogenous Allergies.

Patient no.	Activity in per cent of standard preparation			
	Prekallikrein activator		Kininogenase	BAEe-esterase
1		95	107	95
2		75	105	95
3		89	100	96
4	A.	139	D. 104	D. 78
5		99	113	104
6		73	89	85
Mean		95.0	103.0	92.2
S.d.		24.0 (25.3 %)	8.1 (7.9 %)	9.2 (10.0 %)
7		93	89	109
8		135	128	142
9		128	115	64
10	B ₁	120	B ₂ 122	C. 90
11		109	96	73
12		93	118	93
Mean		113.0	111.3	93.2
S.d.		17.7 (15.7 %)	15.4 (13.8 %)	23.0 (23.3 %)
				27.9 (29.3 %)

Enzyme preparations.

A. Citrated plasma activated with kaolin.

B and C. Citrated plasma activated with acetone 17 per cent (v/v) = HP-kall/17 per cent.

D. Citrated plasma activated with acetone 25 per cent (v/v) = HP-kall/25 per cent.

Plasma pooled from three healthy males was used for the standard preparations.

Substrates.

A. In the prekallikrein activator assay partially purified plasma prekallikrein (11) was used, and BAEe-esterase peak values were recorded.

B₁ and B₂. Citrated plasma (B₁) or citrated EDTA-plasma = HP-kin (B₂) was used in assays intended to estimate whether there were any factors positioned between activator and prekallikrein. BAEe-esterase peak values were recorded after activation with a submaximum amount of activator preparation, HP-kall (17 per cent).

C and D. In the prekallikrein kininogenase assays HP-kin/60° was used.

C and D. In the prekallikrein esterase assays benzoyl-arginine ethyl ester (BAEe) was used.

For further details see text.

Case no. 2 reported chronic infection in nose and sinuses in one of the parents, and asthma in the family. Case no. 9 reported recurrent sinusitis in mother. Case no. 10 reported conjunctivitis of probable allergic origin.

RESULTS

Prekallikrein activator: Table 2, A demonstrates that the average level of kaolin-activated prekallikrein activator in plasma specimens from six allergic patients did not differ significantly from that of a standard preparation based on plasma from three healthy males. The activity of the standard preparation used did not deviate from the average activity of plasma specimens from 12 healthy subjects assayed in our laboratory by the same method (11).

Table 2, B shows that the average level of kallikrein (BAEe-esterase) activated in plasma specimens from six allergic patients by a submaximum amount of prekallikrein activator (acetone-activated) did not significantly exceed that of a standard preparation based on three healthy males. The intention of the assay procedure adopted was to obtain an estimation of prekallikrein plus any factors that might be positioned between activator and prekallikrein. It should be taken into consideration that differences in protease inhibitor levels of standard and test plasma, if present, might influence the results.

Prekallikrein. Kininogenase and arginyl-esterase activated by acetone and arginyl-esterase activated by kaolin: Table 2, C and D shows the results of assays of the kininogenase and the BAEe-esterase activities in HP-kall/17 per cent and in HP-kall/25 per cent based on plasma specimens from 12 patients with allergies. No deviations from the standard preparations used could be demonstrated. Each standard preparation was prepared from the pooled plasma from three healthy males and found to deviate 10 per cent or less from several similar preparations. From Table 3 it is evident that no significant deviations could be demonstrated between healthy subjects and allergic patients when considering the maximum

TABLE 3

Peak Values of Esterolytic Activity in Citrated Plasma (CPL) and in Citrated EDTA-Plasma (HP-kin) After Activation with Kaolin.

Subject no.	BAEe-esterase activity U/ml plasma after kaolin activation			
	A		B	
	CPL	HP-kin	CPL	HP-kin
1	0.82	0.90	0.57	0.61
2	0.76	0.97	0.84	0.86
3	0.69	0.79	0.73	0.72
4	0.79	0.81	0.81	0.79
5	0.64	0.65	0.57	0.66
6	0.64	0.67	0.70	0.80
7	0.76	0.78	—	—
8	0.59	0.57	—	—
9	0.88	0.91	—	—
10	0.55	0.58	—	—
Mean	0.712	0.763	0.703	0.740
S.d.	0.107 (15.0 %)	0.141 (18.5 %)	0.115 (16.4 %)	0.094 (12.7 %)
S.e. of difference A - B:				
		HP-kin	0.0588	$P > 0.8$
		CPL	0.0582	$P > 0.8$

A. Healthy subjects.

B. Patients with specific, exogenous allergies.

Incubates: 0.50 ml CPL or HP-kin and 0.50 ml of kaolin 10 mg/ml in 0.15 M sodium chloride at 25°.

Aliquots of 0.03 ml were withdrawn at frequent intervals and the activities against benzoyl-arginine ethyl ester (BAEe) recorded and given as U/ml plasma. 1 U represents 1 μ mol BAEe split per minute. The maximum activities registered are shown in the Table. Patient nos. 1 to 6 correspond to case nos. 7 to 12 in Table 1.

For further details see text.

arginyl-esterase activities obtained upon kaolin activation of citrated plasma or citrated plasma treated with EDTA (HP-kin).

Kallikrein inhibitors: Table 4 shows the results of immunological determinations of three relevant plasma protease inhibitors. No statistically significant differences were dem-

Table 4

Immunological Determination of Protease Inhibitors in Citrated Plasma.

	Subject no.	Inhibitor activity in per cent of standard preparation		
		CI-inactivator	α_2 -macroglobulin	α_1 -antitrypsin
A	1	112	126	113
	2	118	124	112
	3	88	82	106
	4	102	112	—
	5	72	76	—
	6	86	92	88
	7	74	78	86
	8	100	98	88
Mean		94.0	98.5	98.8
S.d.		16.8 (17.9 %)	20.1 (20.4 %)	12.8 (13.0 %)
B	1	124	138	131
	2	104	93	85
	3	106	125	90
	4	88	92	92
	5	107	137	98
	6	118	115	73
Mean		107.8	116.7	94.3
S.d.		12.5 (11.6 %)	20.5 (17.6 %)	20.3 (21.5 %)
S.e. of difference		7.8	11.0	9.8
A-B		0.2 > P > 0.1	0.2 > P > 0.1	0.7 > P > 0.6

A. Healthy subjects.

B. Patients with specific exogenous allergies.

The data (mean of two assays) are given as percentage values of a standard plasma batch (pooled plasma from three healthy males) assayed in parallel.

Patient nos. 1 to 6 correspond to case nos. 7 to 12 in Table 1.

For further details see text.

onstrable between the plasma levels of the three inhibitors tested in the allergic individuals and in the control group.

Kininogenase and arginylesterase activity during incubation of plasma with hog pancreas kallikrein: In accordance with previous observations (2) it was found that submaximum concentrations of hog pancreas kallikrein induced a somewhat

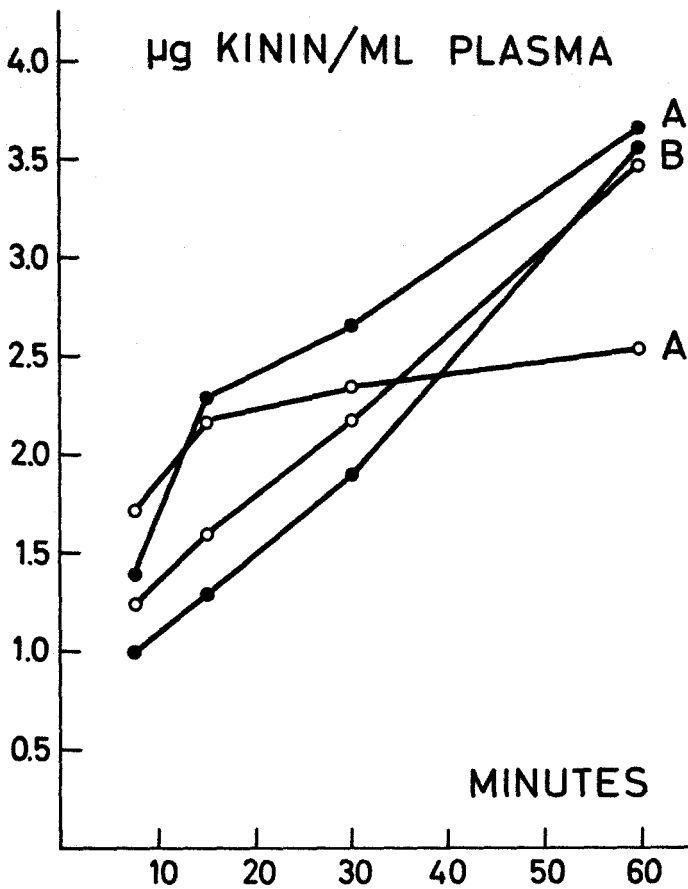


Fig. 1.

Rate of release of kinin in plasma specimens from healthy males and males with specific exogenous allergies.

Enzyme: Hog pancreas kallikrein (Padutin® R), 0.14 units/ml incubate.

● — ●: Release values based on the pooled plasma from three healthy men.

○ — ○: Release values based on the pooled plasma from three allergic patients.

Incubates A: HP-kin 1.00 ml + enzyme 0.60 ml + saline 3.40 ml.

Incubates B: HP-kin 1.00 ml + enzyme 0.60 ml + acetone 0.60 ml + saline 2.80 ml.

The components were added in the above sequences. Temperature: 37°.

Kinin released calculated as μg bradykinin/ml plasma. For further details see text.

higher initial rate of kinin release and a definitely lower maximum release in plasma specimens from allergic patients than in specimens from healthy individuals (Fig. 1, A). It should be pointed out that the kinin release was not accompanied by

an increase in BAEe-esterase activity. The incubation mixtures contained plasma (HP-kin), hog pancreas kallikrein and saline, which was the last component added. If acetone 12 per cent (v/v) was included in the incubate the initial increased rate of kinin release in the pathological plasma specimens was retained, and the rapid halt in release was eliminated (Fig. 1, B). The release of kinin obtained by an excess amount of hog pancreas kallikrein, 4 units/ml plasma, was roughly the same in the two plasma mixtures used, about 4 μ g/ml plasma.

DISCUSSION

Prekallikrein activator (Factor XII): It seems fairly well established that factor XII of the coagulation system in its active state(s) can activate prekallikrein directly (4, 10, 15, 20, 22). In addition, there are reports in the literature of a factor XII-activated protease situated between factor XII and prekallikrein (1, 17, 18, 23). Laake & Vennerød (12, 13) based their method for the determination of factor XII in human plasma on the effect of kaolin-activated plasma on partially purified plasma prekallikrein. Such an assay procedure will be influenced by a factor XII-dependent prekallikrein-activating protease if it is present in rate-limiting concentrations. With the method used in the present work no difference could be demonstrated between the allergic patients and the healthy individuals (Table 2 A).

Factor XII-dependent prekallikrein activator: By assaying the BAEe-esterase activity developed upon activation of prekallikrein in plasma specimens by a submaximum amount of activator (acetone-activated plasma containing XII_F), one should be able to estimate the amount of any prekallikrein activator that may be positioned between active factor XII and prekallikrein, if it is present in rate-limiting concentrations. The results obtained in the present work by such a procedure provided no evidence of a deviation between the plasma obtained from patients with allergies and plasma from healthy individuals (Table 2, B₁ and B₂).

Prekallikrein. The data obtained gave no indication of an abnormal level of plasma prekallikrein in patients with specific, exogenous allergies. The kininogenase activity and the BAEe activity registered in HP-kall/17 per cent and in HP-kall/25 per cent from healthy individuals and from patients were indistinguishable (Table 2, C and D). In addition, experiments carried out on the BAEe-esterase activity obtained upon kaolin activation of plasma, as suggested by Colman et al. (5, 6) for the estimation of prekallikrein, did not demonstrate significant differences between the test and the control material (Table 3).

Kallikrein inhibitors: According to McConnel (14) the three main protease inhibitors effective against plasma kallikrein are C_I inactivator (1), α -2-macroglobulin (9) and α_1 -antitrypsin (7, 8). Immunological assays of the three inhibitors in the present work gave no evidence of higher plasma inhibitor levels in patients with allergies than in healthy individuals (Table 4). The results accordingly did not support the assumption made in a previous report (2) that the lower degree of kinin release observed in plasma specimens from allergic patients as compared with control plasma after activation with HP-kall/17 per cent or with hog pancreas kallikrein, could be ascribed to a higher level of kallikrein inhibitor in the patient plasma. The possible significance of inhibitors other than those tested should not be overlooked, but determinations of the BAEe-esterase activity of hog pancreas kallikrein in the incubates during the kinin release period gave no evidence of an inhibition.

Kininogenase and arginylesterase activities during incubation of plasma with hog pancreas kallikrein: It seems to be well established that dilution of plasma by saline will cause an activation of factor XII. Oh-Ishi & Webster (16) were able to detect both active factor XII and arginylesterase, probably kallikrein in complex with α -2-macroglobulin, upon dilution of citrated plasma with saline. The fact that the addition of hog pancreas kallikrein followed by some dilution with saline induced no BAE-esterase activity in citrated EDTA plasma

from either allergic patients or healthy subjects seems to exclude the possibility that the increased rate of kinin release noticed for the patient plasma could be due to an activation of prekallikrein or other arginylesterases. The observation that the presence of acetone (12 per cent v/v) in the incubates eliminated the halt in kinin release noticed for the patient plasma (Fig. 1) could indicate the inhibition of a kallikrein inhibitor in addition to those assayed in the present work, which is present in plasma from the allergic patients in higher concentrations than in plasma from healthy individuals. However, the increased rate of kinin release regularly observed in plasma from allergic patients, which was retained in the presence of acetone (Fig. 1), could not be due to an increased level of a kallikrein inhibitor. It seems more appropriate to consider the possibility that the differences registered between the patient plasma and the normal plasma as to kinin release might reflect differences in the relative amounts of kininogen fractions present. Some recent publications discuss a previously unknown high molecular weight plasma factor, possibly a high molecular weight kininogen, which is important for the generation of kinin (19, 24). It should also be mentioned that experiments in our laboratory on the release of kinin from a high molecular weight kininogen preparation (S1) by incubation with submaximum amounts of hog pancreas kallikrein yielded time-release curves very similar to the curves obtained with plasma from allergic patients (Briseid et al., in press). In experiments with increasing concentrations of hog pancreas kallikrein it was found that the release of kinin from S1 was dependent on the concentration of enzyme in the incubate, while the release of kinin from low molecular weight kininogen (S2) continued until complete exhaustion of the substrate irrespective of the enzyme concentration.

SUMMARY

Factors of significance for the release of kinin were estimated in plasma from patients with specific, exogenous allergies and

in that from healthy individuals. There was no evidence that the rapid initial kinin release in plasma from allergic patients caused by submaximum concentrations of hog pancreas kallikrein or by acetone-activated human plasma (2) was due to an increased level of prekallikrein activator (activated factor XII), to prekallikrein itself or to a factor possibly positioned between active factor XII and prekallikrein. In addition, the rapid halt in kinin release observed for the patient plasma could not be ascribed to an increased level of α_2 -macroglobulin, C $\bar{\text{I}}$ -inactivator or α_1 -antitrypsin. It is suggested that the differences in kinin release registered between the patient plasma and the normal plasma might reflect differences in the relative amounts of kininogen fractions present.

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