CLINICAL EVALUATION OF THE PARAMETERS OF KININ PRODUCTION

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Many clinical studies of changes in components of the kinin system of the blood in various diseases have been published in recent years. In most of these investigations activation of the blood kinin system was demonstrated irrespective of the stage of the course of the disease. Only in a few investigations [6, 8, 14] was variation in the character of the changes in kininogenesis (intensified, weakened, unchanged) noted in the course of the disease. To evaluate the physiological state of the kinin system levels of prekallikrein, kallikrein, and its inhibitors have most frequently been determined. Usually an esterase method [18] and its modifications [2, 4], an *in vitro* chromatographic method [10], and the kininogenase (biological) method [13, 14] have been used. In an attempt to assess the clinical significance of changes in the blood kinin system in inflammatory-allergic diseases of the respiratory organs in adults and children, the present writers found a number of discrepancies and contradictions in the basic assumptions, interpretations, and final conclusions given in these publications [3, 8, 11, 16, 17].

In the investigation described below parameters of kinin production were determined in 21 sick infants under 3 years of age by the three most widely used methods simultaneously.

EXPERIMENTAL METHOD

At the height of the clinical course of the disease prekallikrein, kallikrein, and kallikrein inhibitors were determined in citrated blood plasma. Determinations were carried out by kininogenase [14], esterase [4], and *in vitro* chromatographic methods [10]. Kallikrein and prekallikrein activity was estimated in the last of these methods by measuring their esterase and kininogenase action. A purified preparation of high-molecular-weight kininogen (HMWK) from human serum was used as the substrate. The specific activity of the preparations corresponded to 2.5-4 μg bradykinin. Kinins in TCA filtrates were determined on the uterine cornu of Wistar rats. The bradykinin triacetate used in the work was from Reanal, Hungary; Tris, ovomucoid, N-benzoyl-L-arginine ethyl ester hydrochloride (BAEE·HCl), and N-benzoyl-L-arginine-p-nitroaniline (BAPNA) were of Soviet manufacture, trypsin was from Spofa, Czechoslovakia, and the DEAE-Sephadex A-50 was from Pharmacia, Sweden.

EXPERIMENTAL RESULTS

Altogether 28 clinically healthy children and 21 with bronchopulmonary disease were tested. Eight children had acute unilateral or bilateral pneumonia, nine had subacute pneumonia, and four had infectious-allergic bronchial asthma. On the basis of investigation of total kallikrein all the sick children were divided into two groups. Group 1 consisted of all children with increased kinin production: seven patients with acute pneumonia, one with subacute pneumonia, and two with bronchial asthma. Group 2 consisted of children with reduced kinin production: eight with subacute pneumonia, one with acute pneumonia, and two with bronchial asthma. Mean statistical parameters obtained by the esterase and *in vitro* chromatographic methods (Table 1) were calculated for each group, respectively. The tests showed that in a stage of clinically manifest disease activation of kinin production (on average by 50%) was observed in 88% of patients with acute pneumonia, in one with subacute pneumonia, and in two with bronchial asthma. In 12, 80, and 50% of children tested with these nosological forms of bronchopulmonary disease, respectively, kinin production was reduced on average by 47%.

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TABLE 1. Parameters of the Kallikrein–Kinin System of Blood Plasma in Infants Determined Simultaneously by Different Methods (M ± m)

Parameter tested	Character of changes in kinin production		
	unchanged	increased	reduced
	clinically healthy infants (n = 28)	patients of	
		group 1 (n = 10)	group 2 (n = 11)
Kininogenase method			
Total kallikrein	1,57±0,08	2,36±0,08*	0,83±0,09*
Kallikrein bound with inhibitors, μg/ml/h	1,05±0,16	1,63±0,12**	0,44±0,03*
Prekallikrein	0,52±0,08	0,73±0,05**	0,39±0,03*
Esterase method			
Spontaneous activity, μmoles BAEE/ml/h	3,08±1,60	6,4±1,65	7,11±1,53
Prekallikrein, μmoles BAEE/ml/h	87,6±6,2	71,5±7,35	75,8±2,23
Kallikrein inhibitor, conventional units	0,66±0,07	0,65±0,11	0,66±0,09
In vitro chromatographic method			
BAEE esterase activity, milli-Units/ml/min			
kallikrein	0—10**	14±1,0**	8,5±0,64**
prekallikrein	318±57**	184±17*	254,6±16,6**
Kininogenase activity, μg/ml/min			
kallikrein	0,1±0,02	—	0,055±0,002**
prekallikrein.	1,9±0,11	—	1,59±0,10**

Legend. *) $P < 0.05$; **) values for P taken from [1]. Number of infants shown in parentheses.

Increased kinin production was due to increased activity of total kallikrein, kallikrein bound with inhibitors, and prekallikrein activated by kaolin and acid. When kinin production was depressed, all the parameters mentioned were significantly reduced.

No significant changes were found in parameters of spontaneous BAEE-esterase activity, prekallikrein, and kallikrein inhibitors between groups which we distinguished by Colman's method in the modification of Gomazkov et al. In patients of both groups there was only a common tendency for spontaneous plasma esterase activityto increase and for the prekallikrein concentration to decrease. Values obtained for kallikrein inhibitor were indistinguishable from normal.

In the group of patients with increased kinin production the chromatographic method revealed a significant increase in kallikrein esterase activity and a decrease in prekallikrein esterase activity, evidence of activation of the blood kinin system. Inhibition of kinin production was discovered only when kininogenase activity of kallikrein and prekallikrein was determined. Values of the esterase activity of these components were changed so that they could be interpreted as increased kinin production.

A clear example of divergence in conclusions drawn from data obtained by Colman's method in the modification of Gomazkov et al. and the kininogenase method is an observation on the sick infant N. (aged 1 year 2 months) with a severe form of acute bilateral pneumonia, in whom considerable activation of the kinin system was discovered by kininogenase and *in vitro* chromatographic methods (total kallikrein 3.53 μg/ml/h, kallikrein bound with inhibitors 2.54 μg/ml/h, prekallikrein 0.99 μg/ml/h, kallikrein, according to Paskhina and Krinskaya 15.0 milli-Units/ml/min, prekallikrein 1.96 milli-Units/ml/min), and by Colman's esterase method in the modification of Gomazkov et al. no pathological disturbances were found: spontaneous plasma BAEE-esterase activity 0, prekallikrein 114.8 μmoles BAEE/ml/h, kallikrein inhibitor 0.62 Units/ml.

A special comparison of BAEE-esterase, BAPNA-amidase, and kininogenase activities of the same samples of plasma showed no correlation between these kallikrein activities (Table 2). Whereas the kininogenase activity of prekallikrein and kallikrein remained unchanged or was actually increased by 30% 30 min after heating plasma acidified to pH 3.0 at 61°C, the BAEE-esterase activity of prekallikrein remained unchanged, whereas that of kallikrein was reduced by 75%. Heating (for 1 h) led to loss of 50% of the kininogenase activity of kallikrein and to total loss of its esterase activity. Heating plasma in a more acid medium (pH 2.0) was accompanied by a decrease in kininogenase activity of kallikrein by almost 80% and an almost fourfold increase in its BAPNA-amidase activity.

TABLE 2. Components of Blood Plasma Kinin System Found in Samples of Plasma Acidified with 1 N HCl to pH 3.0 and Heated at 61°C (pooled plasma from eight blood donors was used in the experiment)

Parameter of kinin system	Time of heating plasma samples, min		
	15	30	60
Prekallikrein			
BAEE-esterase activity, milli-Units/ml/min	109	109	109
kininogenase activity, μg/ml/h	1,28	1,74	0,87
Kallikrein			
BAEE-esterase activity, milli-Units/ml/min	72	18	0
kininogenase activity, μg/ml/h	2,13	2,90	1,45
Kallikrein heated at pH 3.0			
BAPNA-amidase activity, milli-Units/ml/min	1,23	—	—
kininogenase activity, μg/ml/h	1,52	—	—
Kallikrein heated at pH 2.0			
BAPNA-amidase activity, milli-Units/ml/min	4,62	—	—
kininogenase activity, μg/ml/h	0,28	—	—

Potentiation of the plasma BAEE-esterase and BAPNA-amidase activity of the plasma has been explained [12] by the appearance, not of kallikrein, but of active trypsin in the blood. Since our previous investigations [14] showed that plasma kallikrein can be adsorbed on kaolin and that the degree of adsorption depends on the functional state of the enzyme, and is increased in various pathological processes, it can be postulated that the fall in BAEE-esterase activity during incubation of plasma with a large weighed sample of kaolin, as in Colman's method, was due to absorption of kallikrein on the kaolin and not to the effect of inhibitors on this enzyme. The nonspecificity of Colman's method with respect to kallikrein inhibitor is also confirmed by the fact that Gomazkov [5] found 40% less inhibitor in serum than in plasma. Determination of kallikrein inhibitors by the specific kininogenase method [15] showed that the antikallikrein capacity of serum is 10-15% greater than the inhibitory activity of blood plasma on account of its dilution with solutions of anticoagulants (Fig. 1).

To sum up the results given in this paper it can be concluded that Colman's esterase method and its modifications do not reflect the true activity of prekallikrein, kallikrein, and its inhibitor, i.e., the method is nonspecific when used to evaluate activity of the blood kinin system whether under normal or under pathological conditions. Soundly argued criticism of this method is to be found in publications by Kiselev [6], Krinskaya [7], and Orlov [9], who found no correlation between kininogenase and BAEE-esterase activity of kallikrein in experimental and clinical tests. We must agree with the views of those workers [8, 19] who consider that the increase in BAEE-activity of plasma (interpreted as a parameter of prekallikrein) after the addition of substances such as kaolin, celite, or dextran sulfate to the sample, reflects the state of the total of known and unknown factors of contact activation. Krinskaya [7] is therefore right when he states that activation of prekallikrein in native plasma by kaolin is not specific for this proenzyme. Since only about 6% of the arginine-esterase activity of healthy human blood serum is due to kallikrein, and since spontaneous BAEE-esterase activity does not reflect any of the known serum proteinases of the blood [19], the group to which kallikrein belongs, it is impossible to draw any reliable conclusions, in our opinion, on the state of kallikrein production on the basis of parameters of spontaneous plasma BAEE-esterase. Only activation of kinin production, but not weakening of this process, can be detected by the *in vitro* chromatographic method of investigation of BAEE-esterase activity of kallikrein and its precursor by the method of Paskhina and Krinskaya. The true functional state of the blood kallikrein can be established only by determination of kininogenase activity of plasma fractions obtained by chromatography. From our point of view, the esterase-chromatographic method gives incorrect information in clinical in-

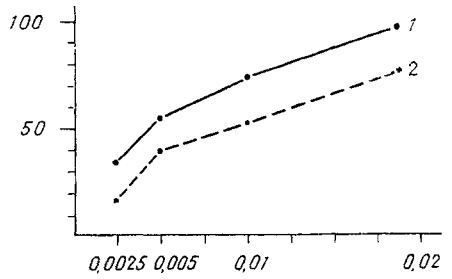

Fig. 1. Kallikrein inhibitor activity in blood serum (1) and plasma (2) of healthy infants up to 3 years of age. Each point represents mean value in eight infants tested. Abscissa, volume of plasma or serum (in ml); ordinate, inhibition of kininogenase activity of kallikrein (in %).

vestigations and is most suitable for tasks requiring evaluation of a preparation in the course of obtaining purified enzymes. The decision of the International Committee on Nomenclature of Vasoactive Peptides (1968) that, to describe the enzymic properties of kallikreins their kininogenase activity must be determined, remains valid. The kininogenase method we have developed, by means of which the state of kinin production can be judged on the basis of determination of levels of three forms of kallikrein in whole plasma [14], is the most objective method. The choice of method of determination of the activity of the components of kinin production in clinical medicine is of great practical importance, for it enables kinin-inhibiting or kinin-stimulating remedies to be prescribed individually for each patient, allowing for the stage of the course of the pathological process.

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