CONTACT ACTIVATION OF KALLIKREIN AND PLASMIN SYSTEMS OF RABBIT BLOOD

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Contact activation of the kallikrein–kinin system by kaolin and activation of plasmin by streptokinase can take place in rabbit blood plasma. Preliminary brief treatment of plasma with chloroform removes the factor which inhibits both these activation processes.

KEY WORDS: kinins; kallikrein; plasmin; contact activation.

Contact between native human blood plasma and kaolin leads to the formation of kallikrein [8]. This reaction lay at the basis of a method of determining the state of the kallikrein–kinin system of plasma in various states of the body [1, 2, 8].

The specificity of contact activation of rabbit blood plasma was investigated and methods of quantitative estimation of the state of the kallikrein system in these animals were evaluated.

EXPERIMENTAL METHOD

Citrated human, rat, and rabbit blood plasma, taken under conditions excluding contact with the glass surface, and then immediately frozen to −20°C, was used. The plasma was activated with kaolin and streptokinase as described earlier [2]. Treatment of the plasma with chloroform was carried out at 4°C, by shaking equal volumes of plasma and chloroform. To study the esterolytic activity of the plasma when activated with kaolin or (and) streptokinase, BAEE was used as the substrate [1, 2]. Kinin-forming activity was investigated in blood plasma incubated with kaolin in the presence of o-phenanthroline ($2 \cdot 10^{-4}$ M). Samples were fixed with an equal volume of 4% CH_3COOH and, after neutralization, they were tested on a segment of guinea pig intestine against standard bradykinin. Fibrinolytic activity was tested on fibrin plates [4]. The area of the zones of lysis was calculated by the equation:

$$S = \pi ab,$$

where a and b are the radii of the zone of lysis.

The following reagents were used: BAEE (N-benzoyl-1-arginine-ethyl ester hydrochloride), Reanal, Hungary; streptokinase, Streptase, Behringwerke, West Germany; SBTI (soy bean trypsin inhibitor), Reanal, Hungary; LBTI (lima bean trypsin inhibitor), Nutritional Biochemicals Corporation, USA; o-phenanthroline, Chemapol, Czechoslovakia; bradykinin triacetate, Sandoz, Switzerland; contrical, Germed, VEB Arzneimittelwerk, East Germany.

EXPERIMENTAL RESULTS AND DISCUSSION

Brief contact between human and rat citrated plasma and kaolin led to a rapid increase in BAEE-esterase activity; the maximal effect was observed at the first minute of incubation (Fig. 1). By the 5th-20th minute activity was reduced by blocking of the enzyme by inhibitor [8, 9]. So far as rabbit plasma is concerned, its arginine-esterase activity reached the maximum only after 3-5 min of incubation. These observations, as a first approximation, agree with those of Paskhina and Gurtovenko [3], who found no con-

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TABLE 1. Effect of Chloroform on BAEE-Esterase Activity of Rabbit Plasma Activated by Kaolin and Streptokinase (M ± m)

Experimental conditions	Quantity of BAEE hydrolyzed (in μmoles/ml plasma/h)		
	without chloroform	plasma + chloroform (1 min)	plasma + chloroform (120 min)
Control	20,65±5,5	22,50±2,42	69,25±18,80
Plasma+kaolin (incubation 1-5 min)	42,2±6,36 †	86,25±10,00*	127,50±22,00*
Plasma+kaolin (incubation 20 min)	20,62±2,42	43,57±8,30	109,00±4,26
Plasma + streptokinase (10 000 units/ml)	33,8±3,17†	68,40±4,25*	—

*Maximum of activity at 1st minute of incubation.

† Maximum of activity at 5th minute of incubation. In each case 4-6 samples of plasma tested.

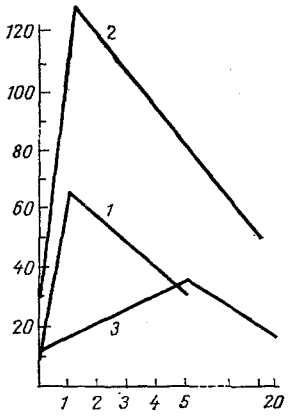

Fig. 1

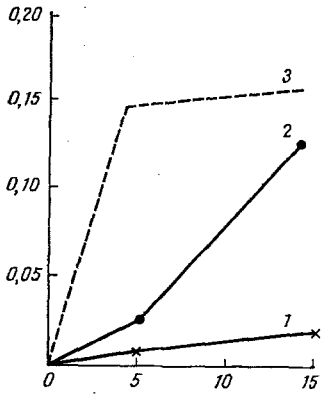

Fig. 2

Fig. 1. Dynamics of BAEE-esterase activity of animal and human blood plasma on activation with kaolin (25°C): 1) human plasma, 2) rat plasma, 3) rabbit plasma. Abscissa, time of activation of plasma by kaolin (in min); ordinate, quantity of BAEE hydrolyzed (in μmoles/ml plasma/h).

Fig. 2. Bradykinin formation in rabbit and rat plasma during activation by kaolin: 1) rabbit plasma +kaolin, 2) rabbit plasma +chloroform (1 min) +kaolin (1 min), 3) rat plasma +kaolin (1 min). Abscissa, incubation of plasma; ordinate, quantity of bradykinin (in μg/ml plasma).

tact activation or subsequent kinin formation in rabbits. Nevertheless, rabbit plasma is known to contain Hageman's factor, prekallikrein activator, and prekallikrein, and several workers have purified them and studied their properties [5-7, 11, 12].

Rabbit plasma also was inert to streptokinase treatment: Whereas the BAEE-esterase activity of human blood is increased by six to eight times on the addition of streptokinase [2], in rabbits the same dose of streptokinase increased this activity by only 60%; evidently plasmin formation under the influence of streptokinase is delayed in rabbit plasma.

Preliminary treatment of rabbit plasma with chloroform for 1 min considerably increased its BAEE-esterase activity after contact with kaolin (Table 1); the activity reached a maximum in this case after only 1-3 min of incubation. During prolonged (120 min) treatment of rabbit plasma with chloroform the original activity increased by 3.5 times. On subsequent contact between this plasma and kaolin, additional esterase activity was exhibited; this activity agreed quantitatively with that found after brief incubation with chloroform and kaolin. The effect of inhibitor (incubation with kaolin for 20 min) was reduced under these circumcumstances. The BAEE-esterase activity of chloroform-treated rabbit plasma was increased threefold after treatment with streptokinase also.

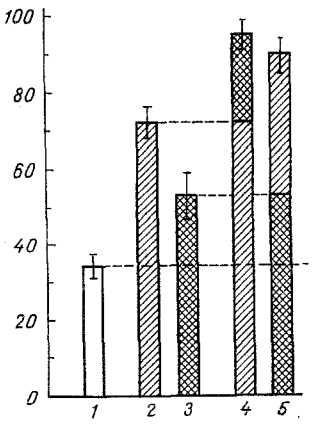

Fig. 3. Dynamics of BAEE-esterase activity of rabbit blood plasma (treated with chloroform for 1 min) during alternate activation with kaolin and streptokinase: 1) original plasma + chloroform, 2) original plasma + chloroform + kaolin (5 min), 3) original plasma + chloroform + streptokinase (5 min), 4) original plasma + chloroform + kaolin (5 min) + streptokinase (5 min), 5) original plasma + chloroform + streptokinase (5 min) + kaolin (5 min); Values of $M \pm m$ from nine experiments are given. Ordinate, quantity of BAEE hydrolyzed (in μmoles/ml plasma/h).

Considering that Hageman's factor and its fragment (prekallikrein activator) in the rabbit do not possess BAEE-esterase activity [12], an attempt was made to distinguish between kallikrein and plasmin arginine esterases. By monitoring kinin formation (Fig. 2), a marked effect of preliminary treatment of rabbit plasma with chloroform was demonstrated. However, the rate and degree of kinin formation in kaolin-activated plasma was still considerably higher in the case of rats. The results of the experiments on fibrin plates showed that if rabbit plasma was treated with kaolin or with chloroform and kaolin, no fibrinolytic activity was present in the samples. However, in samples first treated with chloroform and then activated with streptokinase, large zones of lysis were found ($S = 188$–282 mm^2). Contrical and SBTI largely or completely inhibited the arginine-esterase activity of rabbit plasma treated with chloroform and kaolin. An inhibitor blocking plasmin but not kallikrein activity, LBTI had a much weaker action. However, the results of the experiments with inhibitors varied considerably depending on the concentration of the inhibitor, the initial level of BAEE-esterase activity, the incubation temperature, and so on. In experiments with alternate activation of rabbit plasma with kaolin and streptokinase (Fig. 3), activation of plasma treated with chloroform and kaolin was shown to have no effect on the subsequent action of streptokinase (and vice versa).

It can be concluded from these results that arginine esterase, activated by chloroform and kaolin, is chiefly kallikrein, whereas that activated by chloroform and streptokinase is chiefly plasmin. These results provide a much more accurate basis for developing methods of investigating the level of prekallikrein, plasminogen, and the inhibitors of these enzymes from changes in the arginine esterase activity in rabbit blood plasma. The results indirectly confirm the presence of a functional connection between the kallikrein and plasmin systems of the blood at the level of Hageman's factor [10]. Brief treatment of rabbit blood plasma with chloroform probably removes the factor common to the kallikrein and plasmin systems which inhibits contact activation.

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