INVESTIGATION OF THE ESTEROLYTIC ACTIVITY OF HUMAN BLOOD PLASMA AFTER TREATMENT WITH KAOLIN AND STREPTOKINASE

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Kaolin and streptokinase independently activate two different BAEE-esterases* in human blood plasma, one of which is kallikrein and the other plasmin. The method suggested enables the correlation between prekallikrein and plasminogen to be assessed in various physiological and pathological conditions.

The ability of kallikrein and plasmin, two functionally connected blood enzymes, to hydrolyze the ester bond in N-substituted esters of L-arginine is used to estimate the activity of these enzymes [5, 8, 16, 18]. Colman et al., showed that incubation of native blood plasma with kaolin leads to a sharp increase in esterase activity strictly specific for kallikrein [9]. Incubation of blood plasma with streptokinase leads to the appearance of fibrinolytic, kinin-forming, and esterolytic activities characteristic of plasmin [3, 10, 12, 15].

Considering the functional dependence of the systems of kininogenesis and fibrinolysis it would be useful to have a single method of measuring the esterolytic activity of kallikrein and plasmin treated with kaolin or streptokinase and of assessing correlating changes in the blood levels of prekallikrein and plasminogen.

EXPERIMENTAL METHOD

Citrated human blood plasma obtained without contact with glass surfaces and frozen immediately after centrifuging to −20°C was used. The plasma was activated with kaolin under the conditions described previously [1]. Optimal conditions for the activation of blood plasma by streptokinase were verified in preliminary experiments. The activation was carried out with 1,000 units of streptokinase to 0.1 ml plasma in 0.1 M phosphate buffer, pH 7.6, at 25°C. The composition of the incubation mixture used to determine the enzyme activity (incubation for 20 min at 37°C) was: 0.1 ml of the original plasma or 0.2 ml of plasma activated by kaolin (by streptokinase), or 0.4 ml of plasma activated by kaolin and streptokinase together; 0.7 ml of 0.02 M BAEE (14 μmoles); 0.6 ml 0.1 M Tris-HCl buffer, pH 8.0; 0.9% NaCl solution to a volume of 1.7 ml. The subsequent course of the determination of activity was as described previously [1]. BAEE was used as the substrate for investigation of the esterolytic activity of kallikrein and plasma. Since the Michaelis constants with this substrate for these enzymes are close (1×10^{-4}–1.5×10^{-4} M for kallikrein and 3×10^{-4} for plasmin [11]), their esterase activity could be determined in these experiments by the hydroxamate methods [6] in the writers' modification [1].

Reagents: BAEE hydrochloride (A grade, Calbiochem, USA); streptokinase (Streptase, Behringswerke, West Germany), ε-aminocaproic acid (Chemapol, Czechoslovakia). The other reagents were of Soviet manufacture.

*BAEE: N-benzoyl-L-arginine-ethyl ester.

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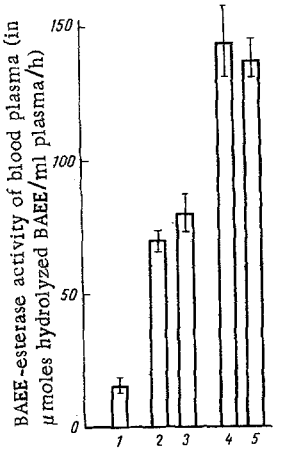

Fig. 1

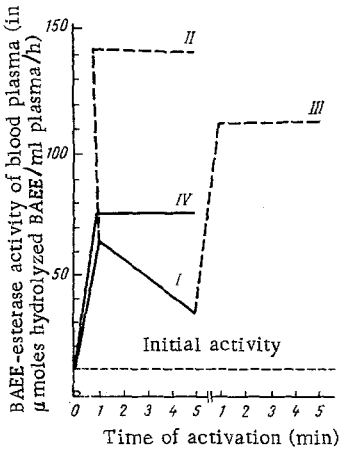

Fig. 2

Fig. 1. Esterase activity of human blood plasma after incubation with kaolin and streptokinase separately and together (mean results): 1) original unactivated plasma; 2) activation by kaolin (1 min); 3) activation by streptokinase (1 min); 4) activation by kaolin (1 min) followed by streptokinase (1 min); 5) activation by streptokinase (1 min) followed by kaolin (1 min).

Fig. 2. Dynamics of esterase activity of human blood plasma treated with kaolin after additional incubation with streptokinase (mean results of four experiments): I) activation by kaolin (control); II) activation by kaolin (1 min) + streptokinase; III) activation by kaolin (5 min) + streptokinase; IV) activation by streptokinase (control).

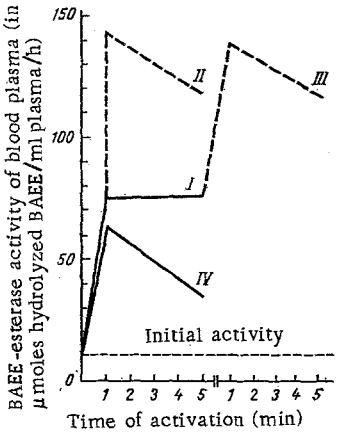

Fig. 3. Dynamics of esterase activity of human blood plasma treated with streptokinase, after additional incubation with kaolin (mean results of four experiments): I) activation by streptokinase (control); II) activation by streptokinase (1 min) + kaolin; III) activation by streptokinase (5 min) + kaolin; IV) activation by kaolin (control).

EXPERIMENTAL RESULTS AND DISCUSSION

Changes in BAEE-esterase activity of the blood plasma were demonstrated after separate treatment with kaolin and streptokinase. During incubation with kaolin the activity reached its maximum after 1 min: after incubation for 5 min the esterase activity was much reduced under the influence of the kallikrein inhibitor. The same pattern was described previously [8]. After treatment of the blood plasma with streptokinase the activity rose very rapidly and remained practically unchanged for 30 min. Only after incubation for 40-60 min did the BAEE-esterase activity fall, probably under the influence of "slow antiplasmins" [2].

During consecutive activation of plasmin by kaolin (incubation for 1 min) followed by streptokinase (and vice versa, streptokinase followed by kaolin) values of BAEE-esterase activity twice as high as those after separate or single activation by kaolin or streptokinase alone were obtained (Fig. 1). This means that treatment of blood plasma with kaolin or streptokinase activates independent BAEE-hydrolytic enzymes. To obtain further proof crossed activation tests were carried out. Streptokinase was added to samples treated with kaolin at the first and fifth minutes of incubation and the total activity was then measured after the next 1 and 5 min of incubation (Fig. 2). Conversely, kaolin was added to samples previously incubated with streptokinase at various measured time intervals (Fig. 3). The results in Fig. 2 show that the samples treated with streptokinase after kaolin, when tested after incubation for 1 and 5 min, still gave the curve characteristic of activation of the plasma by streptokinase only. The difference in absolute values of total ac-

tivity for the first and fifth minutes (Fig. 2, curves II and III) correspond to the degree of inhibition observed in the samples after incubation of the plasma with kaolin only (curve I). When the samples were treated with kaolin after streptokinase (Fig. 3) the character of activation observed during incubation of the plasma with kaolin alone was still maintained. In this case the total values of activity for the first and fifth minutes of incubation were virtually equivalent and the reduction in activity at the fifth minute (compared with the first) corresponds to the degree of inhibition observed in plasma treated with kaolin only (Fig. 3, curve I).

These results show that kaolin and streptokinase independently activate two different esterases in the blood plasma. In accordance with Colman's observations [9] the first is kallikrein, while the second, as many investigations [3, 5, 10, 15] and our own observations have shown, is evidently plasmin. Control experiments with ε-aminocaproic acid, a specific inhibitor of plasminogen activation, showed that this acid, in a concentration of 4-6 mM, abolished the activation of BAEE-esterase of the blood plasma by streptokinase. The original esterolytic activity as well as the esterase already activated were not blocked by these concentrations of ε-aminocaproic acid. Similar results were obtained with the use of other synthetic plasmin substrates [3, 4].

It is important to note the independent character of activation of kallikrein and plasmin by kaolin and streptokinase demonstrated by these experiments. The possibility of kininogenesis through the direct or indirect action of the plasmin formed has been reported by several writers[12, 13, 14, 17].

Buluk et al., [7] postulate the existence of two kallikreins in human blood plasma, one activated by the contact route (contact kallikrein), the other with the aid of plasmin (plasmin kallikrein). Measurements of the esterase activity after incubation of plasma with kaolin or streptokinase provides a means of determining the level of plasmin or kallikrein precursors and of assessing correlations between these two enzymes under various physiological and pathological conditions.

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