ACTIVATION OF KALLIKREIN AND THE KININOGEN LEVEL IN VENOUS AND ARTERIAL BLOOD OF RATS WITH ACUTE MYOCARDIAL ISCHEMIA

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Acute myocardial ischemia was produced in rats by ligation of the coronary artery. The concentration of prekallikrein and of kininogen I, determined by activation of the plasma with glass, was reduced in the venous blood after 30 min by 30%. These changes were preceded by activation of an esterase of nonkallikrein origin for the first minute of ischemia. A marked arterio-venous difference of kininogen I was found in ischemia. The kininogen II level was unchanged after 30 min. A tendency toward normalization of the indices of the kallikrein system was observed 24 h after the beginning of ischemia. The results point to early activation of the kinin system in acute ischemia of the heart.

There is clinical evidence of activation of the kinin system in acute myocardial ischemia and infarction [2-4, 17, 18]. Damage to the tissue and the change in hemostasis accompanying ischemic heart disease could be causes of the activation of kallikrein and the subsequent triggering of the kinin cascade. However, the early phases and the order of the stages of activation of the kinin system cannot be detected by clinical investigations.

Data on the state of prekallikrein, kallikrein, and its inhibitor and also on changes in the kininogen level during the first 30 min after the production of acute myocardial ischemia in rats are described in this paper.

EXPERIMENTAL METHOD

Noninbred rats weighing 200-300 g were used. Myocardial ischemia was produced by ligation of the left coronary artery by Selye's method [14] under acute (ischemia for 30 min) or chronic (24 h) experimental conditions. The onset of acute myocardial ischemia was monitored electrocardiographically and from disturbance of the contractile function of the heart.

Prekallikrein, "spontaneous" esterase activity, and kallikrein inhibitor were determined by Colman's method [7] with some modifications [2]. By this method, when the blood plasma is activated with kaolin, the values of three parameters can be determined simultaneously: 1) the initial "spontaneous" esterolytic activity of the plasma which includes, besides kallikrein, free activity of plasmin, thrombin, and other enzymes with esterolytic properties; 2) prekallikrein, reflecting the concentration of the kallikrein precursor; 3) kallikrein inhibitors. The esterase activity in the samples was determined by the degree of hydrolysis of N-benzoyl-L-arginine-ethyl ester (BAEE), with photometric estimation of the reaction products by the hydroxamate method [6].

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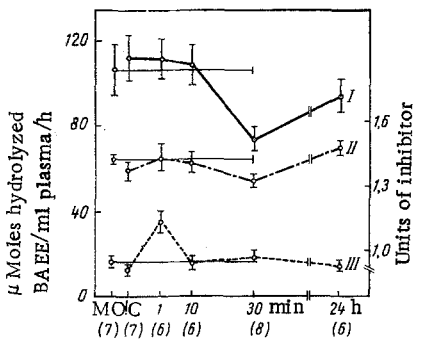

Fig. 1. Changes in prekallikrein (I), kallikrein inhibitor (II), and "spontaneous" esterase activity (III) in blood plasma of rats during development of acute myocardial ischemia (M±m). C and MO represent control animals and animals undergoing mock operation, respectively. Number of experiments at each time shown below in parentheses.

Determination of the level of kininogen, the inactive precursor of bradykinin, was carried out in arterial and venous blood separately. Since rat blood plasma contains two kininogens, the concentration of one of them was measured after treatment of the plasma with glass powder (activation of Hageman's factor), and the other after treatment of inactivated plasma with trypsin [1].

Citrated blood taken by puncture from the right and left ventricles was used in the experiments. Fresh plasma or plasma frozen to −20°C was obtained for the investigation. Blood was taken and centrifuged, the plasma was kept, and the samples were treated in plastic or silicone-treated vessels. Blood was taken once from each rat.

The following reagents were used: BAEE (Calbiochem, USA), bradykinin triacetate (Sandoz, Switzerland), and trypsin and o-phenanthroline (Spofa, Czechoslovakia). Other reagents were of Soviet manufacture.

EXPERIMENTAL RESULTS AND DISCUSSION

The data in Fig. 1 show that 1 min after ligation of the coronary artery the "spontaneous" esterase activity was increased by 2-3 times (P = 0.02). After 10 and 30 min this activity was back to its original level. The prekallikrein concentration was lowered by 30% only after myocardial ischemia had lasted 30 min (P < 0.05). Changes in kallikrein inhibitor were not significant; only a tendency was observed for its activity to decrease after 30 min.

The increase in "spontaneous" activity after 1 min of ischemia while the prekallikrein level still remained unchanged is evidence of activation of an esterase of nonkallikrein origin. The decrease in the prekallikrein concentration after 30 min points to the formation of the active enzyme. Activation of kallikrein coincides with a decrease in the kininogen concentration (Table 1); i.e., it leads to the formation of bradykinin. Allowing for the specific features of Colman's method, the values of the kallikrein inhibitor measured in these experiments can be taken to reflect interaction between kallikrein, plasmin, thrombin, and their common inhibitor [10]; i.e., they ultimately show the content of free inhibitor and its ability to regulate equilibrium in the system of kininogenesis. This evidently explains the absence of sharp changes in "spontaneous" activity and in inhibitor activity after 10 and 30 min of acute myocardial ischemia. These results thus demonstrate activation of the kallikrein system in the first 30 min after the production of acute myocardial ischemia in the rat. After 24 h the prekallikrein concentration rose but still remained below its initial level. Inhibitor activity was increased. These results show a tendency for equilibrium to be restored in the kallikrein system.

As the results given in Table 1 show, 30 min after the onset of myocardial ischemia the concentration of kininogen I activated by glass in the venous blood was reduced by 36% whereas the level of the other

TABLE 1. Concentrations of Kininogens I and II in Plasma of Venous and Arterial Blood of Rats under Normal Conditions and 30 Min after Ligation of the Coronary Artery (M±m)

Blood	Kininogens	Concentration of kininogen (μg bradykinin/ml plasma)		P
		control	acute ischemia for 30 min	
Venous	I (activation of plasma by glass)	0,83±0,12 (8)	0,53±0,07 (7)	<0,05
	II (hydrolysis with trypsin)	1,75±0,36 (4)	1,74±0,17 (12)	>0,1
Arterial	I	0,88±0,14 (8)	0,76±0,10 (7)	>0,1
	II	1,53±0,27 (5)	1,55±0,14 (15)	>0,1

Note. Number of determinations shown in parentheses.

kininogen (II) was not significantly changed. This is evidence of differences in the functions of the two kininogens in rat blood. In other pathophysiological situations similar results have been obtained [5, 8]. Other authors also have postulated differences in the functions of the kininogens in rat blood [11, 13].

One of these kininogens is destroyed by the kallikrein of the plasma during contact activation of Hageman's factor. Since no enzyme specific for the other kininogen has been found in rat blood, it can be assumed that its destruction and bradykinin formation take place during contact with tissue kallikrein; i.e., they are a manifestation not of generalized, but of local activation of the kinin system. It can also be considered that the second kininogen, which remains intact in the presence of ischemia, is a "reserve" form of the first "labile" kininogen. Habermann [9] has emphasized the importance of the tertiary structure of the kininogen molecule for its interaction with the various kininogens. The change in the molecular structure of the kininogen under the influence of specific conditions may be a factor in its conversion from the "reserve" into the "labile" form.

The concentration of kininogen I in the arterial blood after ischemia of the heart for 30 min was not significantly different on the control level and was 30% higher than in the venous blood of rats with myocardial ischemia ($P = 0.05$). The concentration of kininogen II was not significantly changed in all the blood samples. This means that as the blood passes through the lungs the level of kininogen I returns almost to its initial value. A higher kininogen level in the arterial than in the venous blood has also been described by other workers in rats with anoxia and dogs with local ischemia of the limb [15], in tissue ischemia in man [16], and during pregnancy [12]. Since resynthesis of kininogen is known to take place in the liver, and since the rate of this process is slow, this fact has not been satisfactorily explained and further study is required.

LITERATURE CITED

1. O. A. Gomazkov and L. V. Bol'shakova, Byull. Éksperim. Biol. i Med., No. 3, 10 (1971).
2. O. A. Gomazkov, N. V. Komissarova, L. V. Bol'shakova, et al., Kardiologiya, No. 6, 25 (1972).
3. A. A. Dzizinskii and A. D. Kuimov, in: Enzymes in Coronary Heart Disease [in Russian], Kishinev (1971), p. 45.
4. L. T. Malaya et al., in: Enzymes in Coronary Heart Diseases [in Russian], Kishinev (1971), p. 39.
5. D. Armstrong and G. L. Mills, in: E. G. Erdös (editor), Hypotensive Peptides, New York (1966), p. 139.
6. E. A. Brown, J. Lab. Clin. Med., 55, 616 (1960).
7. R. Colman et al., Ann. Intern. Med., 71, 763 (1969).
8. K. M. Gutvik et al., J. Physiol. (London), 197, 22 (1968).
9. E. Habermann, in : E. G. Erdös (editor), Handbook of Experimental Pharmacology, Vol. 25, Berlin (1970), p. 250.
10. P. Harpel, J. Exp. Med., 132, 329 (1970).
11. J. Lecomte, Bull. Acad. Roy. Med. Belge., 9, 563 (1969).
12. P. Periti and F. Gaspari, in: E. G. Erdös (editor), Hypotensive Peptides, New York (1966), p. 536.
13. A. M. Rothschild and A. Castania, Pharmacol. Res. Commun., 2, 45 (1970).
14. H. Selye, Angiology, 11, 398 (1960).
15. K. Scharnagel and K. Greef, Arch. Exp. Path. Pharmak., 253, 81 (1966).
16. F. Sicuteri et al., in: E. G. Erdös (editor), Hypotensive Peptides, New York (1966), p. 522.
17. F. Sicuteri et al., Mal. Cardiovasc., 8, 343 (1967).
18. B. Wiegershausen et al., Dtsch. Gesundh.-Wes., 38, 1785 (1968).