THE KININ SYSTEM OF RAT VENOUS AND ARTERIAL BLOOD PLASMA

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UDC 612.113/-114:612.124:547.962.4

The kininogenase activity and kininogen concentration were studied in rat venous and arterial blood plasma. Venous blood plasma, activated by contact with glass, possesses higher kinin-liberating activity. Two kininogens are found in rat blood plasma: one is activated by contact with glass, the other, after exhaustion of the first, is activated by trypsin. The plasma concentration of the second kininogen is 1.5 times higher than that of the first. No significant difference is found between normal venous and arterial blood in the ratio between their kininogen components.

The suggestion has recently been made [11] that human blood plasma contains two kinin systems. Model experiments have shown that system I is activated specifically by acidification of the medium and by pancreatic kallikrein, while system II is activated by Hageman's factor on contact of the plasma with negatively charged surfaces. It is generally accepted that rat blood plasma contains only system II, for bradykinin is formed by contact of the plasma with glass but is not formed by the action of pancreatic kallikrein [1, 4, 8, 11]. Rat blood plasma can therefore be regarded as a convenient object for study of the characteristics of the kinin system separately in arterial and venous blood.

Because of the role of biologically active polypeptides in a number of pathological processes in the circulatory system, including in central vascular reactions (hypo- and hypertension, shock, etc.), the present investigation was undertaken to investigate the kinin systems separately in rat venous and arterial blood.

EXPERIMENTAL METHOD

Fresh blood plasma from male rats weighing 250-300 g was used in the experiments. Arterial and venous blood samples were taken by puncture from the left and right ventricles respectively. The plasma was separated by centrifugation at 8°C. Activation of the plasma with glass was carried out by allowing contact of the incubation mixture for 10 min with Ballotini P-3 powder (England) with a particle diameter of 0.1 mm. The surface of the glass was previously activated with acid. The incubation mixture was of the following composition: 0.5 ml plasma, 0.7 ml tris-buffer (0.2 M, pH 7.8), 0.3 ml of 1×10^{-2} M solution of o-phenanthrolin (or water). Incubation temperature 25°C. The specimens were fixed by deep freezing or heating in an acid medium, or were treated immediately on guinea pig's intestine.

Before activation with trypsin (Spofa, Czechoslovakia), the plasma was treated by Diniz's method [3] by acidification with acetic acid, boiling for 30 min a water bath, and neutralization with NaOH. The optimal quantity of trypsin (500 μg to 0.5 ml plasma) and absence of appreciable contamination with kinotrypsin were verified by control tests with a gradually increasing quantity of enzymes.

Obtaining the blood and also subsequent procedures were carried out in synchronized vessels. The quantity of bradykinin formed was determined on a segment of guinea pig intestine and compared with standard bradykinin (Sandoz, Switzerland), and expressed in μg/ml plasma.

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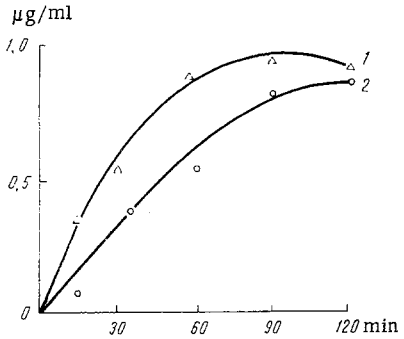

Fig. 1. Liberation of kinin in plasma of venous (1) and arterial (b) blood of rats after activation by contact with glass surface (conditions of incubation given in text). Abscissa, incubation time (in min); ordinate, quantity of bradykinin formed (in μg/ml plasma).

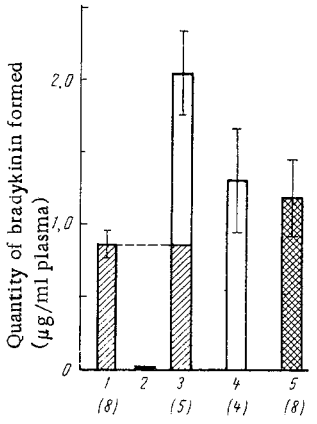

Fig. 2. Kininogen in rat arterial blood plasma when activated under different conditions: 1) plasma activated by glass (+phenanthrolin); 2) plasma activated by glass (without phenanthrolin); 3) plasma activated by glass and trypsin (+phenanthrolin; 4) plasma activated by glass and trypsin (without phenantrholin; 5) plasma treated by Diniz's method (activation by trypsin). Number of experiments given in parentheses.

EXPERIMENTAL RESULTS AND DISCUSSION

Incubation of the blood plasma with glass leads to activation of kininogenase, the enzyme catalyzing the conversion of kininogen into the active product, kinin. To assess the kinetics of this process experiments were carried out with venous and arterial blood plasma activated with glass. The gradual accumulation of kinin in the incubation mixture, reaching a maximum after 90-120 min, is shown in Fig. 1. Destruction of the kinin thus formed, by the specific kinases present in the plasma, was blocked by o-phenanthrolin. The final quantity of kinin accumulating in the incubation medium showed that the content of kininogen in the arterial and venous blood samples was identical. However, the activity of the kininogenase, the enzyme converting kininogen into kinin, was higher in the venous blood. The course of the curves 1 and 2 in Fig. 1 illustrates the kinetics of kininogenase activity of venous and arterial blood when activated by contact with glass.

To verify that all the kinin had been liberated under these conditions, at the end of the incubation period the rat blood plasma was treated with acetic acid, heated on a boiling water bath, and activated with trypsin. This treatment led to the liberation of a large quantity of kinin (Fig. 2: 1 and 3). The quantity of this additional kinin thus formed was roughly the same in the venous and arterial blood.

However, it could be suggested that during incubation of the plasma activated by glass not all the kinin was liberated (because of inhibition of the reaction by inhibitors or by accumulation of the product). The fraction of kininogen remaining untouched was destroyed by trypsin. To test this hypothesis, plasma activated by glass was incubated without phenanthrolin, i.e., the kinin in the incubation medium was formed and destroyed at the same time. In the second stage this plasma was treated with acid, heated, and activated by trypsin. The quantity of kinin thus formed (1.31 ± 0.36 μg bradykinin) was about equal to the difference between the total (2.03 ± 0.29 μg; Fig. 2: 3) and the amount accumulating after activation by glass (0.85 ± 0.105 μg; Fig. 2: 1), i.e., it was equal to the "trypsin" part of the total product (the unshaded part of column 3 in Fig. 2).

At the same time the total quantity of kininogen in the plasma activated by trypsin, but not treated with glass, was determined. The plasma was treated by the method of Diniz [3]. The quantity of kinin obtained in this case (1.14 ± 0.26 μg bradykinin) was much less than the total quantity liberated by glass and trypsin. It was approximately equal to the "trypsin" fraction of the kinin obtained by activation of the plasma with trypsin after treatment with glass (Fig. 2: 5). The kininogen content in the venous and arterial blood was the same.

These experiments thus showed that after complete liberation of kinin as a result of activation of plasma by glass, trypsin liberates a further large quantity of the product. It is evident that two different kininogens and two different kininogenases are present in rat plasma, because neither trypsin nor the kininogenase activated by glass can produce complete exhaustion of the kininogen. In addition, the kininogenase activated by glass is not blocked by trasylol, a powerful inhibitor of trypsin and other kininogenases [2]. Moreover, the kininogen in rat plasma activated by trypsin is not identical with the kininogen I in human blood plasma, for the substrate of rat plasma is not activated by pancreatic kallikrein [4, 11].

Jacobsen postulated the existence of two kininogens in blood plasma [6]. Virtually the same conclusion was reached by Karoti et al. [7]. The facts described above cast doubts on the widely held view that only one kinin system is present in rat blood. The results also show that Diniz's method, which is widely used to determine the kininogen content and to assess the "power" of the kinin system in the body, cannot give a complete picture of the kininogen content in the blood, because the specificity of trypsin extends to only one substrate of the kinin system. This evidently applied also to the kininogen determined in human blood: according to the scheme proposed by Vogt et al. [11], trypsin cannot act directly on kininogen II. It acts on kininogen I or, like Hageman's factor, activates kininogenase II which, however, is destroyed when the plasma is treated by Diniz's method.

The special position of the kinin system in venous blood is mentioned in several publications. Kininogen is formed in the liver [5] and enters the venous circulation. The greater part of the active bradykinin is destroyed by kininases in the lungs, on the borderline between the venous and arterial systems [9, 10]. The present investigation has demonstrated the higher kininogenase activity of venous blood compared with arterial blood, although the content of kininogen activated by glass and by trypsin was equal. The results suggest that the venous system is the chief place of function of kinins in the normal circulatory system. These relationships may be different under pathological conditions or during prolonged physiological adaptations.

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