ROLE OF THE LIVER IN PRODUCTION OF THERMOSTABLE ANTIFACTOR Xa

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KEY WORDS: clotting inhibitors; biosynthesis; liver.

The thermostable inhibitor of thrombin production is a compound of protein nature with mol. wt. 210,000-235,000 daltons, which preserves its activity on heating for 1.5 h at 60°C. Human and albino rat blood serum, if heated under these conditions, inhibits thrombin formation in a system of prothrombin complex–tissue thromboplastin–calcium ions. Inhibition of thrombin formation under these conditions is completely reversible: The values of the velocity constants of formation and breakdown of the enzyme–inhibitor complex are 0.27 and 0.17 respectively. Analysis of the kinetics of inhibition showed that it is incompletely mixed, as indicated by values of the interaction constants: α is greater than unity, but far from infinitely high values, whereas β is equal to zero [2, 3]. Experiments with isolated factors concerned in thrombin production showed that only the action of factor Xa is limited by the test inhibitor. This means that the thermostable serum inhibitor can be regarded as antifactor Xa [6]. According to several properties (thermostability, molecular weight, behavior toward calcium and adsorbent, kinetics of inhibition, character of the change in concentration under external influences) the antifactor Xa described above differs from other inhibitors of thrombin formation hitherto known [5] and, in particular, from antithrombin III [8]. An important distinguishing feature of thermostable antifactor Xa is its dependence on vitamin K [1], and its high sensitivity to dilution, which makes purification of this inhibitor difficult. For instance, by ion-exchange chromatography (on DEAE-cellulose), purification by 114 times could be achieved but with loss of 98.7% of the initial activity [5].

The content of thermostable antifactor Xa in donors' blood serum changes with age and depends on sex [7], and it undergoes significant changes when exposed to procedures leading to hypo- or hypercoagulemia [5]. During partial resection of the liver the content of inhibitor in the blood serum is reduced very significantly [1]. This may perhaps be explained by the role of the liver in the production of antifactor Xa. The investigation described below was devoted to the study of this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes (weighing 120-180 g), kept on a standard diet. When the effect of carbon tetrachloride on the concentration of antifactor in the serum was studied the poison was introduced through a gastric tube (0.15 ml twice, at an interval of 2 days, with samples taken on the 8th day, or a single dose of 0.5 ml, with samples taken on the 2nd day), and by inhalation (0.5 ml in 8 liters air in an airtight chamber, three inhalations each lasting 15 min, at intervals of 2 days, with samples taken on the 8th day). To determine the content of antifactor in the liver and other organs the tissue was minced in a Potter's homog-

TABLE 1. Content of Antifactor Xa in Tissue Extracts of Organs (M ± m)

Organ tested	Content of antifactor Xa	
	units/ml extract	units/g tissue
Liver	0,46±0,09	4,5±0,9
Kidney	0,016±0,002	0,16±0,02
Lung	0,0	0,0
Spleen	0,08±0,01	0,80±0,1
Muscle	0,12±0,07	1,2±0,7

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enizer (0.5 g in 2 ml 0.14 M sodium chloride solution), then diluted to 10 ml with the same solvent, and exposed for 30 min. When the ability of surviving liver slices to synthesize the antifactor was studied, 0.5 g of liver slices was incubated to 10 ml Hanks' medium (medium No. 199), and the content of antifactor in the medium was determined periodically. The possibility of the appearance of antifactor Xa in the perfusion fluid also was investigated during perfusion of the liver with 0.14 M sodium chloride solution through the descending part of the thoracic aorta, by means of an "Elmed" peristaltic pump (at the rate of 5 drops/min). The outflowing fluid was collected from the inferior vena cava. In this series of experiments some of the animals received Pelentan (from "Spofa") in a dose of 7 mg/100 g. Homogenization, preparation of the slices, and perfusion of the liver were preceded by removal of all blood from the blood vessels by perfusion with 10 ml of 0.14 M sodium chloride solution (37°C) into the left jugular vein (blood flowed out from the same vessel on the right side). Extracts of the homogenates, the Hanks' medium, and the perfusion fluid were clarified by centrifugation (15,000 rpm, 15 min) and their pH was adjusted to 7.2. The concentration of antifactor was determined as described above. The circulating blood volume was determined by the isotope dilution method, using albumin-^{131}I.

EXPERIMENTAL RESULTS

After administration of CCl_4 by gastric tube in a dose of 0.15 ml the serum concentration of antifactor Xa fell by 15.1% (control 1.92 ± 0.13, experiment 1.63 ± 0.17 unit/ml; $P < 0.05$). With an increase in the dose (0.5 ml) the serum concentration of antifactor was 29.9% below the control level by the 2nd day (control 0.97 ± 0.06, experiment 0.68 ± 0.08 unit/ml; $P < 0.05$).

In the case of poisoning by inhalation, the concentration of inhibitor was reduced after the 3rd inhalation by 41.1% (control 1.41 ± 0.08, experiment 0.83 ± 0.09 unit/ml; $P < 0.05$), and it still remained 17.2% below the control level 8 days after the last inhalation.

Since CCl_4 damages primarily the functional liver cells [4], this suggests that the decrease in serum concentration of antifactor was due to limitation of its production.

When the results of investigations of serum and of extracts from liver homogenates were compared it was found that, calculated per gram tissue, the content of antifactor Xa in the liver was 3.4 times higher than in the serum (6.0 ± 0.6 and 1.74 ± 0.08 units/ml respectively). The total serum concentration of inhibitor, taking into account the results obtained by determination of the blood volume and hematrocrit index, was 8.91 ± 0.3 units and the total content of antifactor Xa in the liver (the weight of which was determined in 10 animals), was 33.66 ± 2.3 units, i.e., 3.8 times higher.

The concentration of antifactor Xa found in the perfusion fluid from intact animals was 0.82 ± 0.02 unit/ml compared with 0.26 ± 0.08 unit/ml in the animals receiving Pelentan. This confirms yet again the possible role of the liver in the synthesis of antifactor Xa and is further evidence of its dependence on vitamin K.

After incubation of liver slices in Hanks' medium, antifactor Xa was found in the medium in a concentration of 0.41 ± 0.04 unit/ml; consequently, one gram of liver secretes into the medium on average 8.2 units per gram tissue per hour of incubation, i.e., somewhat more than can be extracted from the liver homogenate. Liver tissue under conditions of survival evidently goes on producing antifactor Xa for some time. With an increase in the times of incubation, no increase in antifactor concentration was observed in the medium.

Comparison of the content of antifactor Xa in extracts of homogenates of the liver and other organs revealed that the liver occupies an exceptional place from this point of view (Table 1).

If the content of inhibitor in the liver is taken as 100%, its content in the kidneys, spleen, and muscle is 3.5, 17.7, and 26.7% respectively. Since the blood vessels of the animals had previously been freed from blood by perfusion, there are no grounds for attributing the inhibitor found in the organs to the blood present in them. The only exception is the muscle and spleen, which cannot be completely freed from blood because of their well-developed capillary network.

The results as a whole confirm the view that the liver plays a role in the production of antifactor Xa.

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DYNAMICS OF VASCULAR RESPONSE OF THE SMALL INTESTINE IN ANIMALS AT HIGH ALTITUDES

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Various functional systems are involved in adaptive reactions of man and animals to extremal conditions of life at high altitudes in the mountains, more especially respiration, the circulation, and the blood, which form a single complex responsible for providing the body with oxygen. The main weight of this burden is taken by the cardiovascular system [1, 4, 6, 8, 14]. However, under conditions of natural high-mountain hypoxia only purely hemodynamic changes have been studied [5, 8, 12], and vasomotor reactions at the regional level have been virtually ignored [7].

The aim of this investigation was to study the character and magnitude of responses of resistive and capacitive vessels of the small intestine to regional injection of adrenalin in the course of adaptation to high altitudes.

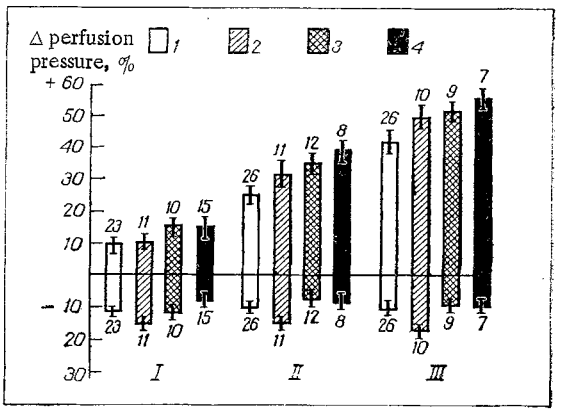

Fig. 1. Changes in responses of resistive vessels of small intestine to intra-arterial injection of adrenalin at different times of adaptation of animals to high-altitude conditions. Ordinate, changes in perfusion pressure (in % of initial level): with + sign, vasoconstrictor, with – sign, vasodilator responses. I) Injection of adrenalin in dose of 0.5 μg, II) 5 μg, III) 10 μg. 1) Control; 2) after adaptation for 3 days, 3) for 15 days, 4) for 30 days. Vertical lines show confidence interval at $P = 0.05$. Numbers inside triangles denotes number of observations.

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