PLASMA ANTICOAGULANT AND NONENZYMIC FIBRINOLYTIC ACTIVITY AFTER INTRAVENOUS INJECTION OF HEPARIN–ANTITHROMBIN III COMPLEX INTO ANIMALS

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When a protective response of the anticlotting system to the appearance of the enzyme thrombin in the circulating blood is excited, the heparin which is released into the blood flow forms a complex with the thrombogenic blood proteins and biogenic amines [1, 7]. Complexes of heparin with thrombin, fibrinogen, fibrin-stabilizing factor, plasminogen, adrenaline, and many other biologically active compounds possess anticoagulant properties and dissolve unstabilized fibrin [3, 8]. It is also known that antithrombin III (AT III), an inhibitor of several of the blood proteinases, neutralizes activity of the enzyme thrombin by forming a complex with it [10]. The reaction of complex formation between thrombin and AT III is considerably accelerated by heparin. The mechanism of action of heparin under these circumstances is varied and may evidently proceed in serveral ways: Heparin may react with AT III, may form a complex with thrombin and, finally, may interact with a preformed complex of thrombin with AT III [7, 10]. Preliminary interaction of the inhibitor with heparin before formation of thrombin–AT III complex includes conformational structural changes in the latter which activate complex formation and thus bring about more rapid neutralization of the enzyme [6]. Complex formation of heparin with antithrombin III (H–AT III) has been effected in experiments *in vitro*. These components take part in the reaction of complex formation in different proportions by weight. The resulting complexes, with components H and AT III in proportions of 1:1 to 1:7 by weight, possessed well marked anticoagulant activity and caused lysis of unstabilized fibrin *in vitro*.

The aim of this investigation was to study the character of action of H–AT III complexes on the anticoagulant potential and on nonenzymic fibrinolytic activity of blood plasma when injected intravenously into animals.

EXPERIMENTAL METHOD

To obtain the H–AT III complex a preparation of AT III was isolated from bovine blood by the method in [5], and a purified preparation of heparin or heparin from Spofa (Czechoslovakia) was used. The H–AT III complex was obtained by the method described previously [3], with components in the ratio of 1:3 by weight. Experiments were carried out on healthy male rats weighing 170-180 g. The animals were divided into four groups: 1) control animals receiving 1 ml of 0.85% NaCl solution intravenously, 2) animals receiving 1 ml of a solution of AT III intravenously, in a dose equivalent to its content in the complex, 3) animals receiving 1 ml of heparin intravenously in a dose equivalent to its content in the complex, and 4) experimental animals receiving 1 ml of a 0.1-0.2% solution of the H–AT III complex intravenously. The intravenous injections of the preparations of the complex and components into the animals was given into, and blood samples were taken from the jugular vein. Blood for analysis was taken with sodium citrate in the ratio of 9:1, 7 and 30 min after injection of the preparations. The thrombin time of the plasma from blood samples taken from the experimental animals was determined in the usual way. To determine the anticoagulant activity of the complex *in vitro*, represented by the time of clot formation after addition of thrombin to the reaction mixture, a 0.2% solution of fibrinogen was used as substrate. Total and nonenzymic fibrinolytic activity (TFA and NEFA, respectively) of the plasma and preparations of the complexes were

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TABLE 1. Anticoagulant and Nonenzymic Fibrinolytic Activity (NEFA) of 0.1% Solution of Heparin–AT III Complex (1:30)

Substances injected	Anticoagulant activity	NEFA, mm^2
H – AT III complex (1:3)	>120 мин	90±8,5
Control solution:		
Phosphate buffer, pH 7.4	19±0,64c	0
AT III in dose equivalent to its content in complex	30±3,9c	7±1,9
Heparin in dose equivalent to its content in complex	26±3,1c	0

TABLE 2. Thrombin Time, TFA, and NEFA of Rat Plasma 7 and 30 min after Intravenous Injection of 0.1-0.2% Solution of H–AT III Complex (1:3)

Substances injected	No. of animals	Time after injection, min					
		7	30	7		30	
		thrombin time, sec		TFA, mm^2	NEFA, mm^2	TFA, mm^2	NEFA, mm^2
0.85% NaCl solution	11	13,6±0,2	13,5±0,2	32,5±4,3	23,5±3,6	28,0±3,6	20,0±3,9
AT III in dose equivalent to its content in complex	5	15,0±0,9	14,1±0,8	32,0±3,4	27,0±3,2	—	—
P		<0,05	>0,05	>0,05	>0,05		
Heparin in dose equivalent to its content in complex	12	34±0,09	25±0,05	45,0±5,6	32,5±3,8	38,0±3,8	25,0±4,5
P		<0,001	<0,001	≤0,05	≤0,05	≤0,05	>0,05
0.1-0.2% solution of H – AT III complex	17	No clotting aft. more than 30 min	No clotting aft. more than 30 min	50,0±4,6	44,0±5,1	48,8±4,0	43,0±6,5
P		<0,001	<0,001	<0,001	<0,001	<0,001	<0,001

investigated by the method in [2]. Solutions of complexes were freshly prepared for injection into the animals. For this purpose, the residue of the complex 20-30 min after its formation was separated by centrifugation. The supernatant was poured off and the residue dissolved in 0.053 M phosphate buffer, pH 7.2-7.4, to yield a 0.1-0.2% solution of the complex.

EXPERIMENTAL RESULTS

Anticoagulant activity and NEFA of the preparations of H–AT III complexes were first determined *in vitro*. As Table 1 shows, the addition of 0.1 ml of a 0.1% solution of the complex to the reaction mixture when determining anticoagulant activity inhibited the formation of fibrin clots for 120 min of incubation at 37°C. NEFA of the complexes, determined on disks of unstabilized fibrin, reached values of 80 to 100 mm^2 for zones of lysis in both the presence and absence of ε-aminocaproic acid, an inhibitor of enzymic fibrinolysis, in the medium.

It will be clear from Table 2 that after injection of a solution of the complex the thrombin time of plasma of the experimental animals (group 4) showed a marked increase compared with its value in the rats of groups 1-3. Injection of heparin in a dose equivalent to its content in the complex also caused some lengthening of the thrombin time, but it was much less marked than the effect of change in experimental animals after injection of a solution of the H–AT III complex. The action of heparin on the thrombin time of plasma clotting 30 min after the injection was much weaker, whereas the action of the complex was completely preserved.

Similar changes after intravenous injection of the complex also were observed with respect to total and nonenzymic fibrinolytic activity of the blood plasma (Table 2) and an increase in the degree of nonenzymic fibrinolysis was discovered in plasma from blood samples taken 7 and 30 min after injection of the complex up to 187 and 215%, respectively, compared with the control animals of group 1. In animals receiving a solution of heparin intravenously, 7 min after the injection some increase in nonenzymic fibrinolysis was observed up to 138% compared with the control (group 1). This is quite easily explained on the

grounds that after injection, exogenous heparin can combine with several blood proteins to form complexes which create an increase in NEFA in the plasma. However, unlike the experimental animals of group 4, the degree of NEFA in rats of group 3 after injection of heparin rose briefly but then began to fall, and 30 min after injection this parameter was much lower in value than in the animals after injection of the complex.

The results are evidence that intravenous injection of the H–AT III complex considerably enhances the anticoagulant background and potentiates the nonenzymic fibrinolytic activity of the animals' plasma. According to our data, intravenous injection of this complex may largely neutralize activity of the enzyme thrombin, unlike injection of AT III alone into the blood stream [5]. These data are evidence that AT III is one of the components of the anticlotting system of the body responsible for controlling the liquid state of the blood through limitation of thrombin production and lysis of unstabilized fibrin clots which have formed in the blood stream.

LITERATURE CITED

1. B. A. Kudryashov, Biological Problems of Regulation of the Liquid State of the Blood and Its Clotting [in Russian], Moscow (1975).
2. B. A. Kudryashov and L. A. Lyapina, Vest. Mosk. Univ., Ser. Biol., No. 4, 3 (1973).
3. B. A. Kudryashov, V. E. Pastorova, and L. A. Lyapina, Biokhimiya, No. 11, 2024 (1981).
4. V. E. Pastorova and S. V. Kovalev, Vopr. Med. Khim., No. 2, 240 (1975).
5. U. Abildgaard, Scand. J. Clin. Lab. Invest., 21, 89 (1968).
6. R. D. Feinman and E. H. H. Li, Fed. Proc., 36, 51 (1977).
7. B. A. Kudryashov (B. A. Kudrjashov), Thrombos. Haemostas., 4A, 459 (1979).
8. B. A. Kudryashov and L. A. Lyapina (V. A. Kudrjashov and L. A. Liapina), Folia Haemat. (Leipzig), 95, 272 (1971).
9. E. Marciniak, Thrombos. Haemostas., 38, 466 (1977).
10. R. D. Rosenberg, Fed. Proc., 36, 10 (1977).

MECHANISM OF THE CEREBROVASCULAR EFFECT OF PYRACETAM

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According to data in the literature [2, 7] pyracetam increases the blood supply to the brain both in intact cats and in animals with mild hypoxia. The increase in the cerebral blood flow produced by pyracetam is also found in patients with chronic cerebrovascular insufficiency [6]. However, the mechanism of the effect of the drug on the cerebral circulation has not yet been established. In particular, we have no information on the effect of pyracetam on nervous control of the cerebral blood supply. At the same time, it has been found that GABA, the natural analog of pyracetam, takes part in the realization of the cerebrovascular effects of GABA-ergic substances [3, 5]. For the reasons given above it was decided to study the effect of pyracetam on nervous control of the cerebral circulation with an analysis of the role of GABA in its cerebrovascular effects. Another aim of the investigation was to study the effect of pyracetam on the cerebral hemodynamics under conditions of hemorrhagic shock.

EXPERIMENTAL METHOD

Experiments were carried out on 31 cats weighing 3-4 kg under general anesthesia (urethane, chloralose) with artificial ventilation of the lungs, and on 10 unanesthetized rabbits

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