ANTIPLASMIN AND ITS ACTION ON THE PROCESS OF FIBRINOLYSIS UNDER EXPERIMENTAL CONDITIONS

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In the very first studies of the fibrinolytic system, it was noted that the plasma and serum may neutralize the characteristic proteolytic effect of the active enzyme plasmin (cited in [6]). However, the nature of these inhibitors of the fibrinolytic process, as well as their effect upon the process of fibrinolysis, is insufficiently clear. In the opinion of Ratnov et al. (cited in [6]), several inhibitors of fibrinolysin, which possess different physicochemical properties and exert different effects upon the process of fibrinolysis, are found in human blood.

Norman and Hill [7] have established that two inhibitor components of plasmin, found in the α_1- and α_2-globulin fractions of the blood, are present in normal plasma.

An investigation of the inhibition of the process of fibrinolysis is just as vital as the study of questions related to its activation. In surgical and obstetric-gynecological practice, states of increased fibrinolytic activity of the blood are frequently encountered, leading to acute "hypofibrinogenemia," which frequently has a lethal outcome as a result of continuous hemorrhage.

In this work, we studied the effect of antiplasmin upon the fibrinolytic activity of the plasma and the activity of a preparation of plasmin.

EXPERIMENTAL PROCEDURE

The antiplasmin preparation was produced from bovine plasma by salting with ammonium sulfate [5] and freeze dried. The preparation, readily soluble in physiological saline, was administered to animals intravenously (v. juglaris) or added in definite concentrations to samples in experiments in vitro.

The plasmin preparation was produced by activating plasminogen, isolated from human plasma, with a solution of streptokinase (commercial preparation of distreptase, with activity 25,000 units per 5 ml of solution). Plasminogen (80 mg) was dissolved in 20 ml of physiological saline at pH 3.0 for 30-40 min, after which the pH was adjusted to 6.8 with 1 N solution of NaOH. Then the plasminogen solution was activated with a solution of a preparation of streptokinase in a dose of 0.1 ml of the streptokinase solution per 10 ml of plasminogen solution at 37° for 15 min. The activity of the plasmin obtained was determined during lysis of a standard plot of fibrinogen (fraction 1 of Kohn) in the following reaction: 0.2 ml fibrinogen solution + 0.2 ml plasmin solution + 0.2 ml thrombin solution (containing no plasminogen, activity 11 sec).

The clotting time of whole blood was determined according to Lee and White. The fibrinolytic activity and fibrinogen concentration in the blood plasma were established according to Bidwell's method. The experiments were conducted on white rats of both sexes, weighing 170-180 g.

EXPERIMENTAL RESULTS

In experiments in vitro, it was established that the preparation of antiplasmin obtained inhibits the fibrinolytic activity of the plasma. Thus, in the investigation of 25 samples after the addition of antiplasmin in a dose of 2 mg per sample, this index is equal to 6.5% (in the control 27%, number of samples 25).

TABLE 1. Fibrinogen Concentration and Fibrinolytic Activity of the Blood in Normal Animals 1, 5, and 30 Min After Injection of Antiplasmin

Group of animals	No. of animals	Fibrinogen concentration (in mg %)			Fibrinolytic activity (in %)		
		1 min	5 min	30 min	1 min	5 min	30 min
Experimental	35	429	425	385	4,7	35,5	27,0
Control (physiological saline)	30	423	411	390	24,0	13,8	9,0

TABLE 2. Clotting Time of Whole Blood (in sec), Taken from Animals Before, After 40-50 Sec, and 5 Min After Intravenous Injection of Antiplasmin

Group of animals	No. of animals	Before injection	After 40-60 sec	After 5 min
Experimental (5 mg antiplasmin)	15	70	56	109
Control (physiological saline)	12	73	74	70

In the experiments in vitro, it was found that, when plasminogen is activated with streptokinase, the activity of the plasmin formed is substantially reduced if the plasminogen solution was preliminarily incubated with a solution of antiplasmin (in various concentrations) at 37° for 1 h. The addition of antiplasmin in the same concentration to active plasmin, followed by incubation at 37° for an h also substantially lowers the activity of the plasmin, which is expressed in a longer time needed for lysis of the standard clot of fibrinogen.

In subsequent experiments, we studied the effects of antiplasmin upon the process of fibrinolysis in the intact organism.

One min after intravenous injection of 5 mg of antiplasmin into normal animals, the fibrinolytic activity is somewhat reduced, as was noted in experiments in vitro, but after 5 and 30 min, the fibrinolytic activity of the blood is already increased in comparison with this index in the control animals, which received injections of physiological solution (Table 1).

Analogous results were also obtained with respect to the general blood clotting. From the data cited in Table 2, it is evident that one min after the injection of antiplasmin, the clotting time of whole blood is appreciably reduced, but after 5 min it already exceeds the original level.

The results obtained show that the administration to normal animals of antiplasmin, a substance that ultimately promotes thrombus formation, is accompanied by a protective humoral reaction, there is an increase in the fibrinolytic activity of the blood and an increase in its clotting time. Preliminary data that we obtained show that this reaction evidently occurs indirectly, through the formation of thrombin, the action of which, as was shown in earlier experiments [1], induces a reflex protective response against thrombus formation in normal animals. In the

TABLE 3. Effects of Intravenous Injection of Antiplasmin on the Fibrinolytic Activity of the Blood of Normal and Irradiated Animals

Group of animals	No. of animals	Dose of preparation (in mg)	Fibrinogen concentration (in mg %)	Fibrinolytic activity (in %)	
				Before injection	After injection
Irradiated + antiplasmin	20	20	611	6,4	7,1
Normal + antiplasmin	15	20	429	20,5	31,3
Normal + physiological saline	15	—	457	15,5	13,8

incubation (37°) of oxalate-stabilized blood, taken from animals one min after their injection with 5 mg of antiplasmin, in six out of 10 experiments, the formation of strands of fibrin and small clots was detected. When the blood of the control animals was incubated, no such phenomenon was noted.

In subsequent experiments, we found that in animals with an inhibited physiological anticlotting system, no protective response arises to the injection of antiplasmin. As a model of depression of the physiological anticlotting system, we used animals irradiated by substantial doses of x rays (500 R). On the eighth to tenth day after irradiation, together with hemorrhage, they exhibited a prothrombic state, characterized by sharply reduced fibrinolytic activity of the blood and a high concentration of fibrinogen. Intravenous injection of thrombin in these animals readily provoked the formation of thrombin [3].

From the data presented in Table 3, it is evident that a protective response to the injection of antiplasmin does not arise in the irradiated animals. The fibrinolytic activity of the blood, as before, remains at a low level, characteristic of this disease, while in the control animals, the fibrinolytic activity of the blood is distinctly raised 5 min after the injection of antiplasmin.

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