ACTION OF ANTIPLASMIN ON COURSE OF EXPERIMENTAL "HYPOFIBRINOGENEMIA"

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A previous investigation [4] showed that the preparation antiplasmin (antifibrinolysin) isolated from bovine blood possesses the ability to depress the fibrinolytic activity of normal plasma in vitro and also, to a lesser degree, to block the activity of the preparation plasmin.

In the present investigation the action of antiplasmin was studied on the course of experimental "hypofibrinogenemia" arising in rats as the result of activation of the anticlotting system. The function of the anticlotting system was activated by intravenous injection of tissue thromboplastin or thrombin. The animals developed a lowered blood fibrinogen level, determined by the thrombin method, and a considerably increased fibrinolytic and anticoagulant activity [2, 3].

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes weighing from 170 to 180 g.

The antiplasmin preparation was obtained from bovine blood by the method of Loomis and co-workers [10]. This preparation had neither thrombin nor thromboplastin activity, nor had it the activity of a fibrin-stabilizing factor. Thromboplastin was prepared from the brain of albino rats; in vitro it clotted normal rat plasma in 19-20 sec. The fibrinogen concentration was determined by Bidwell's method [7] and expressed in mg %. The fibrinolytic activity was determined by the method of Astrup and Mullertz [6], using fibrin discs previously warmed for 30 min at 82-84° to remove traces of plasminogen [9]. The fibrinolytic activity was expressed as the number of square millimeters occupied by the zone of lysis of the fibrin disc, and its value was calculated in percent. Lysis of the control discs was conventionally taken as 100% fibrinolytic activity. The euglobulin fraction of the plasma applied to the surface of the fibrin discs was prepared by the method of Kowarzyk and co-workers [8]. The thrombin time of the plasma was determined by mixing equal volumes of plasma with thrombin solution with an activity of 15-20 sec. Blood was taken from the jugular vein. The preparations were injected into the same vein.

The results obtained were analyzed statistically by the method of quantitative evaluation of the pharmacological effect [1].

EXPERIMENTAL RESULTS

In the experiments of series I the rats received prophylactic intravenous injection of the antiplasmin preparation in a dose of 5-20 mg per animal. Three minutes after receiving the injection of antiplasmin, the extract of thromboplastin was injected intravenously. Blood was taken from the animals for analysis 5-7 min after the injection of thromboplastin.

It is clear from Table 1 that a preliminary intravenous injection of antiplasmin lowered the degree of activation of the anticlotting system produced by intravenous injection of thromboplastin and, as a result of this, caused a less marked decrease in the fibrinogen level and a smaller increase in fibrinolytic activity of the blood in the experimental animals. The thrombin time of the plasma of these animals was also reduced by 60% compared with its value in animals receiving thromboplastin alone.

In the next experiment, in contrast to the first, antiplasmin was injected 3 min after injection of thromboplastin, against the background of activation of the anticlotting system. Blood for analysis was taken 5-7 min and 1 h after injection of the antiplasmin preparation. In some cases, depending on the

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TABLE 1. Fibrinogen Concentration, Fibrinolytic Activity of Blood, and Thrombin Time of Animals with Experimental "Hypofibrinogenemia" Preliminarily Receiving Antiplasmin Preparation in a Dose of 5-20 mg

Substance injected	Number of animals	Dose of preparation (in ml)	Fibrinogen concentration (in mg %)	Fibrinolytic activity (in %)	Thrombin time (in sec)
Thromboplastin	40	0.5	105	202	137
Antiplasmin + thromboplastin	34	20* and 0.5	211	151	57
Physiological saline (control)	35	1.0	360	100	18

*Dose of preparation given in milligrams.

TABLE 2. Fibrinogen Concentration, Fibrinolytic Activity of Blood, and Thrombin Time of Animals with Experimental "Hypofibrinogenemia" Receiving Various Doses of Antiplasmin Preparation

Substance injected	No. of animals	Dose of preparation (in ml)	Fibrinogen concentration (in mg %)	Fibrinolytic activity (in %)	Thrombin time (in sec)
Dose 10-15 mg					
Thromboplastin	25	0.5	107	142	55
Thromboplastin + antiplasmin	25	0.5 and 10-15 *	84	182	80
Physiological saline (control)	25	1.5	294	100	17
Dose 30-40 mg					
Thromboplastin	23	0.5	105	141	57
Thromboplastin + antiplasmin	25	0.5 and 30-40 *	109	125	59
Physiological saline (control)	25	1.5	304	100	16

*Dose of preparation given in milligrams.

dose of the antiplasmin preparation, diametrically opposite results from those obtained in the previous experiment were found.

Table 2 shows that after intravenous injection of antiplasmin in a dose of 10-15 mg into animals with experimental "hypofibrinogenemia", the fibrinolytic activity of their blood rose still further, and the thrombin time was also increased by comparison with its level in the control animals receiving thromboplastin only. This effect was perhaps the result of temporary formation of a heparin–antiplasmin complex in the blood of the experimental animals. Antiplasmin administered from an external source, together with heparin entering the blood stream from the tissues after intravenous injection of thromboplastin, may form a complex possessing anticoagulant and fibrinolytic properties [5].

Injection of antiplasmin in a larger dose (30-40 mg) into animals with experimental "hypofibrinogenemia" was accompanied by a temporary and slight depression of the fibrinolytic activity of the blood. However, in later periods of the experiment (after 1 h), the fibrinolytic activity of the blood of the experimental animals again rose sharply and reached its level in control animals receiving thromboplastin alone. The depression of fibrinolysis, the increase in the fibrinogen level, and the shortening of the thrombin time in animals with experimental "hypofibrinogenemia" in the later periods of the experiment (after 1 h) were observed only after two injections of antiplasmin (total dose 40-60 mg). The first injection of antiplasmin was given 3 min, and the second 15 min, after injection of thromboplastin (Table 3).

TABLE 3. Fibrinogen Concentration, Fibrinolytic Activity of Blood, and Thrombin Time of Plasma of Animals with Experimental "Hypofibrinogenemia" After Two Injections of Antiplasmin in a Total Dose of 40-60 mg

Substance injected	Number of animals	Dose of preparation (in ml)	5-7 min after injection of antiplasmin			1 h after injection of antiplasmin		
			fibrinogen (in mg %)	fibrinolytic activity (in %)	thrombin time (in sec)	fibrinogen (in mg %)	fibrinolytic activity (in %)	thrombin time (in sec)
Thromboplastin	24	0.5	105	133	84	100[1]	185[2]	127
Thromboplastin + antiplasmin	23	0.5 and 40-60[1]	109	113	53	162[1]	147[2]	89
Physiological saline (control)	25	2.5	375	100	17	334	100	17

Note. Statistical analysis of data for fibrinogen concentration and fibrinolytic activity:
[1] $t = 2.00$; $P < 0.05$.
[2] $t = 2.65$; $P < 0.05$.

From the results of these experiments taken as a whole it may be concluded that the preliminary administration of antifibrinolysin preparation before intravenous injection of thromboplastin considerably weakens activation of the anticlotting system: the increase in fibrinolytic activity of the blood and the decrease in the fibrinogen concentration in the experimental animals were less marked than in the controls receiving injections of thromboplastin only. If antiplasmin was injected in a dose of 10-15 mg per animal, after the injection of thromboplastin, a much sharper increase in fibrinolytic activity of the blood and a more marked fall in the fibrinogen level was observed than in the controls, possibly as a result of the action of an antiplasmin–heparin complex formed in vivo in these conditions. When the dose of the antiplasmin preparation injected was increased to 40-60 mg, depression of fibrinolysis and some degree of restoration of the fibrinogen concentration took place in animals with experimental "hypofibrinogenemia".

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