THE ANTIPLASMIN DEFENSIVE REFLEX SYSTEM

B. A. Kudryashov and T. M. Kalishevskaya

Laboratory of the Biochemistry and Physiology of Blood Clotting, Department of Biochemistry of Animals, M. V. Lomonosov Moscow State University
(Presented by Active Member AMN SSSR S. E. Severin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 9, pp. 29-33, September, 1963
Original article submitted September 19, 1962

Investigations undertaken in our laboratory [1,4,5,8] have shown that animals (amphibians and mammals) possess a defensive anticoagulating system which is reflex in nature. The principle of action of this system is that the appearance of a small excess of thrombin in the circulating blood evokes a reflex act, as a result of which agents are secreted into the blood to prevent it from clotting. Among these agents have been identified plasminogen activators and heparin or heparin-like substances [4,6,7,9].

The antagonist of thrombin in the body is plasmin (fibrinolysin). In respect of both plasmin and thrombin, the substrate of the enzymic reaction in the blood is fibrinogen. Interaction between thrombin and fibrinogen leads to the formation of fibrin. When fibrinogen is treated with plasmin it undergoes lysis, in the course of which changes take place, leading in particular to the appearance of products (antithrombin VI) possessing specific properties. An excess of thrombin in vivo is known to bring the risk of thrombus formation, and an excess of plasmin may be associated with a tendency towards increased bleeding, as a result of the ensuing fibrinogen deficit. This phenomenon has been described in the literature [13,16].

On the basis of the experimental study of the function of the physiological anticlotting system, protecting the organism against clotting of the blood should an excess of thrombin appear, we put forward the suggestion that the organism must also possess a defensive system reacting to an excess of plasmin in the blood. The nature of this reaction must also be reflex. We have made an experimental investigation of the defensive reaction of the organism to injection of plasmin into the blood stream.

EXPERIMENTAL METHOD

Experiments in vivo were conducted on rabbits weighing from 2.8 to 3.0 kg, kept on a natural, balanced diet. The veins of the thigh and ear were used for withdrawal of blood for analysis and injection of plasmin solutions. The preparation of plasmin was obtained from Cohn's 3rd fraction, from human blood plasma, by Kline's method [15]. A preparation of streptokinase (400 units per 10 mg plasminogen) was used as plasminogen activator (profibrinolysin). The activity of the resulting plasminogen solutions was tested by their ability to produce lysis of clots obtained by coagulating a 1% solution of purified fibrinogen with thrombin. Lysis of the clot thus formed usually took place in the course of 15-20 min at 37°.

The fibrinogen content and the fibrinolytic activity of the blood of the experimental animals were determined by Bidwell's method [14]. The general clotting time of the blood was determined by a variant of the method of Lee and White, described by Quick and co-workers [17]. The thrombin time was determined from the clotting time (in seconds) of the oxalated test blood mixed with an equal volume of standard thrombin solution at 37°.

EXPERIMENTAL RESULTS

1. Plasmin in vitro retards blood clotting. In the first series of experiments the effect of plasmin on blood clotting was studied in vitro. Blood in a volume of 0.3 ml was drawn directly from a vein into a syringe containing 0.1 ml of plasmin solution. The contents of the syringe were expelled into a test tube, which was placed quickly into a water bath at 37°, and the time taken for the clot to form was noted. For control purposes the same reaction was carried out except that 0.1 ml of 0.85% physiological saline was used instead of plasmin.

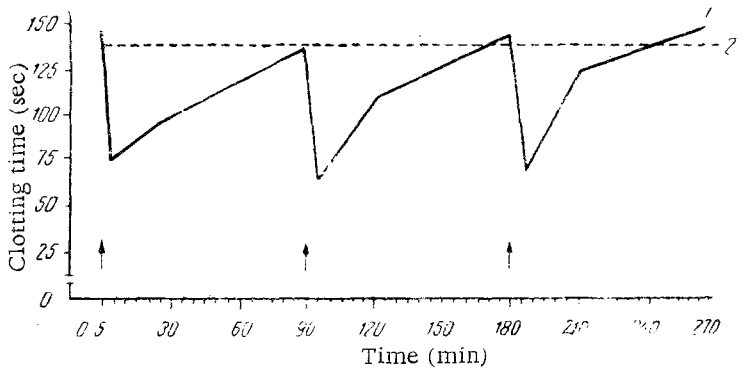

Fig. 1. Changes in the clotting time of the blood after the first, second, and third injections of plasmin (mean data). 1) Experiment; 2) control. The arrows indicate injection of plasmin.

The results of these experiments (Table 1) demonstrate that blood taken from healthy animals takes longer to clot in the presence of plasmin than control blood. Hence, the addition of plasmin to blood in vitro retards clot formation.

The second series of experiments showed (Table 2) that the intravenous injection of from 4 to 8 ml of plasmin solution leads to a marked slowing of blood clotting immediately after the injection. However, a sharp acceleration of blood clotting takes place 3-5 min after the intravenous injection of plasmin. This reveals the difference between the action of plasmin in vivo and in vitro on blood clotting.

2. Plasmin in vivo accelerates blood clotting. It is clear from Table 2 that the injection of moderate doses of plasmin into animals causes an initial, transient prolongation of the clotting time (for 1 min after injection), followed by a marked acceleration of clot formation (5 min after the injection). This acceleration of clot formation persisted for more than 30 min after the injection. The normal clotting time of the blood in animals receiving an adequate dose of plasmin was restored after 60-90 min.

Experiments in which repeated injections of definite doses of plasmin (4 ml 3 or 4 times at intervals of 90 min) were given showed that an appreciable acceleration of blood clotting took place in response to each injection of plasmin (Fig. 1).

Following researches [10,11,12] showing the importance of the autonomic nervous system to the slowing and acceleration of blood clotting, and taking into consideration the fact that the reflex defensive reaction of the anticlotting system is associated with the function of the parasympathetic division of the autonomic nervous system [2,9], we postulated that the acceleration of the blood clotting observed after intravenous injection of plasmin is brought about by the sympathetic division of the autonomic nervous system. It has been shown that if, in animals, the sympathetic chain is divided bilaterally before an intravenous injection of plasmin solution is given, the described effect of acceleration of blood clotting is not observed. This operation leads to a persistent prolongation of blood clotting after intravenous injection of plasmin, similar to that taking place when blood is mixed with plasmin in the test tube.

3. In healthy rabbits the left kidney was isolated from the circulation but its nerve supply was left intact. The renal vessels were perfused with Ringer-Locke solution heated to 37° [2,3]. It was found that there was no difference between the clotting times of the blood taken 5 min after the beginning of perfusion and of the blood taken before

TABLE 1. Action of Plasmin on the Clotting Time of Rabbit's Blood in Vitro (mean data)

No. of blood samples	Experimental conditions	Clotting time of blood (sec)
8	Experiment: 0.1 plasmin + 0.3 ml blood	410
4	Control I: 0.1 ml physiological saline + 0.3 ml blood	216
2	Control II: 0.4 ml blood	222

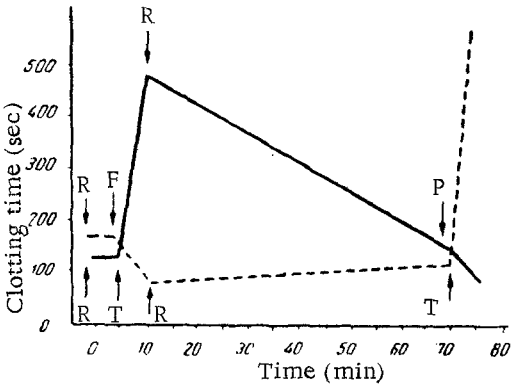

Fig. 2.

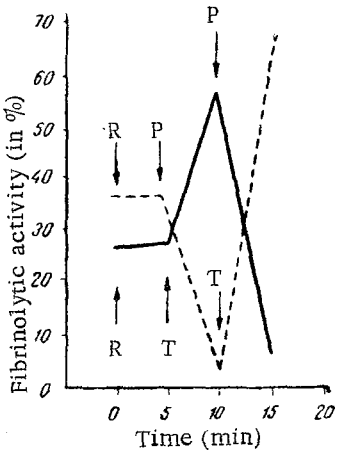

Fig. 3.

Fig. 2. Changes in the clotting time of blood taken from the femoral vein of rabbits after perfusion of the isolated kidney with Ringer-Locke solution (R), and solutions of thrombin (T) or plasmin (P). The arrows indicate the onset of perfusion.

Fig. 3. Changes in the fubrinolytic activity of blood taken from the femoral vein of rabbits after perfusion of the isolated kidney with Ringer-Locke solution (R), and solutions of thrombin (T) or plasmin (P). Legend as in Fig. 2.

perfusion (Table 3). Replacement of the Ringer-Locke solution by plasmin solution caused an appreciable acceleration of clotting and a shortening of the thrombin time of the blood taken from the general circulation 5 min after the beginning of perfusion. A marked fall in the fibrinolytic activity of the blood was observed under these circumstances (4% compared with 29%).

Perfusion of the vessels of the isolated kidney with thrombin caused a sharp increase in the length of the clotting time of blood taken from the general circulation (Fig. 2), and increased its fibrinolytic activity from 27 to 54% (Fig. 3). Subsequent perfusion of the renal vessels with plasmin led to a shortening of the clotting time of the blood and to a lowering of its fibrinolytic activity.

The experimental results indicate that the peripheral blood vessels, especially those of the kidneys, contain receptors transmitting impulses along a reflex arc whenever a relative excess of either thrombin or plasmin appears in the blood stream. This physiological reaction to thrombin was described in our earlier papers [2,3]. In regard to plasmin it can be stated that a slight excess of this substance in the blood stream evokes a reflex act, as a result of which the activity of this enzyme in the circulating blood is blocked. The physiological mechanism of this phenomenon is apparently associated with the secretion into the blood stream of substances possessing antifibrinolytic activity. This reflex reaction, according to preliminary findings (see Table 2), is related to the activity of the sympathetic nervous system.

TABLE 2. Changes in the Clotting Time of the Blood of Normal Animals after Intravenous Injection of Plasmin Solution

Experimental conditions	No. of animals	Dose of plasmin (in ml)	Clotting time (in seconds)			
			before injection	after injection		
				1 min	5 min	30 min
Experiment I: injection of solution of active plasmin	2	4	145	—	77	63
into normal animal	2	8	131	210	79	58
Experiment II: injection of active plasmin solution after	2	4	150	—	251	252
bilateral division of the sympathetic chain in the neck	2			—		
Control: injection of plasmin solution heated to 60° for	2	4	116	—	116	106
60 min into normal animal	2	8	127	—	119	117

TABLE 3. Changes in the Clotting Time of Blood Taken from the Femoral Vein of Normal Rabbits after Perfusion of the Vessels of a Kidney Isolated from the General Circulation but Retaining its Innervation with Ringer-Locke Solution or Plasmin Solution (mean data)

Experimental conditions	No. of animals	Dose of plasmin (in ml)	Clotting time (in seconds)		Thrombin time (in seconds)	
			before	5 min after	before	5 min after
			perfusion of plasmin			
Experiment: perfusion of solution of active plasmin, innervation of organ intact	5	5	185	109	21	14
Control: perfusion of solution of active plasmin, denervation of organ	2	5	163	171	—	—

It follows from previously published findings [1-3,5] and the results of the present investigation that the automatic mechanism of the nervous regulation of the liquid state of the blood in the body and of its power to clot is based on the chemoreceptors of the blood vessels. The receptors are excited by the two key agents of the clotting and anticlotting systems—thrombin and plasmin. These enzymes operate the reflex and humoral processes responsible for maintaining a physiological state in the blood stream.

LITERATURE CITED

1. T. M. Kalishevskaya, B. I. Kotlyar, and B. A. Kudryashov, Byull. éksper. biol., 7, 5 (1961).
2. T. M. Kalishevskaya, in the book: Proceedings of a Conference on Problems in the Physiology and Biochemistry of Blood Clotting and Thrombus Formation, to Commemorate the Centenary of A. A. Shmidt's Studies of the Enzyme Theory of Blood Clotting [in Russian], Tartu (1961), p. 39.
3. T. M. Kalishevskaya and B. A. Kudryashov, Fiziol. zh. SSSR, 48, 5, 1234 (1962).
4. B. A. Kudryashov and P. D. Ulitina, Doklady Akad. Nauk SSSR, 120, 3, 677 (1958).
5. B. A. Kudryashov, Nature, 184, 454 (1959).
6. B. A. Kudryashov and T. M. Kalishevskaya, Doklady Akad. Nauk SSSR, 131, 1, 213 (1960).
7. B. A. Kudryashov and V. E. Pastorova, Doklady Akad. Nauk SSSR, 130, 6, 1382 (1960).
8. B. A. Kudryashov, Thrombos. Diathes. haemorrh. (Stuttg.) (1961), Bd. 6, S. 371.
9. B. A. Kudryashov, G. V. Andreenko, G. G. Bazaz'yan, et al., Vestn. Moskovsk. Univ. Seriya: Biologiya, pochvovedenie, 2, 3 (1961).
10. A. A. Markosyan, Nervous Regulation of Blood Clotting [in Russian], Moscow (1960).
11. G. I. Tsobkallo, Doklady Akad. Nauk SSSR, 66, 4, 765 (1949).
12. G. I. Tsobkallo, Fiziol. zh. SSSR, 1, 84 (1955).
13. R. Battle, G. Camo, J. Lieb, et al., Sang., 27, 80 (1956).
14. E. Bidwell, Biochem. J., 55, 497 (1953).
15. D. L. Kline, J. biol. Chem., 204, 949 (1953).
16. E. Kowalski, Ginek. pol., 29, 43 (1958).
17. A. J. Quick, R. Honorato, and M. Stefanini, Blood, 3, 1120 (1948).