THE REFLEX PATHWAYS OF THE ANTICOAGULATION SYSTEM

T. M. Kalishevskaya, B. I. Kotlyar, and B. A. Kudryashov

Laboratory of the Biochemistry and Physiology of Blood Clotting (Head, Prof. B. A. Kudryashov), Department of Animal Biochemistry, of the Biological Soil Faculty, M. V. Lomonosov Moscow State University
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It has been shown [1-4] that the injection of moderate doses of thromboplastin or thrombin suppresses blood clotting, either partially or completely. A study of the blood shows that in response to the injection of thrombin into the blood stream substances are liberated which increase fibrinolytic activity and markedly reduce the amount of fibrinogen; there is also an increase of the concentration of heparin and heparinlike substances. This defensive reaction against the formation of thrombin is blocked by anesthesia, and if an injection of thrombin is given to an animal which has been deeply anesthetized for 15 minutes, it will usually die from thrombosis, whereas control animals will survive. The defensive reaction has been shown to exist not only in mammals, but in certain amphibia (in Rana temporaria) [5]. The injection of thrombin into the frog ventricle does not cause thrombosis, but actually completely prevents blood from clotting in a test tube when thromboplastin from the lungs from the same species of animal is added [5]. If, however, the spinal cord of the frog is destroyed before injecting the thrombin into the heart, the animals die from generalized thrombosis [5]. Destruction or removal of the brain in Rana temporaria did not prevent the occurrence of the protective action against thrombin injected into the blood stream. All the above results indicate that the protective reaction which maintains the blood in a fluid condition in vivo, is reflex in nature [1-5].

We here report further experiments which also indicate the reflex nature of the anticoagulation system.

METHOD

Experiments were performed on white rats weighing 170-180 g. They were kept on the normal laboratory diet. Blood was collected in a syringe from the jugular vein, and intravenous injections of thrombin were made into the same vein. The thrombin used was prepared by the N. F. Gamalen Institute of Epidemiology and Microbiology. The activity of the thrombin solution was determined from the clotting time of oxalated rat blood at 37°, when 0.3 ml of blood was added to 0.3 ml of thrombin.

RESULTS

As has been shown previously [5], in frogs, the reflex anticlotting system does not operate when the spinal cord has been destroyed. In rats we found that destruction of the cord at or below the VII vertebra causes a rapid reflex reaction which produces an anticlotting effect (Table 1).

It was found that the impairment of the blood-clotting power was of the same kind as was produced by intravenous injection of thrombin (Table 2). There was then a marked increase in the fibrinolytic activity of the blood, and a fall in the amount of fibrinogen. Blood taken after partial destruction of the spinal cord, and after intraperitoneal injection of thrombin, did not clot in vitro, and clotted only very slowly after thrombin had been added. However, the thrombin time of the same blood was greatly reduced if it was first incubated with protamine sulfate (Table 3); this result appears to indicate that there is an increased concentration of heparin or of heparin-like substances in the blood stream even after an intravenous injection of thrombin, and after the spinal cord has been destroyed.

It is possible that this reaction occurs through the formation in the plasma of antithrombin VI [6]. After partial destruction of the spinal cord the normal clotting power of the blood is restored after 60-90 minutes or more, and after this time intravenous injection of thrombin will again produce a complete protective reaction (Figs. 1 and 2).

TABLE 1. Determination of the Mean "Natural" Clotting Time of Rat Blood Before and After Destruction of the Spinal Cord*

Number of animals	Experimental conditions	Time of clotting for 0.5 ml of blood	Thrombin time (0.2 ml thrombin + 0.2 ml oxalated blood)
42	Before destruction of the spinal cord	80 sec	28 sec
42	After destruction of the spinal cord	From 3 min to more than 30 min	From 3-4 min to more than 10 min

* The blood was incubated in a glass tube with thrombin at 37°.

TABLE 2. Measurement of the "Natural " Clotting Time of Rat Blood

Number of animals	Experimental conditions	Clotting time for 0.5 ml of blood	Thrombin time (0.2 ml oxalated blood + 0.2 ml thrombin) *
31	Before intravenous injection of thrombin	On average, 79 sec	On average, 29 sec
31	Four minutes after the intravenous injection of thrombin (0.9 ml active, 5-6 sec)	From 4 min to more than 30 min	From 5 min to more than 30 min

* The blood was incubated in a glass tube with thrombin at 37°.

TABLE 3. Determination of the Thrombin Time of Acidified Rat Blood Incubated with Protamine Sulfate or Physiological Saline*(Mean Values)

Number of animals	Conditions of experiment	After incubating 0.2 ml of blood + 0.2 ml of thrombin with physiological saline	After incubating 0.2 ml of blood + 0.2 ml of thrombin with protamine sulfate
37	Before partial destruction of the spinal cord	28 sec	22 sec
37	4 min after partial destruction of the spinal cord	From 3 to more than 10 min	52 sec
28	Before intravenous injection of thrombin	29 sec	24 sec
28	After intravenous injection of thrombin	From 5 to more than 30 min	81 sec

* Other procedures were followed: 0.3 ml of blood + 0.03 ml of 0.2% protamine sulfate solution or 0.03 ml of physiological saline were incubated for 15 min at 37°.

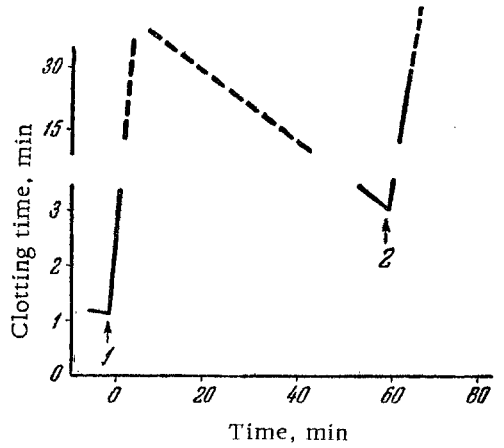

Fig. 1. Changes in the clotting time in rats caused by partial destruction of the spinal cord and subsequent intravenous injection of thrombin (mean results of 11 experiments). 1) Destruction of the spinal cord; 2) injection of thrombin.

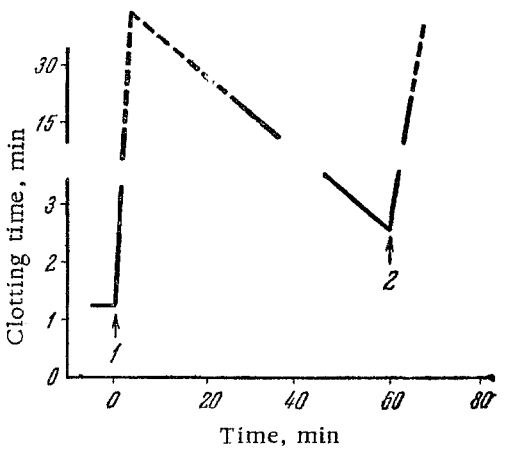

Fig. 2. Changes in the clotting time in rats induced by intravenous injection of thrombin and subsequent partial destruction of the spinal cord (mean values of 5 experiments). 1) Injection of thrombin; 2) destruction of spinal cord.

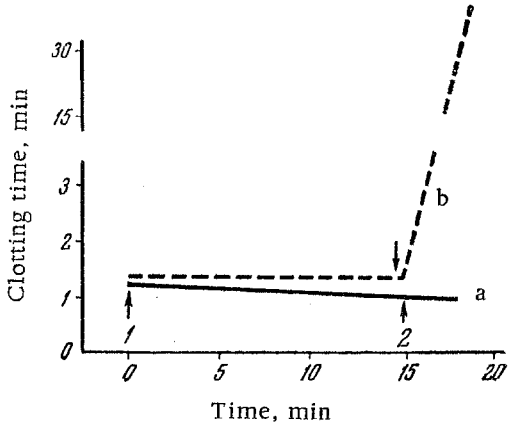

Fig. 3. Effect of ether anesthesia on changes in the clotting power of rat blood caused by destruction of part of the spinal cord (mean results of 24 experiments). a) Experiment; b) control (no anesthesia); 1) anesthesia; 2) destruction of the spinal cord.

The reflex-humoral reaction which leads to the loss of blood clotting power, and which is caused in rats by the partial destruction of the spinal cord, is completely blocked by anesthetizing the animals completely with ethyl sulfate at the time of the operation (Fig. 3).

Destruction of the thoracic and cervical portions of the cord (while respiration is maintained artificially) and destruction of the whole of the brain do not impair the activity of the anticlotting system. Experiments on rats in which the neocortex has been removed have shown that the anticlotting system is not destroyed, although it is somewhat weakened. Intravenous injection of thrombin caused a typical change in the anticlotting system, but unlike the operated controls which received an injection of inactivated thrombin, they frequently died within 20-30 min. Destruction or section in the region of the diencephalon or hypothalamus does not block the action of the anticlotting system.

However, section in the dorsal part of the cerebellum and medulla, while respiration is maintained artificially, immediately eliminates the anticlotting system; in such animals, controls which receive thrombin inactivated by heating survive, whereas those which receive an injection of thrombin die rapidly from a generalized thrombosis. In all cases, the operation completely blocks the anticlotting system. However, at the time at which the brain is sectioned, there is a weak reflex response, which influences the anticlotting system, and there is some increase in the clotting time and an increase in the fibrinolytic activity of the blood; this effect has some influence on thrombin injected subsequently, but it affords no protection from the thrombosis.

In later experiments it was shown that the reflex arc of the anticlotting system involves the medulla. A detailed account of this work will be published later.

SUMMARY

Further evidence has been obtained that the physiological anticoagulation system in rats is of a reflex-humoral nature. The reflex arc involves the medulla oblongata.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
