REFLEX AFIBRINOGENEMIA

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Perfusion of the rabbit carotid sinus, isolated from the rest of the circulation but with its innervation intact, by means of heparin solution slows blood clotting and causes a sharp decrease in the fibrinogen concentration and an increase in the heparin concentration in the blood. The reflex nature of these changes was demonstrated and data are given to show that this reflex hypofibrinogenemia and afibrinogenemia is based on changes in the properties of fibrinogen under the influence of endogenous heparin.

Afibrinogenemia is an important factor in the pathogenesis of many types of bleeding. Data indicating that the hypofibrinogenemia and afibrinogenemia may be reflex in character are given in this paper.

EXPERIMENTAL METHOD AND RESULTS

The carotid sinus of 48 rabbits, anesthetized with urethane, was isolated from the general circulation, and then perfused with Tyrode solution heated to 37°C. At certain time intervals the carotid sinus was perfused with Tyrode solution containing dissolved heparin (from 1 to 1500 units/ml).

During the first minute after the beginning of perfusion the blood fibrinogen level fell sharply. In 17 of 48 experiments the blood lost its ability to clot, and in 28 experiments a definite hypofibrinogenemia was observed (Table 1). The reflex hypo- or afibrinogenemia lasted for 1-1.5 h, after which the blood fibrinogen level was either completely or partially restored. A similar reaction could be produced several times over in the same animal.

In the control tests, perfusion with heparin after division of the nerve to the carotid sinus or preliminary perfusion of the carotid sinus with procaine caused no changes in blood coagulation.

These experiments showed that the hypofibrinogenemia or afibrinogenemia which was produced was in fact of reflex origin, and was evoked by stimulation of the carotid sinus by heparin. The afferent pathway of this reflex is obvious. Its central connections are evidently located in the structures of the reticular formation and hypothalamus, in which, as Markosyan and Yakunin [3], Chepurov [4], and Stepanyan have shown, stimulation of certain zones delays blood clotting.

TABLE 1. Changes in Blood Fibrinogen Level after Perfusion of Isolated Carotid Sinus with Heparin

Index studied	Initial value	After perfusion		P	
		1–5 min.	60 min.	$M-M_1$	$M-M_2$
	$M\pm m$	$M_1\pm m_1$	$M_2\pm m_2$		
Fibrinogen (in mg/0.5 ml)	4,9±0,2	1,8±0,4	2,8±0,7	<0,01	>0,05
Fibrinogen (in mg %)	415±32	232±36	290±66	<0,01	>0,05

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TABLE 2. Effect of Perfusion of Isolated Carotid Sinus with Heparin on Blood Clotting in Intact Rabbits and in Rabbits after Bilateral Vagotomy (M ± m)

Time of determination	Number of experiments	Blood clotting time (in sec)	
Initial values	16	62.5 ± 1.7	114 ± 4.7
Before vagotomy	10	103 ± 7.5	242 ± 51
		P < 0.001	P < 0.05
After vagotomy below diaphragm	8	64.4 ± 2.9	142 ± 5.2
		P > 0.5	P < 0.01
After vagotomy in neck	7	63 ± 3.4	117 ± 9
		P > 0.5	P > 0.5

Subsequent experiments were carried out in order to identify the efferent pathway of this reflex.

In the experiments of series I, the carotid sinus was perfused with heparin 15-20 min after the end of the operation and recovery of the animal from the anesthetic. Perfusion continued for 1 min. The clotting time of the blood was determined by means of a Sitkovskii–Egorov apparatus before perfusion, 1 min after perfusion, and then in the course of 30-60 min until the rate of clotting had returned to its original value. The vagus nerves were then divided below the diaphragm, the carotid sinus was again perfused with heparin for 1 min, and the clotting time was again determined.

After subdiaphragmatic vagotomy, perfusion of the carotid sinus with heparin did not change the time when clotting started, but it delayed the time of its completion (Table 2).

Perfusion of the carotid sinus with heparin after vagotomy in the neck caused no changes in the coagulation properties of the blood (Table 2).

These results indicate that the efferent pathway of the reflex action of heparin on blood clotting runs in the vagus nerves. In the experiments of series I subdiaphragmatic vagotomy removed the innervation and, consequently, the possibility of a response to stimulation, of the abdominal organs and, in particular, of the liver. However, the innervation of the lungs remained intact, and the heparinocytes of the lungs continued to produce heparin and to supply it into the blood stream, where it led to some delay in blood clotting. In series II, division of the vagus nerves in the neck prevented a reflex response of all organs involved in blood clotting.

The next step was to discover the causes of onset of hypofibrinogenemia or afibrinogenemia. To solve this problem, double tests were carried out simultaneously on plasma obtained from unclotted bloood.

Into each of two tubes, 0.2 ml of plasma was placed. Thrombin (activity 5 sec) was added to one tube, and in the other the fibrinogen was salted out with sodium sulfate by Padmore's method [4]. The results showed that whereas addition of thrombin to this plasma did not lead to the formation of a clot, the addition of sodium sulfate caused precipitation of floccules of fibrinogen in the same amount as in normal plasma. Consequently, the inability of the blood to clot was due not to the development of true afibrinogenemia, but to the fact that the fibrinogen, which remained completely intact in the blood, had lost its ability to react with thrombin. These findings confirmed the hypothesis that in reflex hypofibrinogenemia or afibrinogenemia the conversion of fibrinogen into fibrin is disturbed. What is the mechanism of this disturbance?

In all the experiments with reflex slowing of blood clotting, a considerable increase in the heparin concentration (on the average by 100%) was observed. Having regard to the great ability of heparin to combine with certain substances, including proteins, it could be postulated that heparin combines with fibrinogen and thereby prevents the action of thrombin on fibrinogen (Lomazova, 1966). This hypothesis was confirmed by the following results.

The velocity of clot formation in a mixture of reagents (plasma, heparin, thrombin) was dependent on the order in which they were added. If 0.2 ml plasma were treated with 0.1 ml heparin (250 units/ml), and then with 0.6 ml thrombin (activity 5 sec), a clot was formed in 65-1800 sec (mean 394 ± 176 sec). If heparin

were first added to thrombin, and then plasma added in the same proportions, a clot formed in 45-148 sec (mean 51 ± 30.1 sec). In the first case the heparin evidently reacted with the fibrinogen, lowering its coagulating power.

More direct and convincing proof that heparin modifies the coagulating properties of fibrinogen was obtained by subjecting the plasma which had lost its clotting power as a result of this reflex to electrophoresis on paper.

After electrophoresis of normal plasma and staining with amido black, fibrinogen appeared at its characteristic place. After electrophoresis of plasma which had lost its clotting power by this reflex mechanism, no fibrinogen could be detected.

In the experiments of series II an electrophoretic analysis was made of pure fibrinogen and of fibrinogen treated with heparin. Fibrinogen treated with heparin did not appear on the paper.

Data showing that heparin can form a complex with fibrinogen were also obtained by Kalishevskaya [1, 2].

The results described above suggest that the reflex hypofibrinogenemia or afibrinogenemia is due to a change in the properties of the chief substance concerned with blood clotting, fibrinogen, leading to a disturbance of the third phase of coagulation of the blood, resulting in the delaying of this process.

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