Analysis of the TEG showed that suspensions of nuclei and mitochondria from liver, kidney, and small intestine cells from animals treated with $PGF_{2\alpha}$ reduced the reaction time and increased the parameters ma and E (Figs. 2 and 3).

$PGF_{2\alpha}$, injected intravenously into animals with experimental toxic hepatitis, strengthened the hemostatic properties and depressed the fibrinolytic activity of liver, kidney, small intestine, and skeletal muscle tissues. These changes were accompanied by corresponding changes in the subcellular fractions, so that it can be postulated that the hemostatic properties of tissues depend on the nuclear fractions and mitochondria in the cells of these tissues.

Changes in opposite directions were found in tissue from the stomach wall. It can accordingly be concluded that the ultimate effect of injection of prostaglandins is determined not only by dose, exposure, and mode of administration of the substance and the initial functional state of the recipient, but also by the specific character of metabolism of the organ concerned.

LITERATURE CITED

1. R. M. Glants, E. A. Tkach, I. M. Parobetskaya, et al., Probl. Gematol., No. 5, 51 (1980).
2. R. M. Glants and E. A. Tkach, Probl. Gematol., No. 5, 38 (1979).
3. A. I. Shternberg, Probl. Gematol., No. 1, 34 (1963).
4. E. A. Tkach, in: The Blood Clotting and Fibrinolysis System [in Russian], Vol. 1, Saratov (1975), p. 255.

BRADYKININ, THROMBIN, AND PROSTAGLANDINS AS MODULATORS OF MICROTHROMBOSIS AFTER LOCAL INJURY TO THE VESSEL WALL

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UDC 616.16-001-092.9-07:616.16-005.6-02:615.31:577.175.85

KEY WORDS: bradykinin; thrombin; microvessel wall.

Disturbance of the structure of the microvessel wall gives rise to a number of linked compensatory reactions which constitute the complex dynamic picture of repair of the injured microvessel. The writers showed previously by means of their model of microthrombosis (MT), induced by local laser injury to the vessel wall, that this process is based on prostaglandin-dependent aggregate and adhesive reactions of the platelets [5, 6].

The object of the present investigation was to study the role of other humoral factors of regulation of the microcirculation in these processes: the kallikrein–kinin system (KKS) and the blood clotting system. The basis for this approach to the problem was the concept of the role of the "Hageman factor system" in the regulation of the functional relations of the microvessel wall and the rheologic properties of blood flowing along these vessels, elaborated previously [1, 4].

EXPERIMENTAL METHOD

Experiments were carried out on 98 male Wistar rats weighing 190-250 g, anesthetized with pentobarbital. The intravital study of MT after injury to the vessel wall by a laser

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 1, pp. 30-32, January, 1983. Original article submitted June 23, 1982.

0007-4888/83/9501-0038$07.50

TABLE 1. Effect of Infusion of BK (200 µg/kg/min) and T (10 units/kg/min) on Parameters on MT in Rat Mesenteric Venules

Substance injected	Duration of infusion, min	TMT, sec	AT, μ^2	TDE, sec	DE, Oe/5 min
Control (physiological saline)		121±4,1	987±52,7	41,3±0,2	4,1±0,2
BK	5	88,3±5,9*	675,3±87,0**	53,7±3,1*	2,5±0,2***
	12	99,0±14,6	720,0±40,0	46,5±8,5	3,2±0,4
	20	103,9±9,5	747,9±87,2	45,5±3,3	4,4±0,6
		(*n*=23)	(*n*=7)	(*n*=7)	(*n*=8)
T	3	118,1±9,5	921,9±65,1	45,1±3,7	4,6±0,7
	10	150,4±7,2*	1444,2±135,4***	34,6±3,7	7,3±0,4***
	25	93,5±9,3	1119,6±110,3	56,1±4,1*	4,0±4,1
		(*n*=24)	(*n*=7)	(*n*=8)	(*n*=8)
Control (physiological saline)		120,5±6,5	969,9±48,6	44,0±2,8	4,9±0,3

Legend. *P < 0.05, **P < 0.01, ***P < 0.001; n) number of determinations.

beam was carried in a complex experimental situation described previously [5]. Mesentric venules 34.5 ± 0.4 µ diameter were subjected to injury and subsequent observation. The substances were injected by means of an infusion pump at the rate of 0.03-0.07 ml/min through a catheter introduced into the rat's caudal artery. Doses of the substances chosen beforehand did not cause any significant changes in the systemic arterial pressure. The diameter of the vessels studied and the character of the blood flow in them likewise remained unchanged. In the experiments of series I the effect of prolonged infusion of bradykinin (BK, 20 µg/kg/min) on the parameters of MT was studied at the site of injury to a venule by a laser beam, inflicted after 3, 12, and 20 min of infusion. In series II, against the backgrounds of the same infusion of BK and at the same times, blood samples were taken from the left ventricle to study parameter of the KKS. In series III and IV similar investigations of MT and of the above-mentioned biochemical parameters were carried out against the background of infusion of thrombin (T) at the rate of 10 units/kg/min. Corresponding observations were made after 3, 10, and 25 min of infusion. For each series control experiments were carried out with infusion of physiological saline. Control tests for infusion as such also were set up. The process of local MT was assessed quantitatively relative to the following parameters: 1) the time of maximal thrombosis (TMT); 2) the area of projection of thrombus (PT); 3) the time of detachment of the first embolus from the main conglomerate (TDE); 4) the degree of embolization (DE). The state of the blood plasma KKS was assessed on the basis of changes in spontaneous arginine esterase activity and the level of prekallikrein and kallikrein inhibitor, by the methods described in [2, 8]. T was obtained frm Boehringer Mannheim, BK and tosyl-L-arginine methyl ester (TAME) were from Reanal, Hungary, and the remaining reagents were of Soviet origin. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

As Table 1 shows, infusion of BK led during the first few minutes to a decrease in the parameters of MT induced by laser injury to the vessel wall. The time of formation of the platelet conglomerate (TMT) was increased by 26%, its area (AT) was reduced by 25%, and TDE and the intensity of embolization were reduced by 36-38%. However, after 12 min of BK infusion these changes in MT were much weaker, and after 20 min none of the parameters of MT differed from their initial values, i.e., the BK infusion had practically no effect.

During the first few minutes of BK infusion the prekallikrein level fell appreciably below its value in the intact animals (Table 2). However, this change can hardly have been due to injection of the polypeptide, for similar changes also were observed after infusion of physiological saline. None of the parameters differed from normal by the 20th minute of BK infusion.

During prolonged infusion of T changes also were observed in the parameters of MT. Unlike BK, however, injection of T led to an increase in the adhesive and aggregative

TABLE 2. Effect of Infusion of BK and T on Parameters of KKS of Rat Blood Plasma

Duration of infusion, min	No. of determinations	IAE (μmoles TAME/ml plasma/h)	PKK (μmoles TAME/ml plasma/h)	KI, conventional units
Intact control	12	18,4±1,1	77,8±3,5	0,84±0,01
5	10	21,6±1,6	58,5±3,5**	1,1±0,09
20	6	13,5±1,6	74,8±4,0	0,78±0,01
5	6	12,5±1,7	49,2±9,1	0,79±0,15
12,5	6	10,6±3,6	50,2±8,0	0,75±0,19
20	6	16,6±0,9	72,2±8,6	1,06±0,14
3	6	31,9±3,2*	39,4±5,4**	0,89±0,11
10	6	31,5±4,8**	42,2±5,0**	0,26±0,06**
25	6	33,4±2,7**	66,4±4,9	0,83±0,07

Legend. *P < 0.05, **P < 0.01. IAE) Initial arginine esterase activity, PKK) prekallikrein, KI) kallikrein inhibitor level [8].

activity of the platelets after injury to the vessel wall. Differences also were observed in the time course of the process: whereas after 3 min of T infusion there were no marked changes compared with infusion of physiological saline, after 10 min there was a distinct increase in TMT, AT, and DE (by 25, 57, and 49% respectively). These changes continued until the 25th minute of infusion, when the parameters of MT were more indicative of a tendency for MT to be depressed.

The phenomena observed took place against the background of appreciable activation of the blood KKS, which was already distinct after 3 and 10 min of T infusion. The fall in the prekallikrein level was accompanied by an increase in spontaneous arginine esterase activity and a sharp fall in the kallikrein inhibitor level, probably reflecting the presence of increased T concentrations in the blood stream. However, by the 25th minute of T infusion the levels of prekallikrein and kallikrein inhibitor returned to their initial values, demonstrating the stabilization of the system.

The results thus enable relations between the plasma and cellular components of the blood during local injury to the microvessel wall under intravital experimental conditions to be presented in a preliminary form. Previous investigations on the same model definitely showed the importance of the various groups of prostaglandin compounds in the regulation of MT [5, 6]. However, it must be emphasized that besides prostaglandins, an important place in the regulation of platelet aggregation is played by the thrombin component. After injury to the vessel wall, thrombin-activating components are released from the endothelial cells and the platelets themselves. These substances can trigger a thrombin cascade both through the Hageman factor and through the "external" clotting system. It is also known that in the absence of T [9], or if inactive T is used [7], the platelets do not adhere to the surface of an endothelial cell culture. Finally, an increase in the synthesis of thromboxane, the proaggregating factor of the platelets, has also been demonstrated under the influence of T [14].

Meanwhile attention is drawn to data showing an increase in prostacycline synthesis by endothelial cells under the influence of T [10, 12]. This phenomenon, however, is clearly apparent only after the initial addition of T, and later the endothelial cells become sensitive to the adhesive effect of T [11].

These observations, by no means complete and to some extent contradictory, suggest several explanations for the experimental results described here. The appearance of kinins in the blood stream as a result of activation of the KKS or during direct infusion of BK is an important factor preventing MT in the injured vessel wall. Changes in the parameters of MT toward hypoadhesion can be associated with the antiaggregating action of BK on platelets [3]. However, the most important factor here is evidently the probable effect of BK on prostacycline synthesis in the endothelial cells and its release into the blood stream [13, 15]. This process is temporary in character, and previously [6] the writers drew attention to an increase in the parameters of MT after cessation of the action of prostacycline.

During infusion of small doses of T activation of KKS and changes in MT take place at different times. Activation of the kinin system, well marked during the first minutes, may perhaps "neutralize" the prothrombogenic action of the induced T. Since the effect of BK or of T itself on prostacycline formation is temporary in character, the most important factor in the relations between these biochemical shifts is evidently the proadhesive action of T, which is clearly marked by the 10th minute of its infusion. Further neutralization of the action of T may be linked with the effect of antithrombin inhibitors, indirect evidence of which is given by restoration of the kallikrein inhibitor level by the 25th minute.

On the whole the results described above and their analysis indicate that several biochemical regulatory stages are involved in the MT process, and definite interconnection and order exist between them. Besides substances of the prostaglandin series, functioning as a stereotyped final link, another such component, whose importance is established beyond doubt, is T (or other factors of the clotting system), which act as initiators of the initial phase of the process. The role of a factor controlling the intensity of the MT process would be ascribed to KKS. This control can be effected with the direct participation of the Hageman factor, regulating the necessary level of T or of BK and plasmin, which are responsible for limiting the MT process.

LITERATURE CITED

1. O. A. Gomazkov, "Functional role of the kallikrein–kinin system of the blood under normal conditions and in cardiovascular pathology," Doctoral Dissertation, Moscow (1977).
2. N. V. Komissarova, "Cardiovascular disorders and the esterase activity of kallikrein," Doctoral Dissertation, Moscow (1969).
3. R. A. Markosyan and A. V. Suvorov, Byull. Éksp. Biol. Med., No. 8, 139 (1979).
4. A. M. Chernukh and O. A. Gomazkov, Patol. Fiziol., No. 1, 5 (1976).
5. A. M. Chernukh, L. A. Éntsik, and O. A. Gomazkov, Patol. Fiziol., No. 6, 66 (1981).
6. A. M. Chernukh, L. A. Éntsik, and O. A. Gomazkov, Byull. Éksp. Biol. Med., No. 12, 677 (1981).
7. B. J. Awbrey, J. C. Hoak, and W. G. Owen, J. Biol. Chem., 254, 4092 (1979).
8. R. W. Colman, J. W. Mason, and S. Sherry, Ann. Intern. Med., 71, 763 (1969).
9. R. L. Czervionke, J. C. Hoak, and G. L. Frey, J. Clin. Invest., 62, 847 (1978).
10. R. L. Czervionke, J. B. Smith, J. C. Hoak, et al., Thromb. Res., 14, 781 (1979).
11. J. C. Hoak, R. L. Czervionke, G. L. Frey, et al., Fed. Proc., 39, 2606 (1980).
12. S. H. Hong, Thromb. Res., 18, 787 (1980).
13. S. Moncada, K. M. Mullane, and J. R. Vane, Br. J. Pharmacol., 66, 96 (1979).
14. S. Moncada and J. R. Vane, Br. Med. Bull., 34, 129 (1978).
15. K. M. Mullane and S. Moncada, Prostaglandins, 20, 25 (1980).