EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON FUNCTION OF THE TISSUE COMPONENT OF THE ALBINO RAT BLOOD CLOTTING AND FIBRINOLYSIS SYSTEM WHEN PROTEIN METABOLISM IS DISTURBED

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KEY WORDS: prostaglandins; tissues; blood clotting.

Previous investigations in the writers' laboratory showed that prostaglandins of the F group (PGF) have a marked influence on the functional state of the tissue component of the hemostasis system. Intragastric administration of PGF to rats with islet-cell insufficiency (alloxan diabetes) led to depression of the blood-clotting properties of tissues of the liver and gastrointestinal tract and inhibition of their fibrinolytic activity [1]. Other conditions being the same, the ultimate effect of the action of PG is determined by the dose administered, the length of exposure, the mode of administration, and the original functional state of the recipient [2, 3].

This paper describes a study of the effect of prostaglandin $F_{2\alpha}$ on the coagulation and fibrinolytic properties of tissues and subcellular fractions when when protein metabolism is disturbed as a result of protein deficiency and experimental toxic hepatitis.

EXPERIMENTAL METHOD

Experiments were carried out on 80 (40 experimental and 40 control) noninbred male albino rats weighing 200-250 g. The animals were kept on a protein-free diet [4] and were given four subcutaneous injections of a 50% solution of carbon tetrachloride in a dose of 0.5 ml/100 g body weight at intervals of 3-4 days. Rats of the experimental group were given an intravenous (into v. penis) injection of $PGF_{2\alpha}$ (USA origin) in a dose of 20 μg/100 g body weight 7 days after the heat injection of carbon tetrachloride. The rats were killed 30 min after injection of PG. Animals of the control group received physiological saline in the same cases and in the same volume of fluid (1 ml). Tissue extracts from the liver, kidney, stomach, small intestine, and skeletal muscle, homogenized in the usual way and diluted 1:50 with isotonic NaCl solution, were tested. Subcellular fractions (nuclei, mitochondria, nuclear supernatnant containing microsomes, lysosomes, and ribosomes but free from mitochondria) were isolated from cells of the liver, kidney, and small intestine by differential centrifugation in sucrose solutions of different densities. The clotting and anticlotting activity of the tissues and subcellular fractions was determined by the usual biochemical tests (recalcification time, heparin tolerance, and fibrinolytic activity of the substrate incubated with tissue extracts and suspensions of cell organelles), and also by thromboelastography on an instrument of the "Thromb-1" type, by a method developed in the writers' laboratory [5]. Because of the specific character of the thromboelastograms (TEG) obtained — the excursion of the automatic writer was below 20 mm and it was impossible to determine the constants for thrombin — only three parameters were determined: reaction time (r), maximal amplitude (ma), and maximal elasticity of the clot (E). Diluted dry human plasma was used as the substrate. An essential condition of these experiments was that the same dilution of plasma was used in both experiment and control.

EXPERIMENTAL RESULTS

The biochemical tests (Table 1) showed considerable changes in the hemostatic properties of the tissues under the influence of injected $PGF_{2\alpha}$.

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TABLE 1. Effect of Prostaglandin $F_{2\alpha}$ on Hemostatic Properties of Tissues of Albino Rats with Toxic Hepatitis (M ± m, n = 10-12)

Tissue	Recalcification time, %			Plasma heparin tolerance, %			Fibrinolysis time, %		
	experiment	control	P	experiment	control	P	experiment	control	P
Liver	74,60±2,86	62,70±5,05	<0,05	82,80±0,55	79,62±0,81	<0,05	68,01±3,65	81,08±3,42	<0,05
Kidney	86,39±0,90	82,52±1,60	<0,05	90,93±0,47	88,83±0,37	<0,05	81,52±0,94	87,06±0,96	<0,01
Stomach	30,33±3,98	54,16±5,08	<0,01	59,63±2,19	68,08±1,49	<0,05	71,74±2,74	69,82±2,74	>0,05
Small intestine	68,28±2,26	55,46±2,92	<0,01	75,39±4,09	67,33±5,93	>0,05	85,81±1,46	89,17±1,16	>0,05
Skeletal muscle	62,15±2,09	53,49±2,37	<0,05	42,88±5,95	36,07±5,34	>0,05	25,41±3,44	34,49±2,49	<0,05

TABLE 2. Effect of Prostaglandin $F_{2\alpha}$ on Hemostatic Properties of Subcellular Fractions Obtained from Albino Rats with Experimental Toxic Hepatitis (M ± m, n = 10-12)

Subcellular fraction from organ tissue	Recalcification time, %			Plamsa heparin tolerance, %			Fibrinolysis time, %		
	experiment	control	P	experiment	control	P	experiment	control	P
Suspension of nuclei									
Liver	30,93±2,77	24,46±2,50	<0,05	63,20±2,40	54,02±3,63	<0,05	37,55±1,73	45,43±1,47	<0,05
Kidney	57,83±2,02	51,63±2,10	<0,05	78,44±2,29	70,31±1,95	<0,05	64,85±4,18	74,76±2,04	<0,05
Small intestine	36,29±2,85	30,80±1,20	>0,05	62,01±2,13	52,98±2,41	<0,05	58,68±3,97	67,94±3,33	>0,05
Suspension of mitochondria									
Liver	57,50±2,03	50,49±1,46	<0,05	78,89±1,43	73,66±1,43	<0,05	71,68±4,34	68,48±4,70	>0,05
Kidney	74,70±2,64	67,76±1,45	<0,05	86,49±3,11	81,91±1,13	>0,05	86,23±3,61	83,43±2,26	>0,05
Small intestine	51,36±2,08	43,31±1,85	<0,05	68,81±2,47	62,02±1,73	<0,05	88,87±2,21	87,45±1,44	>0,05
Nuclear supernatant									
Liver	72,26±1,38	69,25±1,76	>0,05	83,90±2,13	83,48±2,43	>0,05	39,43±5,56	37,73±4,74	>0,05
Kidney	81,52±0,63	80,67±0,77	>0,05	89,77±1,65	89,11±1,97	>0,05	79,39±3,09	78,91±36,27	>0,05
Small intestine	68,66±5,63	62,97±5,63	>0,05	82,28±3,81	76,03±6,22	>0,05	88,86±1,58	88,47±2,03	>0,05

Tissue extracts of the liver, kidney, small intestine, and skeletal muscle reduced the plasma substrate recalcification time and increased plasma heparin tolerance to a much greater degree after administration of $PGF_{2\alpha}$ than the same tissues taken from rats of the control group ($P < 0.05$). The fibrinolytic activity of the tissues was reduced ($P < 0.05$) after injection of $PGF_{2\alpha}$.

Changes in opposite directions were obtained in a study of the hemostatic properties of stomach wall tissue extracts. The decrease in substrate recalcification time and increase in plasma heparin tolerance in animals of the experimental group was less marked ($P < 0.05$) than in the control group. The fibrinolytic activity of stomach wall tissue was unchanged by injection of $PGF_{2\alpha}$ ($P > 0.05$).

Analysis of the TEG showed that $PGF_{2\alpha}$ has a marked effect on its parameters. Tissue extracts of the kidney, small intestine, and skeletal muscle taken from animals receiving $PGF_{2\alpha}$ reduced the reaction time (r) but the maximal amplitude (ma) and maximal elasticity of the clot (E) both increased ($P < 0.05$). Liver tissue extracts from the experimental animals had no appreciable effect on parameters of the TEG ($P > 0.05$).

Under the influence of stomach wall tissue extracts from experimental animals the reaction time was lengthened and the maximal amplitude and elasticity of the clot were reduced (Fig. 1).

Changes in the TEG parameters, incidentally, coincided in direction with the results of the biochemical tests.

It can be concluded from these results that prostaglandin $F_{2\alpha}$, in this experimental pathological condition, increases the coagulant potential and reduces the fibrinolytic activity of liver, kidney, small intestine, and skeletal muscle tissue. The hemostatic properties of the stomach wall tissues were depressed by $PGF_{2\alpha}$.

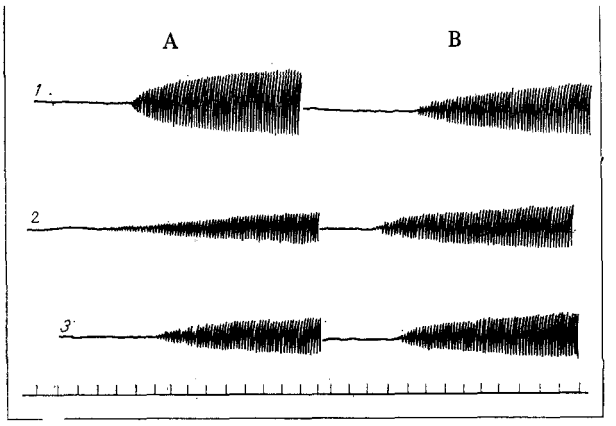

Fig. 1. Effect of tissue extracts on TEG parameters in rats after injection of $PGF_{2\alpha}$. 1) Stomach, 2) small intestine, 3) skeletal muscle. A) Control, B) experiment (the same in Figs. 2 and 3).

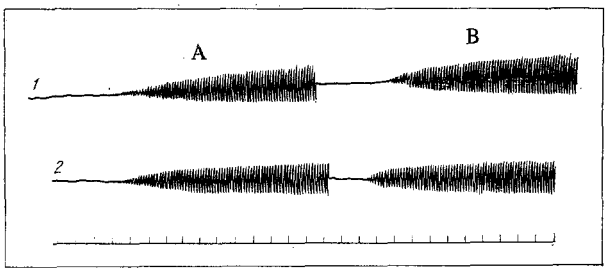

Fig. 2

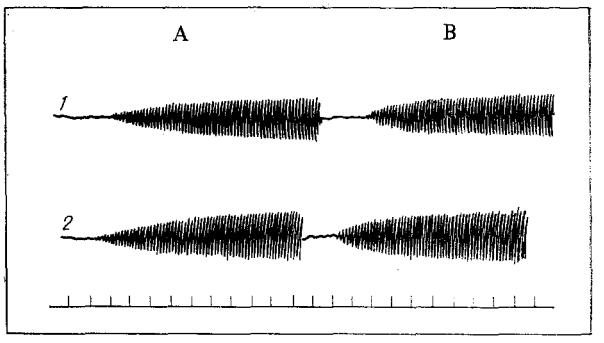

Fig. 3

Fig. 2. Effect of nuclear suspensions of liver and kidney cells on parameters of TEG in rats treated with $PGF_{2\alpha}$. 1) Liver cell nuclei, 2) kidney cell nuclei.

Fig. 3. Effect of suspension of liver and kidney cell mitochondria on TEG parameters in rats treated with $PGF_{2\alpha}$. 1) Liver cell mitochondria, 2) kidney cell mitochondria.

However, the changes in the functional state of the tissue component of the hemostasis system thus revealed do not answer the question of the point of application of the prostaglandins administered. This question can be partly answered by a study of the hemostatic properties of the subcellular fractions. Investigations of the blood-coagulating properties of the subcellular fractions (Table 2) showed that nuclear suspensions of liver and kidney tissue cells of albino rats treated with $PGF_{2\alpha}$ reduced the plasma recalcification time by a much greater degree than cell nuclei obtained from control animals ($P < 0.05$). The plasma heparin tolerance was increased by $PGF_{2\alpha}$ ($P < 0.05$). The fibrinolytic activity of the nuclear suspensions was reduced after injection of $PGF_{2\alpha}$ ($P < 0.05$). Nuclei isolated from cells of the small intestinal wall had weaker hemocoagulant properties than liver and kidney cell nuclei. Changes in fibrinolytic activity of the nuclear suspension of intestinal wall cells also were much less marked.

Nuclear suspensions and whole tissue extracts, it will be noted, induced changes in the same direction although the thromboplastic activity of the former was much weaker.

A suspension of mitochondria from liver, kidney, and small intestine cells of animals receiving $PGF_{2\alpha}$ shortened the recalcification time and increased the plasma heparin tolerance much more than in the control group ($P < 0.05$). The thromboplastic activity of mitochondria from liver and small intestine cells was higher than that of cell nuclei from the same tissues.

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.

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

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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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