Histological examination of the kidneys of animals of the basic group showed a significant decrease in the number of unchanged (i.e., without any manifestations of ischemia, exudation, and so on) glomeruli ($p = 0.023$). No significant changes were observed in the plasma levels of renin, K, and Na, or in the hematocrit index after administration of ANF.

The investigation thus showed that chronic permanent administration of ANF for a week to spontaneously hypertensive rats in equivalent to the natural concentration does not affect the parameters of cardiovascular and excretory function which were tested. Evidently a sufficiently stable electrolyte balance was created in the spontaneously hypertensive rats, and the connections between the electrolyte balance and BP are very strong. Against this background, administration of exogenous ANF could not change the established homeostasis, in agreement with views that stable hypertension is a reflection of a new level of regulatory interaction in the body [1].

Meanwhile the results are evidence that administration of exogenous ANF disturbs the stability of the developed system of homeostasis, as is reflected in the much greater scatter of the data, widening of the confidence limits of correlation, and a change in the character of correlation. As a result of this, some parameters, while sufficiently strongly correlating with one another in the control animals, ceased to have a mutually determinant effect after administration of ANF.

Chronic administration of ANF affects the kidneys, as is reflected in a decrease in the number of unchanged glomeruli.

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LIPOXIN B: A FACTOR ENHANCING SPONTANEOUS PLATELET AGGREGATION IN WHOLE BLOOD

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UDC 612.111.7+612.115].014.46:
612.397.23

KEY WORDS: lipoxin B; reticulocytic lipoxygenase; platelet aggregation

A subject which has recently attracted the attention of research workers is the role of the lipoxygenase metabolites of arachidonic acid (AA) in the regulation of the aggregation state of the blood [4, 9]. It has been shown that 5-hydroxyeicosatetraenoate (5-HETE), if incorporated into phospholipids of endothelial cells, depresses synthesis of prostacycline and prostaglandin E_2 by endotheliocytes [13]. Prostacycline synthesis also is inhibited by 15-hydroperoxyeicosatetraenoate [6]. Leukotrienes C_4, D_4, and E_4 potentiate platelet aggregation and the "platelet factor release reaction" [10], and also potentiate platelet aggregation induced by adrenalin and thrombin [11]. The direct action of lipoxygenase metabolites of arachidonic acid (monohydroperoxy- and monohydroxy-derivatives) on blood clotting has not been established, although it has been shown that they modify eicosanoid metabolism in various tissues and cells, including platelets [5]. Metabolites of arachidonic and eicosapentaenic acid,

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 26-28, July, 1989. Original article submitted April 10, 1988.

0007-4888/89/0007-0932$12.50

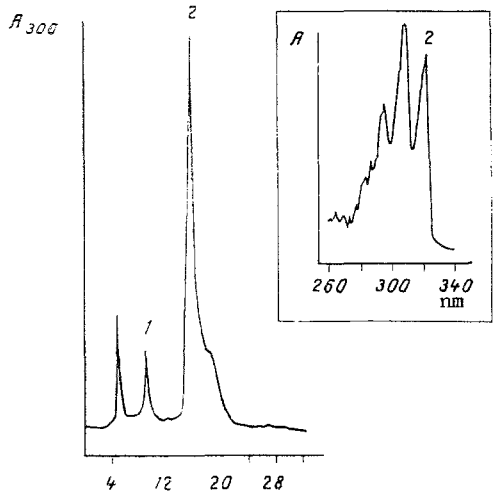

Fig. 1. Fractionation of 5,15-DHETE (1) and lipoxin B (2) by HPLC (for conditions, see text). UV spectrum of lipoxin B is shown separately. Abscissa, elution time, min; ordinate, absorbance.

synthesized by 12-lipoxygenase (the corresponding 12-hydroperoxy- and 12-hydroxy-derivatives) inhibit platelet aggregation and the serotonin "release reaction" from platelets [15]. The action of the recently discovered trihydroxy-derivatives of AA (lipoxins), containing a grouping of four conjugated double bonds, formed with the participation of animal lipoxygenases, on platelet function, however, has virtually not been studied. On this account, it was decided to study effects of 5,14,15-trihydroxy-6,8,10,12-eicosatetraenoate (lipoxin B) and its precursors AA and 5,15-dihydroxy-6,8,11,13-eicosatetraenoate (5,15-D-HETE) on a spontaneous aggregation and procoagulant activity of platelets in whole blood, and also on coagulation of blood and plasma.

EXPERIMENTAL METHOD

Experiments were carried out on 150 male Wistar rats weighing 170 ± 20 g. Blood obtained by cardiac puncture under ether anesthesia was stabilized with 3.8% sodium citrate solution (9:1). Concentrations of the preparations for testing were prepared immediately before use by dilution of the initial solution of the preparation with 0.154 M sodium chloride in ethanol. The final ethanol concentration in the sample did not exceed 0.002%. As preliminary experiments showed, ethanol in these concentrations has no significant effect on the suspension stability or procoagulant activity of platelets, and on coagulation of blood and plasma. The duration of incubation of the preparations with blood or plasma in all experiments was 5 min. Changes in suspension stability of the platelets in whole blood under the influence of the preparations were judged by studying spontaneous platelet aggregation, which was measured by the method in [16] in our own modification. The modification was that in order to determine changes in the number of suspended platelets, we compared not the absolute number of cells, measured by direct counting after sedimentation of erythrocytes, but the optical density of the control and experimental samples. Special experiments demonstrated correlation between optical density and the number of platelets in the blood plasma. The procoagulant activity of the platelets [12], the plasma recalcification time [3], and the stabilized blood clotting time [2] were determined by the usual methods. The results were subjected to statistical analysis, using Student's and the Wilcoxon–Mann–Whitney tests, and also the coefficient of linear correlation.

The AA used in the experiments was obtained from "Sigma" (USA) and its purity was 99%. The 5,15-HETE was obtained by oxidation of AA in 0.1 M borate buffer, pH 9.0, during catalysis with soy lipoxygenase-1, from "Sigma." The 5,15-DHETE was identified by UV spectrophotometry (Hitachi-557 spectrophotometer), with absorption maximum at 242 nm, and by chromatography/mass-spectrometry of the corresponding trimethylsylyl derivative of the methyl ester (M-80A mass-spectrometer, from "Hitachi," Japan) ionization by electron collision at 300°C and with ionizing voltage of 70 eV, capillary column with stationary phase SE-54, m/z peaks 173 (100%), 203, 393, 423, 494 (M^+).

TABLE 1. Changes in Spontaneous Platelet Aggregation in Whole Blood

Preparation	Concentration of preparation, M	Increase (+) or decrease (−) in spontaneous platelet aggregation, % of spontaneous platelet aggregation in control without addition of preparations (M ± m)
AA	$3{,}3\cdot10^{-6}$	+14,1±2,8 ($n = 8$)
	$3{,}3\cdot10^{-5}$	+14,9±1,6 ($n = 10$)
5,15-DHETE	$0{,}6\cdot10^{-9}$	−30,0±8,8 ($n = 4$)
	$2{,}8\cdot10^{-9}$	−30,6±8,8 ($n = 4$)
Lipoxin B	$3{,}3\cdot10^{-11}$	+9,3±2,4 ($n = 7$)
	$3{,}3\cdot10^{-10}$	+12,4±2,4 ($n = 8$)
	$3{,}3\cdot10^{-9}$	+23,8±4,2 ($n = 10$)

Legend: Data on effect of test preparations on suspension stability of platelets differed statistically significantly ($p < 0.05$) from control.

EXPERIMENTAL RESULTS

Lipoxin B was obtained by oxidation of 5,15-DHETE in 0.1 M K, Na-phosphate buffer, pH 7.4, during catalysis with a homogeneous preparation of C-15-lipoxygenase from rabbit reticulocytes, obtained by the method in [14], followed by reduction with $NaBH_4$. The preparations of 5,15-DHETE and lipoxin B were purified by high-performance liquid chromatography (HPLC) on a "Spectra-Physics 8700" chromatograph (USA) on "Lichrosorb SI-60-10" column (250 × 10 mm), with a flow rate of 3 ml/min in a solvent system of hexane–isopropanol–acetic acid 80:20:0.1. The retention time was: 5,15-DHETE 8.3 min, lipoxin B 14.2 min (Fig. 1). The lipoxin B (peak 2) had a characteristic UV-spectrum of conjugated tetraenes: absorption maxima at 287, 301, and 316 nm [7]. The structure of the product obtained in our laboratory was confirmed by chromatography/mass-spectrometry [7]. In the course of our investigations it was shown that lipoxin B, in concentrations of 3.3×10^{-9}-3.3×10^{-11} M reduces the suspension stability of platelets in whole blood. AA gave an effect that was similar in direction and amplitude, but in concentrations several orders of magnitude higher; moreover, in a concentration of 3.3×10^{-7} M it no longer had any statistically significant action (Table 1).

The metabolic precursor of lipoxin B, namely 5,15-DHETE, on the other hand, led to an increase in suspension stability of platelets in concentrations of 0.6×10^{-9}-2.8×10^{-9} M. In a concentration of 2.8×10^{-10} M this preparation had no statistically significant action on the suspension stability of the platelets (Table 1).

No direct effect of the test preparations on blood coagulation could be found with the aid of coagulation tests; meanwhile all preparations, even in minimal concentrations (Table 1), acted on the procoagulant activity of the platelets. Lipoxin B (3.3×10^{-11} M) and AA (3.3×10^{-6} M) increased the procoagulant activity of the platelets by 14.3 ± 0.9 and 11.5 ± 0.4% respectively, whereas 5,15-DHETE (0.6×10^{-9} M), on the other hand, reduced it by 9.5-0.9%. These experiments, incidentally, revealed strong correlation between spontaneous aggregation of the platelets and their procoagulant activity. For instance, the coefficient of correlation between these parameters was 0.995 ± 0.007 for lipoxin B ($p < 0.001$), 0.995 ± 0.044 for AA ($p < 0.001$), and 0.998 ± 0.003 for 5,15-DHETE ($p < 0.001$).

This investigation thus yielded evidence of the opposite actions of lipoxin B and its metabolic precursor, 5,15-DHETE, on platelet aggregation.

The authors are grateful to Dr. H. Kuhn and Dr. R. Wiesner, of the A. Humboldt Berlin University, East Germany, for help with the isolation of the rabbit reticulocyte lipoxygenase and for their interest in the work.

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ANGIOPROTECTIVE EFFECT OF CATHERGEN IN EXPERIMENTAL LIVER DAMAGE

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UDC 616.36-02:615.917]-092.9-06:616.16-008.6-085.31:547.972.3

KEY WORDS: cathergen; microcirculation; mast cell

Disturbances of the microcirculation arising during pathological processes in the liver call for correction by drugs. For the treatment of liver disease, cathergen, a synthetic analog of bioflavonoids, has been used with success. However, the effect of cathergen on the microcirculatory system has not been studied. It was accordingly decided to undertake the investigation described below.

EXPERIMENTAL METHOD

A model of acute toxic liver damage with disturbance of the microcirculation was created by intraperitoneal injection of carbon tetrachloride (CCl_4) in a dose of 1 mg/kg (40 albino rats) or of ethanol in a dose of 6 g/kg (40 rats). Half the animals of both groups received preliminary injections of cathergen in a dose of 120 mg/kg once a day for 2 weeks. The control

TABLE 1. Changes in Number of Mast Cells and Their Degree of Degranulation under the Influence of Cathergen, CCl_4, and Ethanol (M ± m)

Parameter	Control	Cathergen	CCl_4	CCl_4 + cathergen	Ethanol	Ethanol + cathergen
Number of mast cells in 1 m^2	62,3±2,28	68,8±2,43	66,2±2,32	92,9±1,71	51,1±1,52	82,2±2,48
p_1		>0,05	>0,05	<0,001	<0,01	<0,001
p_2			>0,05	<0,001	<0,001	<0,001
p_3				<0,001		<0,001
Index of degranulation of mast cells	1,05±0,02	0,52±0,03	2,20±0,05	0,41±0,04	1,55±0,04	0,46±0,02
p_1		<0,001	<0,001	<0,001	<0,001	<0,001
p_2			<0,001	≤0,05	<0,001	>0,05
p_3				<0,001		<0,001

Legend. p_1) Significance of differences compared with control, p_2) compared with values obtained in animals receiving cathergen, p_3) between values obtained in animals receiving CCl_4 or ethanol and values obtained in rats receiving a combination of CCl_4 or ethanol with cathergen.

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