EFFECT OF BIOLOGICALLY ACTIVE SUBSTANCES ON THE ELECTROKINETIC PROPERTIES OF ERYTHROCYTES

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The effect of adrenalin, acetylcholine, histamine, thrombin, heparin, fibrinogen, and ADP on the electrokinetic potential of human and canine erythrocytes was studied. Heparin increases the ζ-potential and the other substances reduce it. The mechanisms of action of these substances on the electrokinetic properties of the erythrocyte membrane are discussed.
KEY WORDS: heparin; fibrinogen; ADP; electrokinetic potential; erythrocytes.

The dispersed state of the blood cells depends on their interaction with each other; this is determined by the surface properties of the membranes and, in particular, by the surface electric charge [3, 4, 7, 8].

Since the electrokinetic potential reflects the surface properties most closely, it was decided to study the effect of substances active toward blood coagulation on the electrophoretic mobility of the erythrocytes, a function of their electrokinetic potential.

EXPERIMENTAL METHOD

Human and canine erythrocytes obtained by venipuncture were washed three times in phosphate buffer, pH 7.3, and transferred to a modified Abramson's chamber [5]. By means of nonpolarizing $Cu-CuSO_4$ electrodes a constant current of 5 mA was passed through the chamber from a stabilized-voltage source. By using the calibration scale of the microscope ocular, the velocity of movement of 15 erythrocytes in an electric field was recorded. The velocity of movement of 15 electrolytes was then determined when the polarity of the voltage was altered. These measurements were made for each concentration of the preparations studied and also in control experiments on three samples of blood. The mean value of the velocity was thus calculated from 90 observations. The electrophoretic mobility was determined as the rate of migration of the cells per unit of intensity of the electric field and was expressed in microns per second per volt per centimeter. Next, using Smolukhovskii's formula, the electrokinetic potential (the ζ-potential) was calculated:

$$\zeta = \frac{4\pi\eta v}{D},$$

where D is the dielectric permeability of the medium; v the electrophoretic mobility; the viscosity of the solution. The measurements were made at a temperature of 25 ± 0.1°C, the stability of which was maintained with the aid of a water ultrathermostat.

The effect of the following substances was studied on the electrokinetic properties of the erythrocytes: adrenalin, acetylcholine, histamine, thrombin, fibrinogen, ADP, and heparin. Human and canine erythrocytes were incubated in a solution of these substances for 5 min at 37°C, after which the electrophoretic mobility was determined.

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TABLE 1. Effect of Substances Active toward Blood Clotting on ζ-Potential of Erythrocytes (M ± m)

Preparation	Concn. (μg/ml)	ζ-potential of erythrocytes (in mV)			
		canine	P	human	P
Control	—	21,0±0,28		15,8±0,2	
Adrenalin	5	22,7±0,28	<0,2	14,7±0,3	<0,01
	10	19,9±0,43	<0,2	14,6±0,2	<0,01
	50	20,4±0,43	—	14,1±0,3	<0,01
	100	20,6±0,43	<0,2	14,0±0,2	<0,01
	500	20,2±0,28	<0,2	14,1±0,2	<0,01
Control	—	19,7±0,3		16,8±0,3	
Acetylcholine	5	19,4±0,3	—	16,8±0,3	—
	10	19,9±0,4	—	15,8±0,3	<0,05
	50	19,3±0,3	—	16,1±0,4	<0,02
	100	19,7±0,3	—	15,2±0,3	<0,01
Control	—	17,8±0,2		16,8±0,3	
Histamine	0,05	15,4±0,3	<0,01	14,6±0,3	<0,05
	0,10	15,0±0,3	<0,01	14,3±0,3	<0,01
	0,50	14,4±0,2	<0,01	14,1±0,3	<0,01
	1,00	15,1±0,2	<0,01	14,1±0,2	<0,01
	5,00	15,7±0,3	<0,01	14,4±0,3	<0,01
Control	—	18,5±0,3		15,5±0,3	
Thrombin	0,25	17,4±0,4	<0,01	15,4±0,4	—
	0,50	17,6±0,3	<0,01	15,4±0,4	—
	1,00	17,5±0,3	<0,01	15,4±0,2	—
	5,00	16,9±0,4	<0,01	14,4±0,2	<0,01
	10,00	17,1±0,2	<0,01	14,0±0,2	<0,01
Control	—	17,1±0,2		16,7±0,2	
Fibrinogen	50	16,9±0,3	—	16,1±0,3	<0,1
	100	16,5±0,3	<0,1	15,8±0,3	<0,02
	400	16,2±0,4	<0,02	14,8±0,4	<0,01
	4000	14,1±0,2	<0,01	12,2±0,4	<0,01
	10 000	12,2±0,3	<0,01	10,9±0,4	<0,01
Control	—	17,6±0,3		15,5±0,3	
Heparin	0,05	18,6±0,3	<0,02	16,5±0,2	<0,01
	0,10	19,3±0,2	<0,01	16,5±0,2	<0,01
	1,00	20,7±0,3	<0,01	17,9±0,4	<0,01
	25,00	21,7±0,2	<0,01	17,9±0,4	<0,01
	50,00	22,5±0,2	<0,01	19,0±0,4	<0,01
Control	—	18,0±0,3		15,6±0,3	
ADP	5	17,7±0,4	—	14,0±0,3	<0,01
	25	18,4±0,4	—	14,0±0,4	<0,01
	100	18,4±0,3	—	14,0±0,5	<0,01
	250	18,2±0,2	—	14,0±0,6	<0,02
	500	18,6±0,3	<0,05	13,4±0,5	<0,01

Legend: Thrombin and heparin concentrations given in units/ml.

EXPERIMENTAL RESULTS

It can be concluded from analysis of the experimental results (Table 1) that the substances investigated modify the electrical properties of the surface of the erythrocytes. Fibrinogen and heparin had the strongest action on the mobility of the cells. Adrenalin, thrombin, fibrinogen, and histamine in the overwhelming majority of cases significantly lowered the electric charge on the erythrocytes, both human and canine. Acetylcholine and ADP reduced the electrophoretic mobility of the human erythrocytes. So far as the canine cells are concerned, no significant changes were detected under the influence of these substances. Unlike the other preparations, heparin increased the electrophoretic mobility of the erythrocytes.

Analysis of the dependence of the electrokinetic potential on the concentration of the substances tested showed that it is a linear function of the logarithm of the fibrinogen and heparin concentrations (Fig. 1). With an increase in the fibrinogen concentration the mobility of the cells was reduced. An increase in the heparin concentration gave the opposite effect. So far as acetylcholine, adrenalin, and thrombin (for human erythrocytes) are concerned, in these cases the change in velocity was "trigger" in character and only little dependent on concentration. With an increase in the histamine concentration the velocity of the cells fell initially and then rose slightly.

The relationship between the electrokinetic potential and the concentration of these substances can be explained only on the basis of the analysis of the mechanisms of interaction of these substances. The first

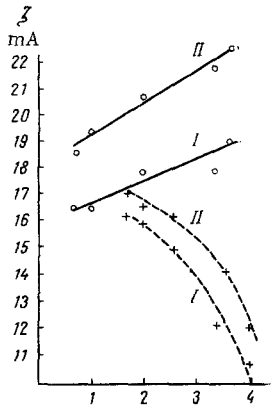

Fig. 1. Electrokinetic potential (ζ-potential) of human (I) and canine (II) erythrocytes as functions of logarithm of concentration of heparin (continuous line) and fibrinogen (broken line).

fact to be noted is that substances with high molecular weight (fibrinogen, heparin) act on the velocity as linear functions of concentration. Substances of low molecular weight (adrenalin, acetylcholine, histamine) give rise to relaxation changes in the electrokinetic potential. This suggests that biopolymers affect chiefly the electric charge as a result of their adsorption on the cell membranes. However, the results cannot be explained entirely by adsorption effects. In all probability, deformation of the membrane structure with consequent changes in permeability with respect to substances capable of neutralizing the surface electric charge plays an important role in this process. Biologically active substances (adrenalin, acetylcholine, histamine) may not only be adsorbed on cell membranes, but they may also interact with their components. In particular, there is evidence that acetylcholine reacts with phospholipids (phosphatidylcholine), inducing conformational changes in the membrane proteins, followed by changes in the permeability of the membrane [6]. It has also been shown that adrenaline, acetylcholine, and histamine can liberate a thromboplastic factor from intact erythrocytes – the "rebound effect" [1, 2]. The action of thrombin can be attributed, first, to adsorption on the surface of the erythrocytes and, second, to its proteolytic properties, affecting the surface of the membranes.

Changes in the electrokinetic properties of the erythrocytes under the influence of substances affecting blood clotting must be regarded as due not only to adsorption effects, but also to the effect of the substances on the permeability of the blood cells.

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