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ROLE OF ENDOPEROXIDES OF THE PROSTAGLANDINS IN PLATELET AGGREGATION

G. Ya. Levin, Yu. A. Sheremet'ev, and S. V. Petrov

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Phospholipase A and lysolecithin stimulate the reaction of liberation of thromboplastic factor and aggregation of erythrocytes and platelets. Polarographic investigations have shown that these aggregating agents cause absorption of O_2 in medium containing platelets, possible evidence of the formation of these conditions of intermediate products of prostaglandin synthesis, namely endoperoxides. Albumin does not prevent the liberation reaction and the absorption of O_2 caused by phospholipase and lysolecithin but it completely inhibits their aggregating action. Aspirin, on the other hand, blocks O_2 consumption by platelets although its action on the aggregating effect of lysolecithin is only very slight. It is suggested that the aggregation of the blood cells is connected with perturbation of the lipid–protein structure of their membranes and not with endoperoxide synthesis.

KEY WORDS: *blood cells; aggregation; liberation reaction; prostaglandins.*

Reports have recently been published on the important role of intermediate products of prostaglandin synthesis (endoperoxides, prostaglandins G_2 and H_2) in platelet aggregation [8]. The suggestion has been made that aggregating agents (such as collagen, ADP, thrombin) can activate phospholipase activity and liberate arachidonic acid, the common precursor of the prostaglandins [11, 12]. The endoperoxides subsequently formed "trigger" platelet aggregation [15].

The investigation described below was carried out to study this problem.

TABLE 1. Effect of Phospholipase and Lysolecithin on Aggregation of Blood Cells (M ± m)

Conditions of aggregation	Plasma enriched with platelets		Suspension of washed erythrocytes	
	Ma, mm	T, min	Ma, mm	T, min
Phospholipase	39,8±1,36	18,8±0,84	38,0±1,36	22,2±0,90
Phospholipase + albumin	5,5±1,37	11,3±2,89	3,8±1,08	11,9±2,68
P	<0,001	<0,01	<0,001	<0,02
Phospholipase + aspirin	10,9±1,71	14,5±1,80	11,4±1,91	18,9±1,92
P	<0,001	<0,05	<0,001	>0,1
Lysolecithin	46,0±2,48	22,6±1,25	41,7±2,74	24,7±1,47
Lysolecithin + albumin	2,5±1,04	7,2±3,05	4,0±1,87	6,8±2,94
P	<0,001	<0,01	<0,001	<0,02
Lysolecithin + aspirin	36,0±2,99	22,4±1,42	35,8±1,95	21,2±1,01
P	<0,05	>0,5	>0,05	>0,05

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TABLE 2. Dynamics of O_2 Consumption in Suspension of Platelets and Erythrocytes during the Action of Phospholipase and Lysolecithin (M ± m)*

Conditions of aggregation	After incubation for 5 min	After incubation for 12 min
Platelet suspension + phospholipase	93,4±4,25	92,1±3,42
Platelet suspension + albumin + phospholipase	113,0±6,90	109,5±6,94
Platelet suspension + aspirin + phospholipase	0	0
Platelet suspension + lysolecithin	140,4±5,83	162,1±8,15
Platelet suspension + albumin + lysolecithin	189,0±7,94	215,5±8,57
Platelet suspension + aspirin + lysolecithin	0	0
Erythrocyte suspension + phospholipase	0	0
Erythrocyte suspension + lysolecithin	0	0

*The data given show the ratio between the decrease in the partial pressure of O_2 in the suspensions of platelets and erythrocytes in the experimental (with addition of phospholipase and lysolecithin) and control (with physiological saline) groups, expressed as percentages.

EXPERIMENTAL METHOD

Aggregation of the blood cells was investigated by a photometric method. The degree of aggregation — the maximal amplitude of the aggregation curve (Ma) — and its rate — the time required for Ma to be reached (T) — were estimated. Washed erythrocytes (1:400), platelets (300,000-400,000/mm^3), and also citrated plasma enriched with platelets were used. Aggregation was stimulated by lysolecithin and phospholipase A. The venum of the cobra *Naja naja* (33 µg/ml), heated to 90°C for 10 min to inactivate the proteolytic enzymes, was used as the source of phospholipase A. Platelet-free plasma, rich in lysolecithin, previously incubated at 37°C for 24 h, was used in the experiments [7]. Albumin (10-20 mg/ml) and aspirin [14] were used to inhibit aggregation. To study the reaction of liberation of thromboplastic factor suspensions of erythrocytes and platelets were preincubated with aggregating agents for 1 h at 37°C. After incubation, the blood cells were washed twice and then disintegrated by repeated freezing and thawing. The venom of *Vipera lebetina* (lebetox) was used for the investigation. The reaction of endoperoxide formation, accompanied by oxygen consumption [9, 15], was studied by a polarographic method. By means of this method the formation of prostaglandins can be determined with sufficient accuracy; comparative investigation of prostaglandin synthesis by polarographic, radiometric, spectrophotometric, chromatographic, and biological methods yielded identical results [5]. All the experiments were carried out in an airtight electrolyzer, using a gold electrode of semicovered type. Electrolysis was carried out under intermittent conditions by short-circuiting the electrodes (pulse duration 20 sec). The magnitude of the residual current before and after the experiment and also between the series of experiments was stable and it averaged 1.2 ± 0.13% of the readings in physiological saline. Aggregation and oxygen consumption also were investigated in an oxygen-free medium containing platelets or erythrocytes. The medium was deprived of oxygen by means of sodium sulfite [1] under polarographic control.

EXPERIMENTAL RESULTS AND DISCUSSION

Phospholipase A and lysolecithin induced aggregation of both erythrocytes and platelets (Table 1). Preincubation of the blood cells with albumin led to complete inhibition of the aggregating action of phospholipase and lysolecithin. Aspirin, which largely inhibits aggregation of erythrocytes and platelets when stimulated by phospholipase, blocked the aggregating action of lysolecithin to a lesser degree.

During incubation of phospholipase and lysolecithin with the blood cells the concentration of thromboplastic factor in the latter fell considerably ($P < 0.001$). Albumin had no appreciable effect on this process.

On the addition of phospholipase and lysolecithin to suspensions of erythrocytes and platelets, preincubated with sodium sulfite, aggregation of the blood cells was undisturbed.

The polarographic investigations showed that phospholipase and lysolecithin induce considerable absorption of O_2 in medium containing platelets (Table 2). This phenomenon was not found on the addition of aggregating agents to the suspension of erythrocytes. Albumin not only did not inhibit O_2 absorption by the platelets induced by phospholipase and lysolecithin, but in some cases it actually stimulated it. Meanwhile aspirin completely prevented O_2 consumption by platelets when stimulated by aggregating substances.

It can be postulated on the basis of these results that endoperoxide synthesis is not the determining factor in aggregation of the blood cells. For instance, aggregation of erythrocytes, stimulated by phospholipase and lysolecithin, takes place under conditions when prostaglandin synthesis is absent. Meanwhile, endoperoxide synthesis and platelet aggregation induced by lysolecithin are blocked, though not parallel to one another: Albumin, inhibiting aggregation, does not prevent endoperoxide synthesis, whereas aspirin has the opposite effect. In addition, platelet aggregation can take place in an oxygen-free medium.

The mechanism of aggregation of blood cells induced by phospholipase and lysolecithin is evidently common at least to platelets and erythrocytes. Treatment of erythrocytes with phospholipase A and lysolecithin is known to cause significant changes in the surface layers of their membrane [10, 13]. According to one view [7], this causes aggregation and lysis of the erythrocytes. Substantial changes in platelet membranes have also been observed under the influence of arachidonic acid [11].

According to the mosaic—liquid theory of membranes, the shape of erythrocytes is determined by two of their properties: fluidity and contraction of the proteins of the inner surface of the membrane [6]. During contraction of these proteins the erythrocytes become "hard" in the zone of contraction and "soft" around it. The same contractile protein (thrombostenin) is also found in the membrane of platelets. It is ascribed the leading role in dynamic conversions of the blood platelets [3].

According to recent observations [4], aggregation of the proteins of the membrane is connected with disorganization of its lipid components, which takes place in particular under the influence of lysolecithin. This process (phospholipase → hydrolysis of membrane phospholipids of the blood cells → lysolecithin → fatty acids → perturbation of lipid components → aggregation of proteins of the inner surface of the membrane → appearance of "stellate" and "needle-shaped" forms of cells → liberation reaction → aggregation) is evidently common to both erythrocytes and platelets and it is the main cause of their aggregation under the influence of various aggregating agents. The validity of the suggested scheme is also confirmed by the writers' earlier observations [2].

Phospholipase and lysolecithin thus stimulate the liberation reaction and aggregation of the blood cells and cause the formation of endoperoxides in medium containing platelets. The interconnection between these phenomena is due to the fact that during the action of aggregating agents the platelets liberate a cyclooxygenase which catalyzes the conversion of arachidonic acid into endoperoxides. At the same time, aggregation is probably connected with preceding perturbation of the lipid—protein structure of the membrane of the blood cells.

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