ACTION OF PHOSPHOLIPASE A ON AGGREGATION OF RED CELLS AND PLATELETS

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The effect of plasmin and trypsin on the activation of phospholipase and the action of phospholipase A (cobra venom) on the response of liberation and aggregation of red cells and platelets were studied. Trypsin and fibrinolysin activate phospholipase, and this is accompanied by the accumulation of nonesterified fatty acids in the blood serum. Phospholipase A induces liberation of thromboplastic factor from red cells and platelets and also their aggregation. Aggregation is inhibited by albumin and EDTA. It is postulated that the action of proteolytic enzymes on the blood cells is mediated through activation of phospholipase.

KEY WORDS: proteolytic enzymes; phospholipase A; aggregation; blood cells.

Several pathological states accompanied by marked features of hypoxia, acidosis, and hyperadrenalinemia (shock, terminal states, acute cardiovascular failure, etc.), are accompanied by significant activation of proteolysis and by thromboembolic complications [3]. Trypsin and fibrinolysin have been shown to potentiate the liberation of platelets and their aggregation and to change their shape [8, 10]. Meanwhile, their action on red cells has been inadequately studied [1, 2]. The urgency of these investigations is also determined by the fact that proteolytic enzymes are widely used clinically for the treatment of thrombosis.

An essential factor in the mechanism of action of proteolytic enzymes on blood cells could be phospholipase activation. It has been shown, in particular, the trypsin activates prophospholipase, which is present in the serum and in membranes of red cells [6, 11]. Activation of phospholipase causes hydrolysis of the membrane phospholipids of platelets and their aggregation [4]. Treating red cells with phospholipase A modifies the surface layers of the membrane and includes the polar lipids within the hydrophobic lipid region [14].

The object of the investigation described below was to study the action of proteolytic enzymes (plasmin, trypsin) on phospholipase and the effect of the latter on the liberation and aggregation of red cells and platelets.

EXPERIMENTAL METHOD

Phospholipase activity was estimated from the accumulation of nonesterified fatty acids (NEFA) in the serum [5] after its incubation for 45 min at 37°C with trypsin (0.5 mg/ml) or fibrinolysin (1000 units/ml). Aggregation of platelets (in platelet-enriched plasma, with a mean concentration of 200,000-300,000/mm^3) was studied photometrically in an aggregometer. The degree of aggregation was estimated from the maximal amplitude of the aggregation curve (MA) and its rate from the time required to reach the maximal amplitude (T). Aggregation of red cells also was investigated in an aggregometer. Washed red cells, diluted with physiological saline in the ratio of 1:400, were used. The source of phospholipase A was the venom of the cobra Naja naja (33 μg/ml), preliminarily heated to 90°C for 10 min to inactivate proteolytic enzymes. The inhibitors of aggregation were albumin (10-20 mg/ml) and EDTA (2 mg/ml). The reaction of liberation of thromboplastic factor was studied with the aid of Lebetox (venom of the viper Vipera lebetina). For this purpose, a suspension of washed red cells (1 ml) and platelet-enriched plasma (0.8 ml) was first incubated with phospholipase A for 40 min at 37°C. The platelets and red cells were then washed twice, resuspended in 0.3 ml physiological saline, and disintegrated by repeated freezing and thawing.

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EXPERIMENTAL RESULTS AND DISCUSSION

Under the influence of trypsin and fibrolysin NEFA accumulated in the serum, evidence of the activating action of these enzymes on phospholipase. With an increase in the calcium concentration in the incubation medium this effect increased (Table 1).

Addition of phospholipase to platelet-enriched plasma or to the red cell suspension led to significant aggregation of the blood cells. EDTA and albumin inhibited aggregation of both platelets and red cells, stimulated by phospholipase A (Table 2).

Phospholipase A also caused liberation of thromboplastic factor from red cells and platelets (Table 3).

Activation of proteolysis was thus accompanied by an increase in phospholipase activity. This, in turn, caused aggregation of the blood cells. The aggregating action of phospholipase A was blocked by substances binding calcium (in these experiments, EDTA).

The mechanism of the aggregating action of phospholipase has not been explained. According to Gleen et al. [7], the addition of phospholipase to platelets stimulates prostaglandin synthesis. The cyclic endoperoxides formed under these circumstances are at present ascribed an important role in the mechanism of platelet aggregation [9]. The present results suggest that prostaglandin synthesis is not the decisive factor in the action of phospholipase on aggregation, for it aggregates both platelets and red cells equally. Two factors may play the main role in the mechanism of its action on the blood cells. First, phospholipase A causes hydrolysis of the lipids which are the main components of the membrane. The liberation reaction takes place as soon as less than 5% of the membrane phospholipids have been hydrolyzed [13]. The alteration of the membrane structure leads to the appearance of "needle" or "spinous" forms of cells which, in turn, promotes aggregation of the red cells and platelets. Second, hydrolysis of membrane phospholipids by phospholipase A causes the appearance of lyso fractions: lysolecithin and lysophosphatidylethanolamine. These hydrolysis products

TABLE 1. Effect of Trypsin and Fibrinolysin on NEFA Concentration (in μeq/ml) in Blood Serum (M ± m)

Initial level	Incubation with trypsin	Incubation with trypsin and calcium	Incubation with fibrinolysin	Incubation with fibrinolysin and calcium
0,46±0,02	0,76±0,02	1,04±0,04	0,83±0,02	1,13±0,03
P	<0,001	<0,001	<0,001	<0,001

TABLE 2. Effect of Phospholipase A on Aggregation of Plateletes and Red Cells (M±m)

Substances added	Platelet-enriched plasma		Suspension of washed red cells	
	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 + EDTA	2,1±0,67	10,1±3,00	3,7±1,86	5,0±2,51
P	<0,001	<0,01	<0,001	<0,001
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	>0,1

TABLE 3. Concentration of Thromboplastic Factor in Red Cells and Platelets after Their Incubation with Phospholipase A (M ± m)

Time of testing	Activity of thromboplastic factor, %	
	platelet-enriched plasma	suspension of washed red cells
Before incubation	100	100
After incubation	73,6±2,0	75,2±2,2
P	<0,001	<0,001

also promote aggregation, changes in the membrane structure of the cells, and hemolysin of red cells [12]. This mechanism is evidently fundamental to the action of phospholipase, for aggregation was inhibited by albumin, which binds the hydrolysis products of phospholipids.

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