PHOSPHOINOSITIDE BREAKDOWN AND DIACYLGLYCEROL FORMATION IN HUMAN PLATELETS UNDER THE INFLUENCE OF LIPOPOLYSACCHARIDE TOXIN

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Lipopolysaccharide toxins (LPST) can activate the coagulation system of the blood, and one possible pathway of this effect may be by their direct action on the platelet component of this system [1]. It was shown previously that treatment of platelets with LPST does not cause their direct aggregation, but it induces increased ability to aggregate in response to the subsequent action of various aggregation inducers [1]. The molecular mechanisms of the effect of LPST on platelets still remain largely unexplained. Investigators consider that the toxin acts through specific receptors of the cell membrane [12]. There is also evidence that LPST exerts its action through a change in the dynamic structure of the membrane lipid bilayer [7]. It can be tentatively suggested that interaction between LPST and platelets involves the functioning of cell regulatory systems such as the phosphoinositide cycle and biosynthesis of various arachidonic acid metabolites [9].

The aim of this investigation was to study the effect of LPST on the structure of the plasma membrane, on the phosphoinositide cycle, on endogenous phospholipase activity, and on thromboxane B_2 synthesis in human platelets.

EXPERIMENTAL METHOD

Platelets were isolated from donated blood by the method in [4]. The suspension of washed platelets was used during 3-4 h after isolation. The functional state of the platelets was verified with respect to aggregation initiated by thrombin (from Merck, West Germany). The Aggregometer was based on a "Spectromil" spectrophotometer (Bausch and Lomb, USA). LPST from *Salmonella typhimurium* was obtained from Difco (USA), the spin probe I from Syva (USA), and probes II and III were generously provided by A. B. Shapiro (Institute of Chemical Physics, Academy of Sciences of the USSR). Electron paramagnetic resonance (EPR) spectra were recorded on a Brucker ER 200D spectrometer (West Germany). Concentrations of polyphosphoinositides [10], phospholipids [3, 11], and diacylglycerol [5] were determined by thin-layer chromatography (TLC; silica-gel HPTLC plates, from Merck, West Germany), using a Camag densitometer (Switzerland) and Du Pont SP 4100 recording integrator (USA). Thromboxane B_2 biosynthesis from endogenous ^{14}C-arachidonic acid was determined by TLC and preliminary incorporation of label into platelets as described previously [6].

EXPERIMENTAL RESULTS

Changes taking place in the platelet plasma membrane under the influence of LPST were determined by the spin probe EPR method [2]. Dependence of changes in the rotational motion of lipid probes I-III during titration with the toxin is shown in Fig. 1. As these results show, with an increase in the LPST concentration the microviscosity of the lipid bilayer increased (parameters $2A_{\parallel}$ and τ); starting with a concentration of 150 μg LPST/10^9 cells, moreover, saturation of the effect took place. Consequently, LPST increases the microviscosity of the plasma membrane, in agreement with data obtained in [7, 8] in a study of the action of LPST on model systems and cells.

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TABLE 1. Effect of LPST on Content of Main Phospholipids of Platelets

Phospholipid	In absence of LPST		After incubation with LPST (10 min at 37°C)		
	moles, % of total	nmoles/10^9 cells	moles, % of total	nmoles/10^9 cells	% of control
Phosphatidic acid	1,8	8,0	2,7	11,4	141,9
Monophosphoinositide	4,7	17,5	4,2	14,8	84,5
Phosphatidylserine	8,9	35,9	9,2	35,1	97,7
Phosphatidylcholine	39,4	158,8	38,3	146,4	92,2
Sphinogomyelin	17,0	67,0	19,4	72,2	107,8
Phosphatidylethanolamine	27,6	118,3	25,2	101,2	85,6
Lysophosphatidylcholine	0,6	3,5	1,0	5,6	160,0

Legend. Relative error of determination did not exceed 10%.

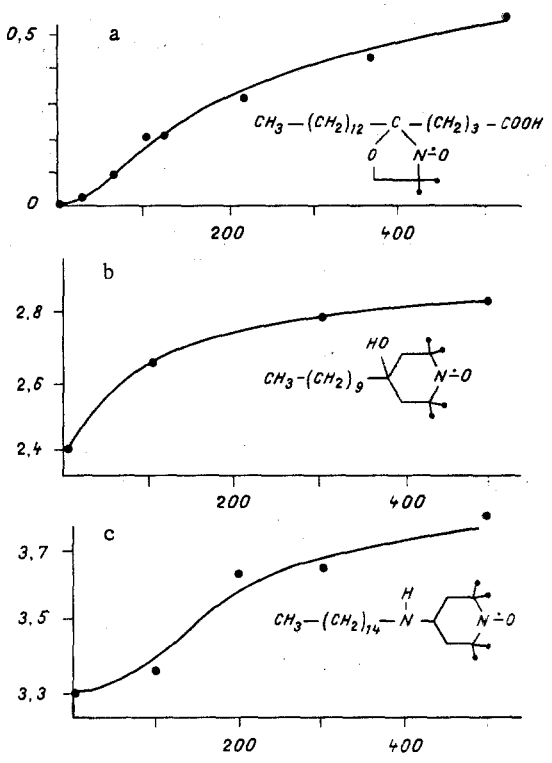

Fig. 1. Changes in EPR parameters of lipid probes during titration of platelets with LPST. Abscissa, LPST concentration (in μg/10^9 cells); ordinate: a) $\Delta A_{\parallel}$ (in G), b, c) $\tau \cdot 10^9$ (in sec). Cell concentration 10^9/ml, temperature 37°C.

The effect of LPST on changes in the concentration of the main components of the phosphoinositide cycle depending on incubation time is shown in Fig. 2. The measured absolute values of concentration of polyphosphoinositides and diacylglycerol, incidentally, varied quite considerably from one sample of platelets to another (up to 30-40%). However, for each platelet population (from one donor) the changes in these concentrations were qualitatively and quantitatively very close (between 5 and 10%). During incubation with the toxin the concentration of mono-, di- and triphosphoinositides fell (15-30%), and by the 10th minute the effect reached saturation (Fig. 2).

Under these circumstances the phosphatidic acid concentration increased significantly (Table 1). Changes in the diacylglycerol concentration were complex in character: by the 1st minute of incubation the diacylglycerol concentration fell, and then rose, and by the 10th minute it was 20% higher than initially. This complex dependence of the changes in diacylglycerol concentration with time may be connected with the fact that initially phospholipase A_2 was activated, leading to a decrease in the diacylglycerol concentration as a

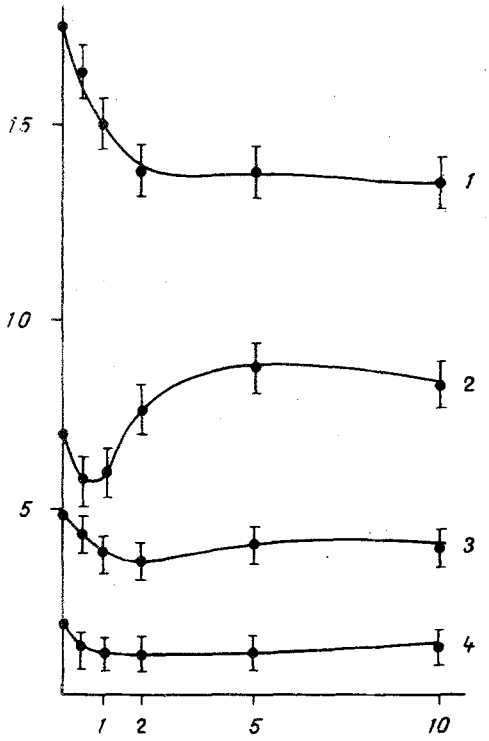

Fig. 2

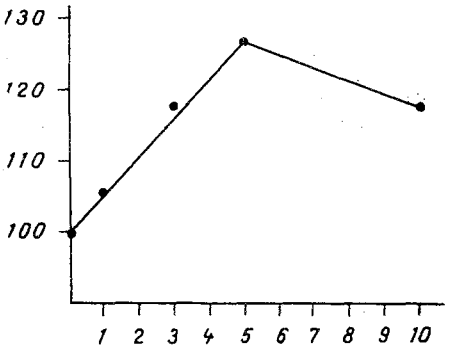

Fig. 3

Fig. 2. Change in concentrations of phosphoinositides and diacylglycerol during incubation of platelets with LPST. Abscissa, incubation time (in min); ordinate, concentration (in %). 1) Monophosphoinositol; 2) diacylglycerol; 3) diphosphoinositol; 4) triphosphoinositol. LPST concentration 1 μg/10^6 cells.

Fig. 3. Changes in thromboxane B_2 level during incubation of platelets with LPST. Abscissa, incubation time (in min); ordinate, thromboxane B_2 concentration (in % of initial). LPST concentration 1 μg/10^6 cells.

result of its conversion into monoglyceride. This change in the diacylglycerol concentration correlates with changes in the level of thromboxane B_2 (Fig. 3), whose concentration initially increased a little, and then began to fall. A further increase in the diacylglycerol concentration was evidently connected with the fact that hydrolysis of phosphoinositides by phospholipase C predominates over processes leading to a fall in the diacylglycerol concentration (hydrolysis by phospholipase A_2, phosphorylation with the formation of phosphatidic acid). The increase in the thromboxane B_2 concentration until the 5th minute may also have been due to the formation of arachidonic acid through the direct action of phospholipase A_2 on phosphoinositides. Simultaneously with a change in the phosphoinositide concentration, under the influence of LPST there was a change in the phospholipid concentration also (Table 1). Under the influence of LPST the phosphatidylcholine and phosphatidylethanolamine concentrations fell, probably due to hydrolysis by phospholipase A_2; this conclusion is supported by the proportional increase in the lysophosphatidylcholine concentration which was recorded (Table 1).

These results are evidence that incubation of platelets with LPST leads to activation of both phospholipase A_2 and phospholipase C. The increase in microviscosity of the plasma membrane bilayer which we observed may have been due, on the one hand, to the more "rigid" organization of the phospholipid molecules, in connection with the introduction of the lipid moiety of the toxin into the lipid bilayer and/or the lateral phase division of the phospholipids induced by LPST, and on the other hand, to a change in the fatty acid composition of the phospholipids, for the action of phospholipase A_2 leads to removal of unsaturated fatty acids, which reduces the index of unsaturation of the phospholipids and, correspondingly, the flowability of the bilayer.

To conclude, it can be said that although LPST leads to breakdown of a significant part of the phosphoinositides (15-30%) and to the formation of considerable quantities of diacylglycerol (20%), platelet aggregation does not take place. This suggests that activation of the phosphoinositide cycle is probably not a necessary and sufficient condition for the induction of platelet aggregation.

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COMPARATIVE STUDY OF THE PROPERTIES OF NATIVE AND MODIFIED COLLAGENASE PREPARATIONS

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KEY WORDS: collagenase; aldehyde-dextran; hyaluronidase; modification; catalytic activity and stability; distribution among organs.

Elimination of metabolic disturbances requires an increase not only in the selectivity, but also in the many-sideness of action of the therapeutic agent on the pathological focus [9]. Potential remedies for this purpose are preparations with combined action obtained by the chemical modification of enzymes [2]. The simultaneous presence of several types of therapeutic activity in these derivatives ensures strengthening and prolongation of their action on an affected organ or tissue.

In fibrosis collagen and proteoglycan synthesis is activated, with the consequent formation of connective tissue [4]. Collagenase, hyaluronidase, and elastase are its natural depolymerizing agents. It was shown previously that hyaluronidase, stabilized with dextran [3], has a marked inhibitory action on the development of fibrosis in the lungs in experimental silicosis [1]. It can be tentatively suggested that the use of preparations with a series of depolymerizing activities (collagenase, hyaluronidase, elastase, etc.) will be effective in the treatment of fibrotic processes. Thus the synthesis of derivatives with dual enzyme activity (collagenase and hyaluronidase, for example) may be very useful for the treatment of fibrosis.

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