ABILITY OF LIPOPOLYSACCHARIDE TOXIN TO ENHANCE THE FUNCTIONAL RESPONSE OF HUMAN PLATELETS TO THROMBIN STIMULATION

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Previously the writers showed that incubation of lipopolysaccharide toxin (LPST) with human platelets in the absence of aggregation causes reorganization of the plasma membrane lipids, stimulates polyphosphoinositide breakdown and diacylglycerol formation, and also leads to intensive hydrolysis of phosphatidylcholine and phosphatidylethanolamine, probably due to activation of endogenous phospholipases A_2.

The aim of the present investigation was to study changes in the functional response of platelets to the action of thrombin after preliminary short-term incubation with LPST.

EXPERIMENTAL METHOD

Platelets were isolated from donor's blood by the method in [4]. Platelet aggregation was recorded by measuring the increase in light transmittance of a suspension at wavelength 600 nm on an aggregometer, assembled on the basis of a "Spectronic" spectrophotometer (Bausch and Lomb, USA), in a constant-temperature cuvette at 37°C. LPST from *Salmonella typhimurium* (Difco, USA), spin probes (Syva, USA), and thrombin (Merck, West Germany) were used. EPR-spectra were recorded on a Bruker ER-200D spectrometer (West Germany). Polyphosphoinositide, phospholipids, and diacylglycerol were determined by thin-layer chromatography (PLC) [3, 5, 13, 14], using a "Camag" densitometer (Switzerland). Thromboxane B_2 biosynthesis was determined by TLC after preliminary incorporation of ^{14}C-arachidonic acid by the method in [7].

EXPERIMENTAL RESULTS

In the absence of LPSP addition of thrombin to a suspension of washed platelets led to a considerable and rapid rise of the levels of di- and triphosphoinositides, phosphatidic acid, and diacylglycerol, accompanied by a significant fall in the monophosphoinositol concentration (Table 1). Parallel with the changes in concentration of products of the phosphoinositide cycle mentioned above, an increase was observed in the thromboxane B_2 concentration (Fig. 1), also in agreement with data in the literature [2, 6]. This was due to a combination of biochemical processes taking place in response to platelet stimulation by thrombin, namely activation of phospholipases C and A_2, of the phosphoinositide cycle, and of thromboxane B_2 biosynthesis, etc. Changes in concentrations of phospholipids [9, 10], polyphosphoinositides [1, 8], and diacylglycerol [12] induced in platelets by thrombin have been studied by several workers, who previously obtained results on the whole sufficiently close to our own, although it should be pointed out that in some investigations levels of di- and triphosphoinositides were more than doubled [8], whereas in others they were almost unchanged [1].

The use of the EPR method with the carbon chain of steric acid spin-labeled in different positions showed that thrombin induces reorganization of membrane lipids during total aggregation: the microviscosity of the lipid bilayer of the plasma membrane is reduced (its flowability is increased). For instance, the orderliness parameter S falls during aggregation from 0.671 to 0.642. Similar results were obtained by the use of the same spin probes in [11]. However, preliminary incubation for 10 min at 37°C with LPST led to appreciable

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TABLE 1. Concentrations of Principal Phospholipid Fractions of Human Platelets (in nmoles/10^9 cells) before and during Aggregation in the Absence of LPST and after Incubation with LPST for 10 min

Phospholipids	Without LPST			After incubation with LPST		
	aggregation					
	initially	15 sec	5 min	initially	15 sec	5 min
Diacylglycerol	7,0	—	7,4	8,2	—	9,0
Monophosphoinositol	17,5	15,3	11,6	14,8	12,5	9,7
Diphosphoinositol	4,8	5,6	6,8	4,3	3,3	4,4
Triphosphoinositol	2,3	2,5	3,8	2,0	1,8	2,5
Phosphatidic acid	8,0	12,3	14,3	11,4	12,2	14,7
Phosphatidylcholine	158,8	149,3	142,8	146,4	142,1	131,7
Phosphatidylserine	35,9	35,3	34,1	35,1	35,2	33,7
Sphingomyelin	67,0	69,5	69,0	72,2	70,9	65,6
Phosphatidylethanolamine	118,3	108,6	104,7	101,2	98,3	94,1
Lysophosphatidylcholine	3,5	7,9	9,9	5,6	8,3	11,1
Total phospholipids, %	100	97,1	93,9	94,6	92,6	87,6

Legend. LPST concentration 1 μg/10^6 cells. Relative error of determination for each platelet population did not exceed 10%.

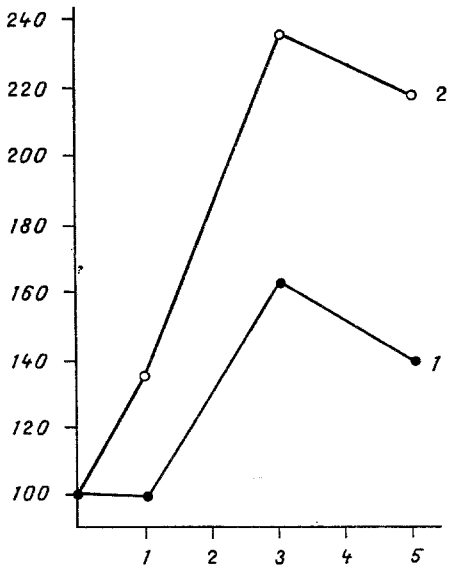

Fig. 1. Changes in thromboxane B_2 concentration under the influence of thrombin (1 U/ml). Abscissa, time (in min); ordinate, thromboxane B_2 level (in %). 1) Thrombin; 2) LPST + thrombin. LPST (1 μg/10^6 cells) was incubated with platelets for 10 min at 37°C.

changes. For instance, the diphosphoinositide concentration no longer rose steadily, but fell significantly toward the 15th second of aggregation, and was restored to its initial level by the 5th minute. Similar changes affected the triphosphoinositide concentration. In the presence of LPST, thrombin also caused a more marked increase (toward the 5th minute of aggregation) in the concentration of diacylglycerol (by about 10%, compared with about 6% in the absence of LPST). Exposure of the toxin-treated platelets to thrombin also revealed

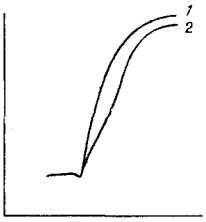

Fig. 2. Platelet aggregation induced by thrombin (1 U/ml). Abscissa, time (in min); ordinate, transmittance. 1) With LPST (1 μg/10^6 cells) for 10 min at 37°C, 2) without addition of LPST.

significant stimulation of thromboxane B_2 biosynthesis, which exceeded that in the control (without LPST) by 80%. According to the EPR data, treatment of platelets with LPST had hardly any effect on the thrombin-induced increase in flowability of the plasma membrane.

These facts may be evidence that preliminary interaction between platelets and LPST (before thrombin-induced aggregation) converts them into a certain state of "latent activation." As a result of the action of thrombin on platelets in such a state, their stimulation is more effective. This effect is illustrated by aggregation curves illustrated in Fig. 2. These aggregation curves show that the initial rates of aggregation of toxin-treated cells was appreciably greater than that of the "initial" control platelets. Incidentally, under these circumstances LPST had virtually no effect on the maximum of aggregation, i.e., after 4-5 min the level of aggregation in the control and toxin-treated platelets was virtually the same. This hypothetical state of "latent aggregation" of platelets may activate the accumulation of physiologically active substances such as diacylglycerol, phosphatidic acid, and precursors of thromboxane B_2 biosynthesis, in up to threshold concentrations.

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