ACTIVATION OF RABBIT PLATELETS BY 1-O-ALKYL-2-O-ACETYL-Sn-GLYCERO-3-PHOSPHOCHOLINE (PLATELET ACTIVATION FACTOR)

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Platelet aggregation can be induced by many physiologically active compounds. Among them two groups can be distinguished: 1) compounds causing release of ADP from cells; 2) substances activating arachidonic acid metabolism. Recently derivatives of membrane phospholipids with high aggregating activity (threshold concentrations 10^{-8} M or below) have been discovered. It has been shown that the natural phospholipid platelet activating factor (PAF) released from basophils [7], macrophages [8], leukocytes [5], and platelets [6] has the structure of 1-O-alkyl-2-O-acetyl-Sn-glycero-3-phosphocholine. This compound has attracted the attention of investigators mainly by its high activity, and also by the specificity of the mechanism of its interaction with cells [10].

This paper presents new data on the characteristics of the mechanism of interaction of a semisynthetic PAF with rabbit blood platelets.

EXPERIMENTAL METHOD

Rabbits weighing 3-4 kg were used. Blood was obtained by cardiac puncture and stabilized with sodium citrate in the ratio 1:9. Platelet-enriched plasma (PEP) was separated by centrifugation. The PEP was diluted with buffered physiological saline (pH 7.35). Aggregation of the platelets was recorded on an EEL-169 apparatus. The effect of PAF on serotonin granules of the platelets was studied by the fluorescent marker method [2, 3, 9]. Acridine orange (AO), the concentration of which was recorded fluorometrically on an SPF-500 spectrofluorometer (Aminco, USA), was used as the label. PAF was obtained by acetylation of alkyllysophosphatidylcholine with acetic anhydride [10] and purified by column chromatography on silisilica-gel. Working solutions of PAF were prepared by successive dilution of the alcoholic mother solution with physiological saline. The final ethanol concentration in the sample did not exceed 0.03%.

EXPERIMENTAL RESULTS

Aggregating Activity of Synthetic PAF. On addition of the synthetic PAF to the PEP, it exhibited high aggregating activity. The threshold concentration inducing irreversible aggregation of rabbit platelets was 55 ± 30 pM, and that inducing reversible aggregation 4.2 ± 2.1 pM. When the aggregation reaction of the cells was recorded after incubation, rather than immediately after the addition of PAF, a decrease in amplitude of the curve was observed, or even its complete disappearance (Fig. 1). This phenomenon may be connected with the presence of phospholipases and (or) esterases in the plasma causing destruction of the PAF, and also, evidently, with incorporation of the PAF into the composition of low- and high-density lipoproteins and serum albumin [4]. Another cause of this phenomenon may be the development of refractoriness of the cells to PAF.

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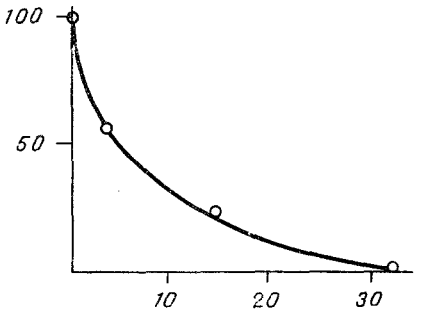

Fig. 1. Amplitude of aggregation of platelets as a function of duration of incubation of PAF in plasma. Abscissa, time (in min); ordinate, aggregation (in %). 44 nM of PAF added to plasma contained in cuvette of aggregometer, mixed once, incubated, after which the mixer of the aggregometer was switched on and aggregation of the cells recorded. Amplitude of aggregation curve obtained without incubation taken as 100%.

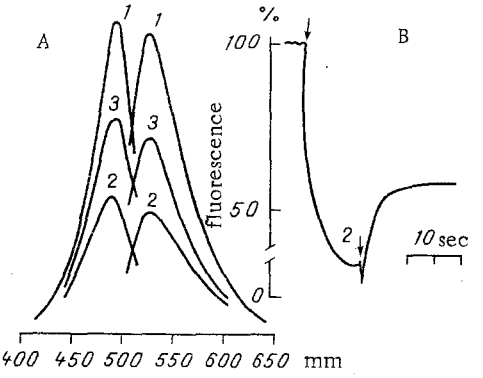

Fig. 2. Changes in spectra of excitation and emission of fluorescence of AO in PEP on addition of PAF (A) and kinetics of the process (B). A: 1) AO solution (1 μM), 2) AO solution (1.5 ml) + 0.3 ml PEP (525,000 cells/mm^3), 3) AO + PEP + PAF (90 nM). B: Vertical axis — fluorescence (rel. %). Experimental conditions the same as for A.

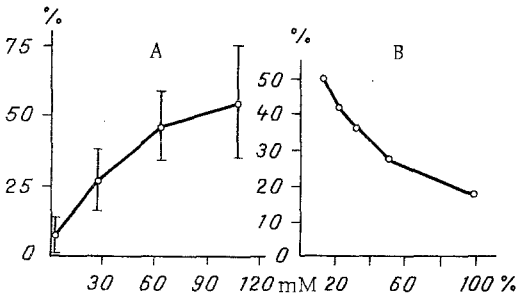

Fig. 3. Dependence of AO release on PAF concentration (A) and concentration of platelet-enriched plasma (B). Abscissa: A) concentration of PAF (in nM); B) plasma concentration in mixture (in %); ordinate, AO release (in %). A) Cells stained with AO (1 μM) for 10 min, PAF added, incubated for 5 min and fluorescence measured, amount of AO released calculated as a percentage of total content in cells; B) cells stained in a mixture of PEP + physiological saline for 10 min, PAF added, incubated for 5 min, and percentage of label released determined.

Effect of PAF on Serotonin Granules (5-HT-organelles) of Platelets. It was shown previously [10] that the synthetic analog of PAF causes release of serotonin from granules in human platelets. This suggests that PAF interacts with the 5-HT-organelles of platelets. It has also been shown that AO can be used as marker of the serotonin granules of platelets [2, 9]. It was later established that certain fluorochromes of the acridine series (9-aminoacridine, atebrin, as well as AO) can serve as indicators of the membrane pH gradient [1, 11].

These fluorescent labels thus provide information not only on changes in intracellular organelles, but also on pH changes on their membranes (for platelets this refers to 5-HT-organelles).

The effect of PAF on platelets treated beforehand with AO was investigated. Figure 2A shows spectra of excitation and emission of fluorescence of a cell suspension with AO before and after addition of PAF. Addition of the platelets to the AO solution led to a decrease in amplitude of the excitation and fluorescence spectra without any appreciable changes in their frequency characteristics. This decrease in amplitude was connected with AO accumulation inside the 5-HT-organelles [1-3, 9, 11]. After addition of PAF to the cell suspension treated with AO, the amplitude of the fluorescence spectra increased again, evidence of release of the label from the granules and cells into the surrounding medium. The kinetics of this process was recorded. For this purpose fluorescence was excited at 495 nm and recorded at 530 nm (Fig. 2B).

Dependence of the intensity of release of the fluorescent marker on the PAF concentration is illustrated in Fig. 3A. Maximal release of the marker under these conditions was observed in a PAF concentration of about 100 pM, when it reached 40-45%. A study of the quantitative characteristics of release of the label under the influence of PAF showed an interesting result: release was more intensive as PEP was diluted (Fig. 3B). This may have been due to the presence of enzymes destroying PAF or of substances interacting with it in the plasma [4, 10]. On dilution of the plasma their concentration was reduced, so that effects due to interaction between PAF and the cells could be observed more clearly.

The semisynthetic PAF obtained as described above thus possesses marked aggregating activity. These data are evidence that plasma contains substances destroying and (or) binding it. Induction of AO release from the 5-HT organelles of the platelets under the influence of PAF indicates that this factor either interacts directly with the granules or induces a release reaction, by acting on the cell receptors. This reaction in accompanied by a fall of ΔpH on membranes of the 5-HT-organelles. The results of the present experiments indicate that the use of fluorescent labels for investigating the character of interaction between PAF and platelets is promising.

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