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Effect of PAF on human lymphocyte membranes: A fluorescence study

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Abstract

We have studied the effect of platelet-activating factor (PAF) on the physico-chemical organization of human lymphocyte plasma membranes by measuring the steady-state fluorescence anisotropy and the fluorescence decay of 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) incorporated in lymphocyte plasma membranes. PAF induced a time-limited and significant increase of the lipid order in the exterior part of the membrane and a decrease in membrane heterogeneity. These changes were blocked in the presence of PAF antagonist L-659,989. The results indicate that the observed changes in the physico-chemical properties of the lymphocyte plasma membranes may be attributed to a PAF–receptor interaction.

Introduction

Recent evidence suggests that PAF is involved in the regulation of cellular immune responses in a number of cell types, including lymphocytes [1]. It has been shown that PAF is synthesized and metabolized by lymphocytes [1, 2]. In this study we have investigated the effect of PAF on the physico-chemical properties of lymphocyte plasma membranes by studying the steady-state fluorescence anisotropy and the fluorescence decay of TMA-DPH incorporated into the plasma membranes.

Materials and methods

Lymphocytes were prepared from 3 ml of heparinized blood using lymphocyte separation medium (ICN-Biomedicals). Lymphocytes were suspended in Krebs–Ringer phosphate (KRP) solution at a final concentration of 10^5 cells/ml. Steady-state fluorescence anisotropy (r_s) measurements were performed at 37 °C with a Perkin Elmer Spectrofluorimeter MPF-66 equipped with a Series Professional Computer for data acquisition, using TMA-DPH (Molecular Probes Inc., Eugene, OR, USA) as a hydrophobic probe at a final concentration of 10^{-6} *M* as previously described [3]. After incubation of lymphocytes with TMA-DPH for 10 min, PAF (final concentration 10^{-7} *M*) was added in the presence or absence of PAF antagonist, L-659,989 (10^{-6} *M*) (kindly donated by Dr. W. H. Parsons, Merck Sharp & Dohme Research Laboratories, NJ, USA). The effect of PAF addition on TMA-DPH fluorescence decay was determined using multifrequency phase fluorometry as previously described [4]. The fluorescence lifetime measurements were analysed by a model that assumes a continuous distribution of lifetime values characterized by a Lorentzian shape centred at a time *C* and having a width *W*. For this analysis, the program minimizes the reduced chi-square defined by an equation reported elsewhere [4].

Results

The fluorescence intensity of TMA-DPH in KRP, in the absence of lymphocytes, was negligible and did not increase upon the addition of PAF or L-659,989 at the concentrations used in the study. In Fig. 1, r_s of TMA-DPH in lymphocyte plasma membranes before and after PAF addition is shown. PAF induced a time-limited (10 min) and significant increase ($p<0.01$) in the r_s value. In the control sample (without PAF), a stable and lasting r_s value was maintained during the measurements. In the presence of L-659,989, no significant changes ($p>0.5$) in the r_s value were observed after the addition of PAF (10^{-7} *M*).

TMA-DPH lifetime distributions in lymphocyte plasma membranes in the absence and presence of PAF are reported in Fig. 2A and 2B, respectively. In the absence of PAF, a two-component distribution has been found: a long component with an average lifetime value of 5.42 ns and fractional intensity of 0.83, and a short component with an average lifetime value of 0.52 ns and fractional intensity of 0.17. The distribution width was 0.20 ns for the long component and 0.05 ns for the short component. The addition of PAF induced a significant decrease in the distribution width of the long component (0.05 ns), but no effect on the average lifetime value and fractional intensity was observed.

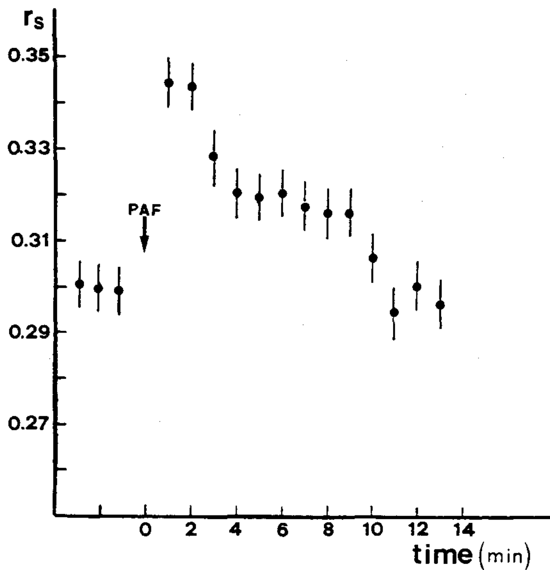

Figure 1
Steady-state fluorescence anisotropy (r_s) of TMA-DPH at 37 °C in lymphocyte plasma membranes before and after PAF addition (10^{-7} *M*). Values are expressed as the mean ± SD of ten samples. The r_s increase after PAF addition is significant ($p<0.01$).

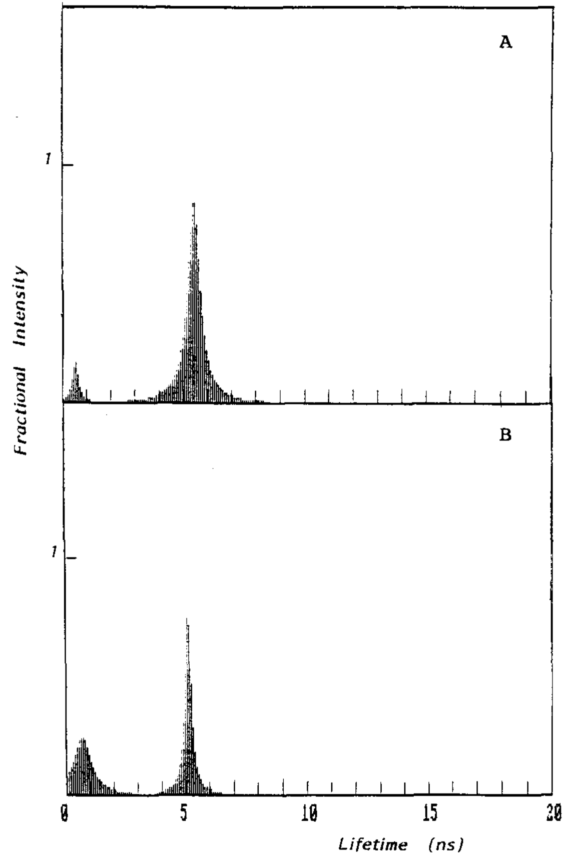

Figure 2
TMA-DPH lifetime distribution in lymphocyte plasma membranes before (A) and after PAF addition (10^{-7} *M*) (B).

Discussion

The fluorescent probe TMA-DPH has been widely used in the study of physico-chemical organization of biological membranes [3–5]. Because of its hydrophobic structure, TMA-DPH incorporates into the membrane but remains at the lipid–water

interface region with its cationic residue up to 30 min [5]. TMA-DPH r_s value reflects the packing of membrane lipid fatty acid chains and can be related to the order parameter S, if certain precautions are taken [4].

Our results indicate that PAF induces a time-limited increase in lipid order in the exterior part of the lymphocyte plasma membranes. In the presence of a PAF antagonist L-659,989, the described effect of PAF is totally abolished. These findings suggest that the observed changes can be attributed to a PAF-receptor interaction.

We have recently shown that PAF induces a time-limited increase of lipid packing in the plasma membranes of platelets [6], granulocytes [7] and erythrocytes [3]. The duration of this increase was different, depending upon the cell type.

TMA-DPH fluorescence lifetime distribution reflects the membrane heterogeneity [4]. Our data show that PAF induces no changes in the average lifetime value, whereas a narrowing in the distribution width of the long component is observed after PAF addition, indicating a decrease in membrane heterogeneity. A similar effect was reported in membranes of platelets [6], granulocytes [8] and erythrocytes [3].

Further studies are in progress to investigate the interaction of PAF with different lymphocyte cell lines.

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