COMPARISON BETWEEN PAF-ACETHER RECEPTORS ON INTACT WASHED HUMAN PLATELETS AND HUMAN ENDOTHELIAL CELLS IN CULTURE

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INTRODUCTION

Paf-acether (paf, formerly platelet-activating factor) (Benveniste et al., 1972), with the structure 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (Benveniste et al., 1979; Demopoulos et al., 1979; Blank et al., 1979), is a biologically active phospholipid which is released by several cell types upon stimulation (for review Pinckard et al., 1982; Vargaftig et al., 1981). The mediator activates platelets, polymorphonuclear neutrophils, macrophages and endothelial cells most probably via a receptor-dependent pathway (Valone et al., 1982; Valone and Goetzl 1983; Lambrecht and Parnham, 1986; Korth et al., 1987; Valone, 1988). Serum albumin acts as a necessary phospholipid carrier for both paf release (Benveniste, J. et al., 1972; Benveniste, J., 1974) and specific paf binding (Korth and Benveniste, 1987) and seems to inhibit the paf-degrading enzyme, acetylhydrolase (Warlow et al., 1986). Different specific antagonists, such as the paf analogue CV 3988 (Terashita et al., 1983), the naturally occurring kadsurenene (Shen et al., 1985) and Ginkgolides (Nunez et al., 1986; Korth and Benveniste, 1987; Korth et al., 1988 c; Braquet and Godfr nails, 1987), as well as synthetic hetrazepines and calcium-blocking agents, inhibited platelet activation and paf binding (Casals-Stenzel and Weber, 1987; Korth et al., 1987 and 1988 b; Wade et al., 1986). Binding studies with radiolabelled antagonists supported the existence of paf receptors in platelets (Hwang et al., 1986 a). A close correlation between inhibition of paf-induced platelet aggregation and binding of labelled paf suggested the importance of the putative paf receptor for platelet activation (Korth et al., 1988 c). Paf receptors were coupled with modulation of phospholipase C activity (Morrison and Shulka, 1988). Stimulation of paf receptors upregulated fibrinogen receptors that represent the final operating stage of human platelet aggregation (Kloprogge et al., 1986). Intact washed human platelets aggregated with paf in the absence of paf catabolism (Korth et al., 1988 a). However, they released their cytoplasmatic acetylhydrolase during platelet aggregation with thrombin, ADP and paf itself, as well as after platelet damage (Suzuki et al., 1988; Korth et al., 1988 a).

In cultured human endothelial cells (HEC), paf induced shape changes (Grigorian and Ryan, 1987), the release of tissue-type plasminogen (Emes and Kluft, 1985) and prostacyclin (D'Humière et al., 1986), and modulated Ca^{2+} flux in HEC as well as in vascular smooth muscle cells (Bussolino et al., 1985; Brock and Gimbrone, 1986; Doyle et al., 1986). Putative paf receptors seem to be involved in HEC activation since specific paf antagonists inhibited the paf-induced effects, and preincubation of HEC with low paf concentrations decreased the functional responses such as increase in intracellular Ca^{2+} concentration and prostacyclin synthesis upon a second exposure to the mediator (Bussolino et al., 1985; D'Humière et al., 1986). Paf antagonists inhibited in vitro peripheral vascular effects, such as hypotension and increased vascular permeability (Casals-Stenzel et al., 1987; Hwang et al., 1986 b).
Stimulated endothelial cells from different sources produced paf, which remained strongly cell-associated (Whatley et al., 1988). Paf can be formed from lyso paf by a lyso paf: acetyl CoA acetyltransferase present in many other cell types (for review: Ninio, 1987; Snyder, 1985). Lyso paf originates either from membranous alkyl-acyl-glycero-phosphocholine (AAGPC) by a Ca²⁺-dependent phospholipase A₂ activity or from paf itself by acetylhydrolase action (Kramer et al., 1988). Paf is metabolized into lyso paf via a Ca²⁺ independent cytosolic acetylhydrolase by intact rabbit platelets (Pieroni and Hanahan, 1983; Lachachi et al., 1985; Touqui et al., 1983) and lysed human platelets (Kramer et al., 1984 and 1988; Korth et al., 1988 a,b). Lyso paf is further metabolized by a cellular acyltransferase to AAGPC (Kramer et al., 1984). Acetylhydrolase activity was demonstrated in human polymorphonuclear neutrophils and eosinophils (O'Flaherty et al., 1986; Chilton et al., 1983 a,b; Lee et al., 1982), cultured venous endothelial cells (Blank et al., 1986) and cultured rat hepatocytes (Okayasa et al., 1986). Paf is also rapidly metabolised to lyso paf by a lipoprotein-bound plasma acetylhydrolase (Farr et al., 1980; Wardlow et al., 1986), which shares most of the properties of a purified acetylhydrolase isolated recently from plasma lipoproteins (Stafforini et al., 1987 a,b).

The purpose of our study was to demonstrate that paf activates platelets and HEC via specific binding of intact paf and not via paf metabolism.

**METHODS**

**Preparation of human platelets**

Venous blood was taken from healthy male volunteers into acid-citrate dextrose (ACD, citric acid 0.8%, trisodium citrate 2.2%, dextrose 2.45%, pH 4.8%) 7:1 and platelets were prepared according to a method (modified) of Lalau Keraly (1984). In short: platelets were enriched in plasma using centrifugation (100 x g, 15 min) and washed three times in Tyrode's buffer (900 x g, 10 min). Platelets were kept in the same buffer containing 0.1 mM aspirin lysin salt for 1h (Aspegic R, Egiec Laboratory, Amilly, France). After the last centrifugation, the platelets (1 x 10⁹/ml) were kept in Tyrode's buffer without ACD (pH 6.4) in the presence of 0.25% fatty acid free bovine serum albumin (BSA from Sigma Chemical Co., St. Louis, MO, USA).

**Specific paf binding to platelets**

Specific paf binding was performed as described (Korth et al., 1986). Platelet suspension (5 x 10⁹/500 ul) was incubated with ³H-paf at final concentrations of 0.325-6.5 nM (1-O-³H-octadecyl-2-acetyl-sn glycero-3-phosphocholine, 80 Ci/mmol, Amersham, Bucks, UK) at 20°C in the presence of 0.25 % BSA (pH 7.4). The binding of ³H-paf to platelets was measured in duplicates, in the absence and presence of unlabelled paf, at the final concentration of 50 nM (1-octadecyl-2-acetyl-sn-glycero-3-phosphocholine, Bachem, Bubendorf, Switzerland). After 30 min incubation, platelets were separated from the solution by vacuum filtration using Whatman GF/C filters (Molsheim, GFR). The filters were then washed with 10 ml cold Tyrode's buffer (pH 6.4). Filter-bound radioactivity in the absence of platelets was subtracted from filter-bound radioactivity in the presence of platelets. ³H-paf bound to 5 x 10⁹ platelets was calculated in fmol.

**Platelet aggregation with paf**

Fifty microliters of washed platelet suspension was diluted with 300 ul pH 7.4 Tyrode's buffer to which were added 1.3 mM CaCl₂ and 0.16 mg x ml⁻¹ fibrinogen (final concentrations), (a gift of B.B. Vargaftig, Pasteur Institute, Paris, France). The platelets (3 x 10⁸ x ml⁻¹) were then aggregated at 20°C or 37°C in the presence of 0.25 % BSA under stirring with different concentrations of paf.