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Receptor-independent metabolism of platelet-activating factor by myelogenous cells

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Human neutrophils incorporate and metabolize platelet-activating factor (PAF). We dissociated these events from PAF binding to its receptors. Cells were pretreated with either pronase, a PAF antagonist (L652731), or excess PAF. This reduced PAF receptor numbers by 70 to almost 100% but had no comparable effect upon the neutrophil's ability to metabolize PAF. Furthermore, HL-60 cells efficiently metabolized, but did not specifically bind, PAF. Thus, PAF receptor availability did not correlate with PAF metabolic capacity and we conclude that myelogenous tissues can process this bioactive ligand by a receptor-independent pathway.

Platelet-activating factor; Receptor; Phospholipid metabolism; (Polymorphonuclear leukocyte, HL-60 promyelocyte)

1. INTRODUCTION

Many cellular stimuli bind with plasmalemmal receptors to form complexes that not only stimulate function but also trigger their own internalization. The complexes commonly internalize in vesicles which traverse cytosol and merge with Golgi/granules where entrapped ligand is deposited and soon degraded [1]. PAF may be processed by such a receptor-mediated endocytotic pathway. This unique 1-*O*-alkyl-2-acetyl-GPC uses plasma membrane receptors to activate diverse cell types. These same target cells deacetylate PAF, acylate the 1-*O*-alkyl-2-lyso-GPC intermediate, and store the final product, 1-*O*-alkyl-2-acyl-GPC, in Golgi/granules [2,3]. Furthermore, Lachachi et al. [4] reported that a PAF antagonist almost completely blocked rabbit platelet metabolism of PAF. Homma et al. [5], however, found that other antagonists lacked this effect; the authors suggested that PAF bypasses rabbit platelet receptors to reach intracellular metabolizing enzymes. This controversy bears not only on the existence of a novel, stimulus processing pathway but also on therapeutic issues. In particular, antagonists, by blocking receptor-mediated metabolism, could worsen acute diseases (e.g. thrombosis, allergy, inflammation, and shock) involving the pathologic actions of endogenously formed PAF. Accordingly, we here employ several techniques to evaluate the receptor dependency of PAG metabolism in myelogenous tissues.

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Abbreviations: PMN, polymorphonuclear neutrophil; PAF, platelet-activating factor or 1-*O*-alkyl-2-acetyl-GPC; GPC, *sn*-glycero-3-phosphocholine; [^{3}H]PAF, 1-*O*-[9,10′-$^3H_2$]hexadecyl-2-acetyl-GPC; BSA, bovine serum albumin

2. MATERIALS AND METHODS

2.1. *Reagents and buffers*

[^{3}H]PAF and 1-*O*-[^{3}H]hexadecyl-2-lyso-GPC (56 Ci/mmol), PAF, 1-*O*-alkyl-2-lyso-GPC, 1,2-diacyl-GPC, L652731, delipidated BSA, silicone oil, TLC plates, pronase and modified Hanks' buffer were obtained as in [3,6,7]. PAF and analogs were taken up in buffer containing 2.5 mg/ml BSA; 50 μl of this was added to 950 μl cell suspension.

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2.2. *Binding*

PMN were isolated from normal human donors [3]; HL-60 cells were purchased (American Type Culture Collection, Rockville, MD). We conducted [^{3}H]PAF binding at 4°C using centrifugation through silicone oil to separate cells from medium [6].

2.3. *Metabolism*

Cells were incubated in buffer for 20 min at 37°C and treated with [^{3}H]PAF, ± unlabelled PAF, for the indicated intervals. Reactions were stopped by adding 1.4 vols methanol/chloroform (2:1, v/v). Chloroform layers were removed, 0.7 vol. chloroform added to reaction mixtures, and the second chloroform layers isolated. Pooled chloroform layers were applied to TLC plates (preheated for 3 h at 180°C) and developed in chloroform/methanol/glacial acetic acid/water (50:25:8:4, v/v). Plates were scraped (5 mm zones) and counted for tritium. Material migrating with lyso-PAF (R_f = 0.25), PAF (R_f = 0.35), and 1,2-diacyl-GPC (the standard for 1-*O*-alkyl-2-acyl-GPC; R_f = 0.6) was calculated as percentage of total recovered radiolabel (recovery >92% of that added to cells). The percentage of added PAF metabolized after 0, 2.5, 5, and 10 min was fitted to a straight line by the method of least squares. These lines were used to estimate the average rate of PAF metabolism during the initial 10 min of incubation.

3. RESULTS AND DISCUSSION

PMN and HL-60 promyelocytes converted [^{3}H]PAF to 1-*O*-[^{3}H]hexadecyl-2-lyso-GPC and 1-*O*-[^{3}H]hexadecyl-2-acyl-GPC (the latter always represented >80% of formed product). This conversion increased with time and cell concentration (fig.1, upper panels). Its initial rate in PMN, but not HL-60 cells, also increased with PAF concentrations up to 6000 pM, above which metabolism slowed in both cell types (fig.1, lower panels). We emphasize that our results are given as the percentage of added PAF converted to TLC-identified products. The absolute mass of PAF metabolized (and products formed) approached a maximum of ~ 1 pmol/10^7 PMN per min (60000 molecules/PMN per min) at 2 μM PAF.

Three types of experiments evaluated the receptor requirements for PAF metabolism. First, HL-60 cells did not specifically bind [^{3}H]PAF (fig.2D). We examined binding to 2.5 × 10^7 HL-60 cells under conditions in which binding to 2.5 × 10^5 PMN was readily detected. Relative to PMN, therefore, promyelocytes have less than 1% of the specific binding capacity but more than 4-fold greater metabolic capacity (cf. upper panels in fig.1) for PAF. Second, 10^7 PMN incubated with 200 fmol 1-*O*-[^{3}H]hexadecyl-2-lyso-GPC

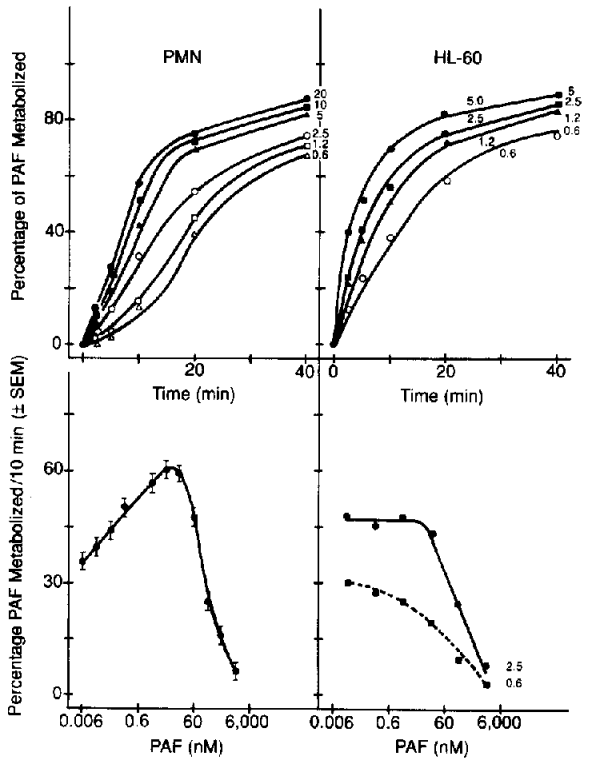

Fig.1. Cellular metabolism of PAF. Upper panels: the indicated number (× 10^6/ml) of PMN or HL-60 cells were incubated with 200 pM [^{3}H]PAF for the indicated period. Lower left panel: 10^7 PMN/ml were incubated with varying amounts of [^{3}H]PAF + PAF (radiolabeled and unlabeled ligand added separately but simultaneously). Lower right panel: 5 × 10^6 (——) or 1.25 × 10^6 (---) HL-60 cells were similarly incubated with radiolabeled and unlabeled ligand. Results are presented as the percentage of added ligand metabolized at the indicated time (upper panels) or the average percentage of added ligand metabolized per 10 min (see Section 2) (lower panels) for PMN from 4–11 different donors or for HL-60 cells obtained on two separate occasions.

formed 60, 80, 100, 140, and 160 fmol 1-*O*-[^{3}H]hexadecyl-2-acyl-GPC in 2.5, 5, 10, 20, and 40 min, respectively. Under the same conditions, PMN converted 200 fmol [^{3}H]PAF to 30, 60, 110, 150, and 160 fmol products (lyso + acylated derivatives) at these times. PMN therefore metabolize [^{3}H]PAF and its lyso analog at similar rates although the latter compound does not interact with PAF receptors [3]. Third, PMN treated with pronase, a competitive PAF antagonist (L652731; ID_{50} = 30 nM [7]), or excess PAF exhibited 70 to nearly 100% decreases in high- and low-affinity receptor numbers. These cells, nevertheless, show-

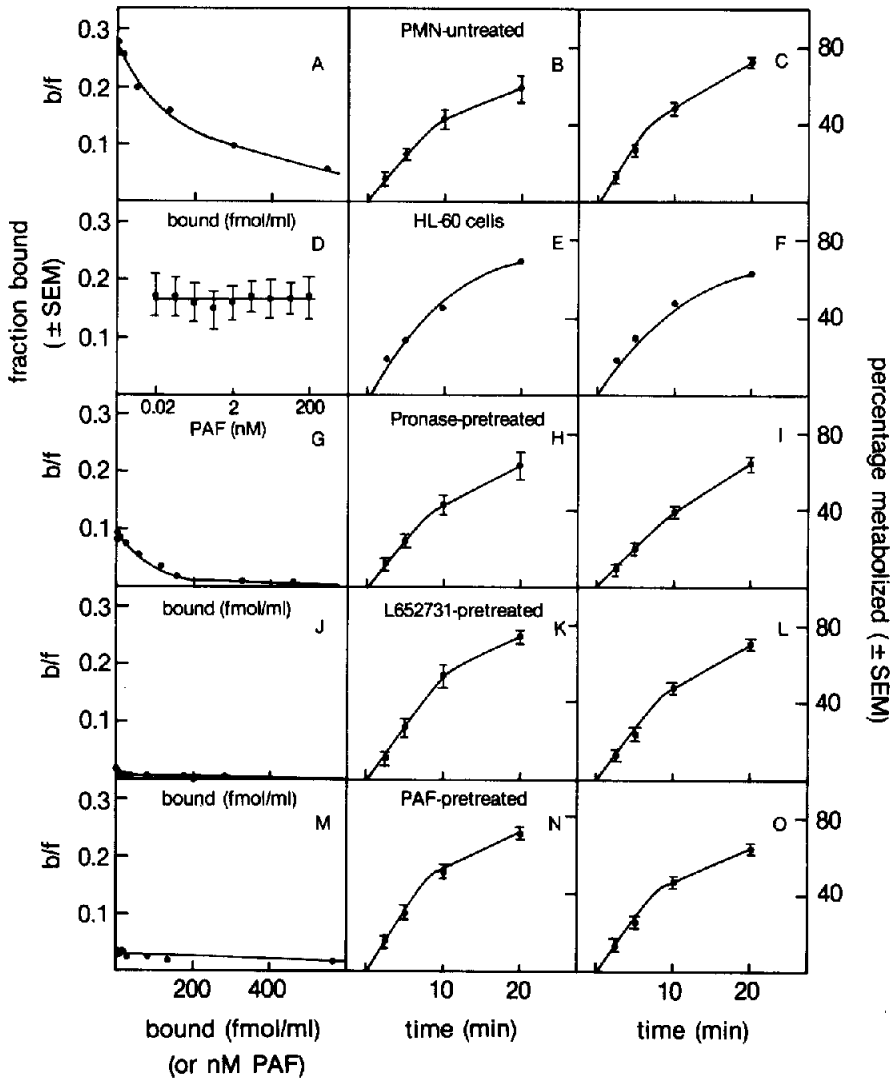

Fig.2. Cellular binding and metabolism of PAF. Where indicated, PMN were pretreated with 860 U/ml pronase for 30 min (G–I) or 100 nM PAF for 30 min (M–O) at 37°C and then washed twice before accessing binding. These cells retained full viability and bound [^{3}H]phorbol myristate acetate normally [6]. Alternatively, PMN were incubated with 500 nM L652731 for 5 min at 4°C (J) or 37°C (K,L) before assessing PAF binding and metabolism, respectively. These cells also were fully viable; they responded to and bound leukotriene B_4 normally [7]. Binding results are corrected for non-specific binding [6] and reported as Scatchard plots from which high-affinity (K_d = 0.5–1 nM) binding sites per PMN were extrapolated to be: 12000 in control, 2500 in pronase-pretreated, 900 in L652731-pretreated, and ~ 0 in PAF-pretreated cells. For low-affinity (K_d = 100–400 nM) binding sites, receptor numbers were: 300000, 80000, 20000, and 170000, respectively. (D) Total uncorrected binding by 2.5 × 10^7 HL-60 cells/ml at the indicated concentration of PAF. In metabolic studies, PMN (10^7/ml) or HL-60 cells (2.5 × 10^6/ml) were incubated at 37°C for the indicated time with 63 pM (B,E,H,K,N) or 63 nM PAF (C,F,I,L,O). Results are for the mean of ≥6 (binding) or ≥4 (metabolism) studies.

ed no comparable losses in their ability to metabolize PAF (fig.2G–O, and legend). We did observe that pronase and L652731 decreased by <20% and PAF pretreatment increased by <30% the rate of PMN metabolism of 6–2000 pM PAF. However, the regimens did not influence metabolism of ≥6 nM PAF (not shown). Thus, PAF metabolic capacity and receptor availability were clearly dissociable. We therefore suggest that mature and immature myelogenous cells can

metabolize PAF and 1-*O*-alkyl-2-lyso-GPC through pathways that do not directly involve PAF receptors.

1-*O*-Alkyl-2-lyso-GPC enters erythrocytes by a receptor-independent route [8]. Furthermore, this GPC as well as 1,2-diacyl- and-1-*O*-alkyl-2-acyl-GPC are shuttled intermembranously by cytosolic exchange proteins [9–12]. Accordingly, we propose the following model for GPC processing. Exogenous PAF, 1-*O*-alkyl-2-lyso-GPC, and other GPCs penetrate to the inner leaflet of the plasmalemma by a diffusional process that may be facilitated by integral membrane proteins [13,14] but does not involve specific receptor binding. Inner leaflet GPC is metabolized and then transferred to Golgi/granules by exchange proteins or, alternatively, associates first with exchange proteins and becomes metabolized during or after transcytosolic movement. Further study of this model and other possible routes for PAF metabolism (e.g. receptor-independent endocytosis) obviously will be required. Regardless of the exact processing pathway used, however, we find that interference with PAF receptors has relatively little influence upon myelogenous cell inactivation of PAF. PAF antagonists, we suggest, may not appreciably increase the in vivo half-life or toxicity of PAF by this mechanism.

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