Influence of platelet activation factor and prostaglandins on cholesterol esterification in human plasma

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Prostaglandin E_1 (PGE_1) and platelet activation factor (PAF) were found respectively to activate or to inhibit cholesterol esterification in whole plasma but not in lipoprotein-deficient plasma. It is suggested that these effects are mediated by the interaction of PGE_1 and PAF with high-density lipoproteins [(1984) FEBS Lett. 173, 291–294]. Possible physiological implications of these findings are discussed.

Platelet activating factor *High density lipoprotein* *Cholesterol esterification*

1. INTRODUCTION

Investigations carried out in these laboratories using phospholipid fluorescence probes demonstrated that low amounts of PAF and PGE_1 caused oppositely directed changes in the surface structure of HDL [1,2]. Analysis of the binding of PAF to various lipoprotein classes revealed that PAF binds unspecifically to very low density lipoproteins whereas HDL exhibit high affinity for PAF binding sites [3]. The existence of specific PAF binding sites on and the reorganization of the HDL surface caused by PAF and PGE_1 indicate that both these lipid effectors could influence the physiological functions of HDL. Since an important function of HDL is to serve as substrate and activator of LCAT, we investigated the influence of PAF and various PGs on the esterification of cholesterol in human plasma. The results demonstrate that PAF inhibits and PGE_1 activates plasma cholesterol esterification in the presence of lipoproteins but not in lipoprotein-deficient plasma. Some possible physiological implications of these findings are discussed.

Abbreviations: PAF, platelet activation factor, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PG, prostaglandin; HDL, high-density lipoproteins; LCAT, lecithin : cholesterol-acyltransferase

2. MATERIALS AND METHODS

1α,2α-[^{3}H]Cholesterol (43 Ci/mmol) was purchased from Amersham (England). Prostaglandins E_1, E_2, $F_{2\alpha}$, cholesterol and cholesteryl oleate were obtained from Serva (FRG).

1-*O*-Alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine was obtained by acylation of alkyllysophosphatidylcholine with acetic anhydride according to [4].

Human blood plasma (blood group O(I) or AB(IV) was prepared by centrifugation (5000 rpm, 15 min) of platelet-rich plasma.

Cholesterol esterification assays were carried out by incubation of [^{3}H]cholesterol (5–10 μCi/ml) with plasma at 37°C. [^{3}H]Cholesterol ester formation was determined by thin-layer chromatography of the lipid extracts on F 254 silica gel plates (Merck) with petroleum ether-diethyl ether-acetic acid (90:10:1, v/v) as developing solvent and measurement of the radioactivity of the cholesterol ester fraction.

Lipoprotein-free plasma was obtained by precipitation of lipoproteins with phosphotungstic acid and $MnCl_2$ [5]. After centrifugation lipoprotein-free plasma was dialyzed against Na-citrate buffer, pH 7.4.

Liposomes prepared by brief sonication were composed of phosphatidylcholine and cholesterol in a molar ratio 7:2.

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LCAT activity of lipoprotein free plasma was determined by measurement of [^{3}H]cholesterol ester formation after incubation of lipoprotein-free plasma (0.3 ml) and liposomes (3.23 μM [^{3}H]cholesterol) at 37°C for 1–2 h.

3. RESULTS AND DISCUSSION

Fig.1 shows that preincubation of plasma with small amounts of PAF (10^{-10}–10^{-8} M) results in significant inhibition of esterification, whereas PGE_1 in the same concentration range enhances the reaction. When the two compounds were applied in the same concentration (10^{-9} M) the stimulating effect of PGE_1 was stronger than the inhibiting effect of PAF. In the presence of both PAF (10^{-9} M) and PGE_1 (10^{-8} M) the esterification reaction was inhibited to the same extent as with PAF alone (not shown). Thus, there is no competition between PGE_1 and PAF. Nevertheless, the effect of PGE_1 is highly specific: as can be seen from fig.1 PGE_2 in the same concentration range (10^{-10}–10^{-8} M) caused only insignificant activation, whereas $PGF_{2\alpha}$ was completely ineffective. It should also be noted that in contrast to PAF and PGE_1, the effect of PGE_2 was concentration independent and thus probably nonspecific.

In principle, the influence of PAF or PGE_1 on the esterification of cholesterol could be attributed to direct interaction of these substances with LCAT or to their influence on the HDL structure. To solve this question esterification experiments were carried out with lipoprotein-deficient plasma using egg phosphatidylcholine-cholesterol liposomes as substrates for LCAT. As can be seen from fig.2 under such conditions neither PAF nor PGE_1 had any effect on the cholesterol esterification. It follows that the influence of PAF and PGE_1 on the plasma cholesterol esterification is mediated by their interaction with plasma lipoproteins, most probably HDL. In any case the results of this work demonstrate unequivocally that PAF and PGE_1 oppositely shift the balance between free and esterified cholesterol in plasma. This fact could be one of the possible causes of the opposite effects of PAF and PGE_1 on platelet aggregation. Human platelets have been shown not to be able to synthesize cholesterol de novo [6], and at the same time to be sensitive to the plasma level of free cholesterol: increase in plasma free cholesterol is known to result in formation of hypersensitive, more readily aggregating platelets [7]. Thus PAF and PGE_1 could enhance or inhibit platelet aggregation by respectively inhibiting or enhancing cholesterol esterification. Experiments designed to check this hypothesis are in progress.

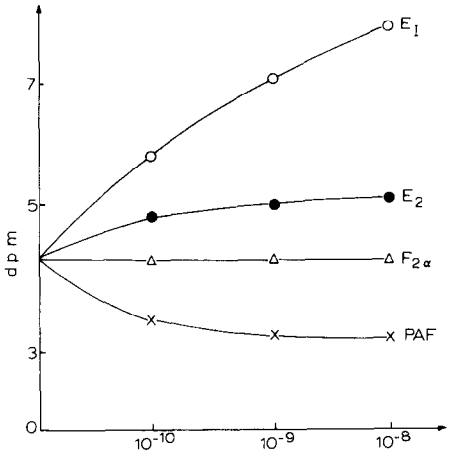

Fig.1. Influence of PAF and prostaglandins E_1, E_2 and $F_{2\alpha}$ on the esterification of cholesterol in human plasma.

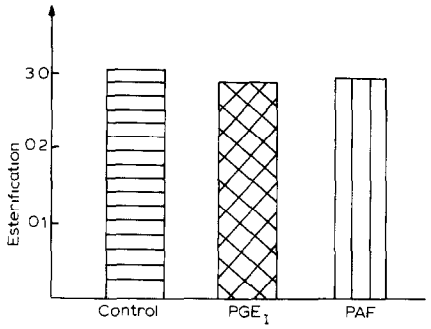

Fig.2. Influence of PGE_1 and PAF on the esterification of cholesterol in lipoprotein deficient plasma.

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