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CONVERSION OF ^{3}H-PAF ACETHER BY RABBIT PLATELETS IS INDEPENDENT FROM AGGREGATION : EVIDENCES FOR A NOVEL METABOLITE

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^{3}H-PAF-acether (Alkyl-[1',2'-^{3}H]-2-acetyl-sn-glyceryl-3-phosphorylcholine) was time-dependently transformed by plasma-free rabbit platelets into an unknown product, "PX", which was distinct from lyso-PAF-acether. This effect was specific for platelets since plasma only converted ^{3}H-PAF-acether into lyso-^{3}H-PAF-acether and platelets were not effective in metabolizing lyso-^{3}H-PAF-acether. Platelet aggregation did not interfere with the formation of "PX". The latter is a novel platelet metabolite of PAF-acether with as yet unknown biological properties.

Stimulated leukocytes and macrophages release platelet-activating factor (PAF-acether), a potential mediator of anaphylaxis and inflammation (1,2) which induces platelet aggregation and secretion (3,4). The chemical structure of PAF-acether has been determined as 1-O-alkyl-2-acetyl-3-sn-glycerophosphorylcholine (5,6). Stimulation of rabbit platelets by thrombin, by collagen or by the Ca^{2+} ionophore A23187 induces the formation of PAF-acether and of its deacylated product, lyso-PAF-acether. The latter may be precursor and/or the metabolite of PAF-acether (7,8). We studied the metabolism of PAF-acether by rabbit platelets, and report on the presence of a new metabolite, distinct from lyso-PAF-acether and which is formed independently from the occurence of aggregation.

MATERIALS AND METHODS

Blood was collected from the central ear artery of adult New Zealand white rabbits on a mixture of disodium and tetrasodium salt of EDTA (final concentration 0.2 mM). Platelet-rich plasma was obtained by centrifugation at 375 g for 20 min and washed platelets were prepared according to Ardlie et al. (9). Final suspension (5 x 10^{8} platelets ml^{-1}) was in Tyrode's buffer (pH 7.4) containing 1 mM $MgCl_2$, 2 mM $CaCl_2$ and 0.25 % bovine serum albumin (BSA, Sigma). The platelet-suspensions and the appropriate controls were incubated at 37°C with 1 ng ml^{-1} of PAF-acether (alkyl-2-acetyl-sn-glyceryl-3-phosphorycholine), to which were added around 10^{4} cpm.ml^{-1} of the labelled material (1-O-alkyl-[1;2'^{3}H]; 45 Ci/mmol. C.E.A. Saclay). In order to avoid aggregation, the platelet suspension was not stirred. Immediately after the addition of ^{3}H-PAF-acether, the suspension was divided into 10 samples of2 ml each, i.e., five controls and five ex-

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perimental samples, which were stirred magnetically with a metal bar. In a few experiments ^{3}H-PAF-acether was added to platelets after chloroform-methanol for controlling the yields of recovery.

Aliquots were collected at the times indicated in figure 2 and were extracted according to Bligh and Dyer (10). The lipid extracts were redisolved with chloroform-methanol 1:2 and subjected to TLC on plastic silicagel plates (Merck), using chloroform-methanol-ammoniac (70:35:7) as a solvent. The chromatogram was divided into 34 bands (0.5 cm width), which were directly scraped into scintillation vials containing 8 ml of Aquasol 2 (NEN). Platelets were also incubated with around 10^{4} cpm.ml^{-1} of lyso-PAF-acether, together with 1 ng.ml^{-1} of unlabelled lyso-PAF-acether. The lyso derivative was obtained by exposing ^{3}H-PAF-acether or the unlabelled material to a 5 % dilution of rabbit plasma in Tyrode's solution for 120 min. The resulting incubate was extracted and chromatographed as indicated above, and the lyso compound was thus recovered.

RESULTS

The incubation of ^{3}H-PAF-acether with the washed platelets led to the formation and to the accumulation of new metabolite (metabolite PX, P standing for platelets) with an Rf of about 0.65 (figure 1). Concomitantly, the recovery of PAF-acether dropped whereas that of lyso-PAF-acether remained constant (figure 2). The transformation of PAF-acether was time-dependent, the maximal formation of PX occuring 120 min after starting the incubation, when it represented 60 % of the total radioactivity (figure 2). The addition of ^{3}H-PAF-ace-

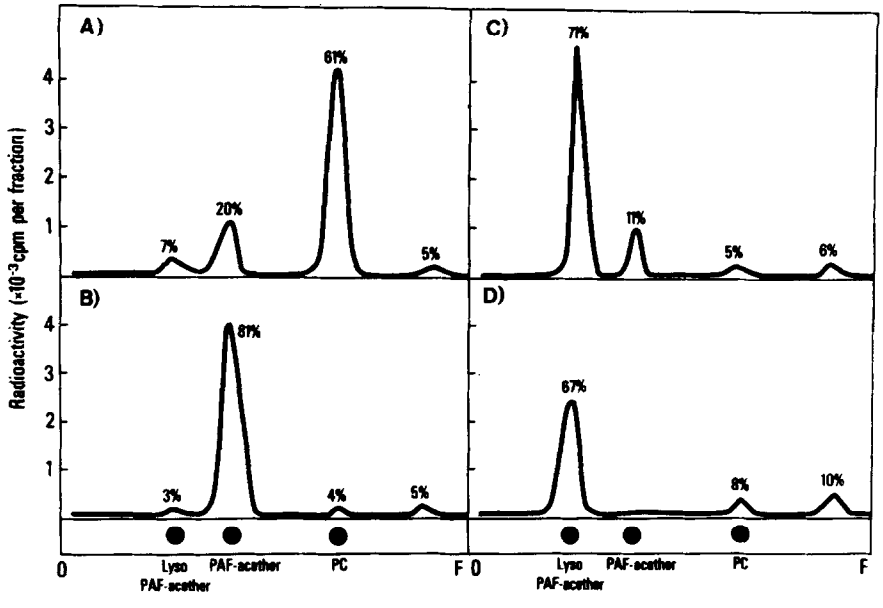

Fig. 1: Thin layer chromatography of PAF-acether metabolites formed by washed platelets and by diluted plasma

^{3}H-PAF-acether (1 ng ml^{-1}, 10^{4} cpm ml^{-1}) was incubated at 37° C during 120 min with the platelet suspension (A), with Tyrode's buffer containing BSA (B) and with 5 % rabbit plasma (C). Lyso ^{3}H-PAF-acether was incubated under the same conditions with the platelet-suspension (D). The extraction was performed as indicated in "Methods".
O, P.C., F. refer to origin, Phosphatidylcholine and solvent front, respectively.

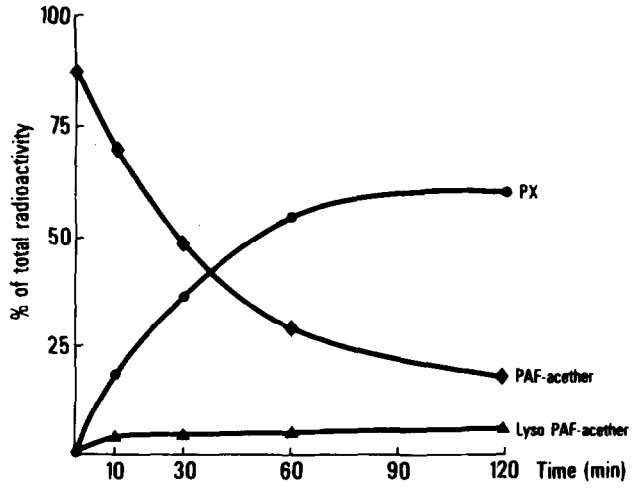

Fig. 2: Kinetics of transformation of ^{3}H-PAF-acether by platelets

The platelet suspension was incubated at 37° C with 1 ng.ml^{-1} of ^{3}H-PAF-acether (10^{4} cpm.ml^{-1}). Aliquots were collected at the times indicated in the figure, and extracted as indicated in "Methods".

ther to platelets after chloroform-methanol was not followed by its transformation and the yield of recovery was of about 80 %.

Generation of PX was the same, whether platelets underwent aggregation or not (Table 1). When EDTA (5 mM final concentration) was added to the platelets 30 min before ^{3}H-PAF-acether, the formation of PX was reduced by about 25 % as compared to the control, even though aggregation was totally blocked.

The effect of diluted plasma on ^{3}H-PAF-acether differed markedly from that of platelets since lyso-PAF-acether now represented 70 % of the total radioactivity after 120 min incubation (figure 1). Furthermore, when lyso-^{3}H-PAF-acether was added to the platelets no PAF-acether was formed whereas PX represented only 8 % of the platelet radioactivity.

TABLE 1 METABOLISM OF ^{3}H-PAF-ACETHER IN WASHED PLATELETS

	PAF-ACETHER	PX	LYSO-PAF-ACETHER	FRONT
Aggregated Platelets	56 ± 3.2	21.1 ± 0.8	3 ± 0.6	4.6 ± 1.4
Non-aggregated Platelets	63 ± 1	17.4 ± 1.2	3.7 ± 0.6	2.6 ± 0.8
Tyrode's Buffer containing BSA (control)	82 ± 1.3	4 ± 1.3	4.4 ± 1.8	5 ± 1.3

Figures are % ± S.E.M. of radioactivity of each indicated component as compared to total plate radioactivity. ^{3}H-PAF-acether (1 ng/ml) was incubated with plasma-free platelets or with the appropriate control during 10 min as indicated in "Methods". Aggregation was obtained by adding a metal bar to the platelet suspension and stirring magnetically.

DISCUSSION

Our results demonstrate that PAF-acether is metabolized by plasma-free platelets and by plasma alone by two different systems.

On one side, plasma converted PAF-acether into lyso-PAF-acether, which may be accounted for by phospholipase A_2 or by acetyl-hydrolase activities (11, 12, unpublished observations of D. Rotilio). On the other side, platelets converted PAF-acether into a new metabolite presently coded as PX. The latter was specific for PAF-acether and its formation was independent from aggregation. This is indicated by:

1) EDTA blocked aggregation but reduced only by about 25 % the formation of PX
2) generation of PX was the same whether platelets underwent aggregation or not
3) aggregation was maximal within 3 or 4 min after the addition of PAF-acether whereas formation of PX was maximal within 120 min.

Since PX was not a derivative of lyso-PAF-acether, the existence of a new metabolic pathway for PAF-acether is presently shown. Further studies of PX shall demonstrate whether it is an end-product and whether it is endowed with cell-stimulating properties comparable to those of PAF-acether.

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