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PLATELET ACTIVATING FACTOR (PAF-ACETHER) IS RELEASED INTO RAT PULMONARY ALVEOLAR FLUID AS A CONSEQUENCE OF HYPOXIA

Marie-Claude PREVOST, Clotilde CARIVEN, Marie-Françoise SIMON, Hugues CHAP, and Louis DOUSTE-BLAZY

INSERM Unité 101, Biochimie des Lipides, Hôpital Purpan, 31059 Toulouse, France

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Summary : Hypoxia provokes pulmonary constriction and because PAF-acether is a very strong pulmonary constrictor, we looked for PAF-acether in lung alveolar lavage (LAL) with a biological method based on the measurement of rabbit platelet aggregation. We first demonstrated a PAF-acether secretion during bronchoalveolar lavage with sterile isotonic NaCl (pH 7.2). PAF-acether secretion was completely suppressed with isotonic NaCl containing 5 mM EDTA but lyso-PAF-acether was still present (1.9 ± 0.55 nmoles). Upon hypobaric hypoxia, PAF-acether was detected in LAL (1.05 ± 0.25 10^{-2}nmoles). The amount of lyso-PAF-acether increased by 6 times (12.1 ± 4.1 nmoles). These results are given for 10^4 nmoles phospholipids of LAL. They indicate that alveolar macrophages might be activated by hypobaric hypoxia, so they produce PAF-acether in the alveole. Such a process could be involved in the well-known bronchoconstriction accompanying hypoxia.

A relationship between oxygen pressure and pulmonary surfactant has been established under various physiological and pathological conditions (1-4). In this respect, we have previously shown that hypobaric hypoxia provoked a 15 % decrease of phosphatidylcholine with a concomitant increase of lysophosphatidylcholine in rat lung alveolar lavage (LAL). These changes could be related to the appearance of a phospholipase A_2 in LAL occurring under the same conditions (5).

Platelet activating factor (PAF-acether or 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a powerful phospholipid mediator able to provoke platelet (6-9) and neutrophil activation (10-11), hypotension (12) and bronchoconstriction (13). As hypoxia induces pulmonary vasoconstriction (14-16), PAF-acether might thus represent a potential mediator involved in this biological response. Moreover, several lines of evidence indicate that PAF-acether biosynthesis might involve a phospholipase A_2 acting on 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine followed by a specific acetyl transferase (17-22).

Abbreviations : LAL, lung alveolar lavage ; GPC, *sn*-glycero-3-phosphocholine ; PAF-acether, platelet activating factor (1-O-alkyl-2-acetyl-GPC) ; lyso-PAF-acether, (1-O-alkyl-GPC) ; CP, creatine phosphate ; CPK, creatinine phosphokinase ; indo, indomethacin ; PLA_2, phospholipase A_2 ; PLA_1, phospholipase A_1.

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These various considerations led us to look for the presence of PAF-acether and of its deacylated derivative, lyso-PAF-acether or 1,0-alkyl-*sn*-glycero-3-phosphocholine,in rat LAL upon exposure to hypobaric hypoxia.

MATERIALS AND METHODS

Isolation of pulmonary surfactant

Six week-old male Wistar rats weighing 200 to 250 g were placed in an altitude simulator chamber for 4 hours as described previously (23). Air pressure was reduced to 370 mm Hg, corresponding to altitude 6,000 m. Control animals were maintained under normal air pressure (760 mm Hg). As soon as the animals returned to normal pressure, pulmonary surfactant was removed as described elsewhere (24).

Phospholipid analysis

Lipids were extracted according to (25), lipid phosphorus was determined according to (26) and phospholipids were separated by one dimensional thin-layer chromatography on silica gel plates using $CHCl_3$:CH_3OH:glacial acetic acid:H_2O (75/45/12/6 by volume). In this solvent, PAF-acether was separated from lyso-PAF-acether as shown by comparison with pure standards. Two compounds (A and B) were eluted from areas corresponding respectively to PAF-acether and lyso-PAF-acether with $CHCl_3$:CH_3OH:H_2O (1/2/0.8) 200 ml for 10 µmoles of total phospholipids. The acetylation of compound B was performed as described previously (27). For some experiments, compound A was submitted to hydrolysis by *Crotalus adamanteus* phospholipase A_2(PLA_2) and by *Rhizopus arrhizus* lipase (PLA_1) as already described (17).

Quantification of PAF-acether and lyso-PAF-acether

Washed rabbit platelets were prepared as described elsewhere (28) and suspended in Tyrode buffer (pH 7.35) containing 0.35 % (w/v) bovine serum albumin. Platelet aggregation was measured at 37°C by the turbidimetric method of Born (29). PAF-acether and acetylated lyso-PAF-acether were added directly to the platelets as ethanolic solution under a minimal volume (2 µl for 250 µl of platelet suspension). PAF-acether concentrations were calculated by comparing the aggregation obtained with synthetic PAF-acether at well-known molarity.

RESULTS AND DISCUSSION

Characterization of PAF-acether in LAL

As shown in Fig. 1, a purified sample obtained from LAL (compound A) corresponding to PAF-acether area induced a dose-dependent aggregation of washed rabbit platelets,which was similar to that obtained with pure PAF-acether. On the contrary, compound B, corresponding to lyso-PAF-acether area, was without effect. However, acetylation of compound B promoted the same platelet aggregating activity.

Fig. 2 illustrates some properties of compound A. Aggregation was not inhibited by 5 mM creatinine phosphate (CP), 20 units creatinine phosphokinase (CPK) or 10 µM indomethacin ; under the same conditions, the aggregation induced by ADP (0.1 mg/ml) and arachidonic acid (500 µM), respectively, was completely suppressed (not shown). Moreover compound A remained insensitive to *Rh. arrhizus* lipase, whereas PLA_2 completely destroyed its activity which was restored upon reacetylation. A similar effect to that of PLA_2 was obtained upon alkaline methanolysis of compound A which remained fully

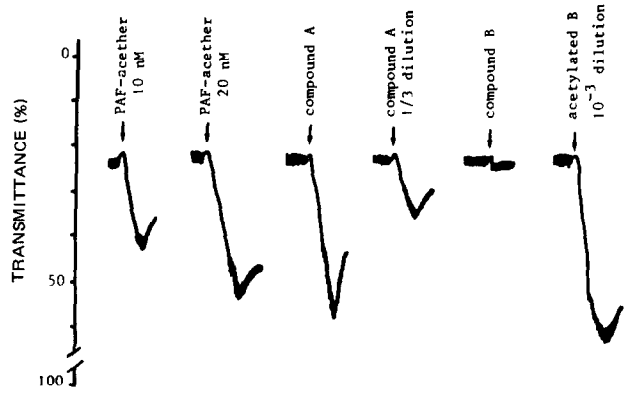

Fig. 1 : Quantification of compound A and compound B in phospholipid extract of rat LAL in comparison to synthetic PAF-acether.
Curves 1 and 2 : Rabbit platelet aggregation with two concentrations of synthetic PAF-acether
Curve 3 : Aggregation with 2 µl compound A in 500 µl ethanolic solution containing 10 µmoles total phospholipids of hypoxic rat LAL.
Curve 4 : Aggregation with 2 µl compound A in 500 µl ethanolic solution containing 4 µmoles total phospholipids of hypoxic rat LAL
curve 5 : Absence of aggregation with compound B.
curve 6 : Aggregation with compound B (dilution 1/1000) after acetylation of the same phospholipid extract as in curve 3.

stable during acidic treatment according to (30) (not shown). At last, previous exposure of washed rabbit platelets to authentic PAF-acether under non-aggregating conditions suppressed the response to a second addition of compound A but not of arachidonic acid (Fig. 2).

Taken together, these data indicate that compound A is identical to PAF-acether. Indeed its thin-layer chromatography behavior is the same as

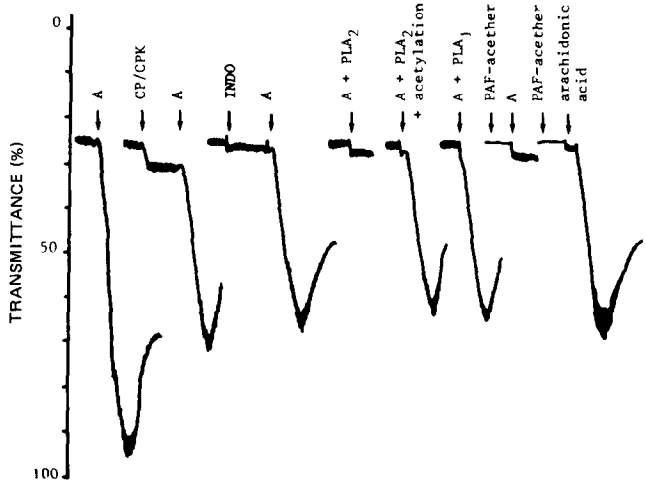

Fig. 2 : Characterization of compound A in phospholipid extract of LAL of hypoxic rats as PAF-acether. In the last two curves, preincubation with PAF-acether was performed in the absence of stirring, which was restored just prior addition of the second compound (A or arachidonic acid).

that of PAF-acether. Moreover, the sensitivity to PLA_2 (or to alkaline methanolysis), together with the restoration of activity upon reacetylation claim for the presence of a 2-acetyl group which was found to be essential for activity (7-9,11). Finally, its stability towards *Rh. arrizus* lipase, as well as to acidic treatment allows to exclude an acyl-ester and an alk-1-enyl-ether bound in the 1 position. Further evidence of identity between compound A and PAF-acether is brought by comparing their biological activity. Rabbit platelet aggregation by compound A was not inhibited by CP/CPK and indomethacin (31) and a specific cross desensitization between PAF-acether and compound A was observed (32-33). This conclusion can be extended to compound B which is a lyso-PAF-acether since it presented the same properties upon chemical acetylation.

Quantification of PAF-acether and lyso-PAF-acether in LAL : effect of EDTA

Using platelet aggregation as a biological method of quantification, both compounds were found in significant amounts in LAL obtained using sterile isotonic NaCl (Table I). However, addition of increasing quantities of EDTA to the washing fluid progressively decreased the amounts of PAF-acether and lyso-PAF-acether, the former one being undetectable in the presence of 5 mM chelator (Table I). Arnoux et al. (34) have previously reported that, under certain conditions of *in vitro* stimulation, alveolar macrophages from various origins secrete PAF-acether, the production of which was completely inhibited by EDTA. It is thus tempting to postulate that some stimulation of alveolar macrophages occurs during pulmonary lavage in the absence of EDTA, leading to the production of PAF-acether.

However, one cannot exclude that other cell types like type II cells could be also able to produce PAF-acether under those conditions. Anyway, our data show that the presence of EDTA is absolutely required in the washing fluid in order to eliminate any production of PAF-acether during the lavage procedure. If such a condition is fulfilled, one should be able to conclude that any amount of PAF-acether and lyso-PAF-acether found in LAL has accumul-

TABLE I - Effect of EDTA and hypoxia on PAF-acether secretion in pulmonary alveole

EDTA	Hypoxia	PAF-acether	Lyso-PAF-acether
–	–	$1.1 \pm 0.4 \times 10^{-2}$	3.2 ± 0.7
2 mM	–	$0.44 \pm 0.17 \times 10^{-3}$	2.6 ± 0.5
5 mM	–	0	1.9 ± 0.6
5 mM	+	$1.04 \pm 0.25 \times 10^{-2}$	12.1 ± 4.1

Results (means ± s.e.m., 5 experiments) represent the total nmoles of PAF-acether and lyso-PAF-acether per 10^4 nmoles of total phospholipids. After F test and t independent Student test $p < 0.05$.

ated prior to the lavage procedure in a non-artefactual way. These conditions were thus chosen to explore the effect of hypoxia on PAF-acether secretion in pulmonary alveole.

Effect of hypoxia on PAF-acether secretion

Data of Table I also indicate that, even in the presence of 5 mM EDTA, significant amounts of PAF-acether were measured in LAL following hypoxia. In this case, the increase of lyso-PAF-acether was 6 times higher compared to the control. So our study gives evidence that PAF-acether can accumulate in lung alveoli during hypoxia. It is noteworthy that the amount of PAF-acether appearing under these conditions still remains 1000 times lower than those of lyso-PAF-acether. However similar results have been obtained upon *in vitro* stimulation of various cell types (35-37) and support the view that PAF-acether production follows the activation of a phospholipase A_2. Such a process provides relatively large amounts of lyso-PAF-acether, a small proportion of which becomes thus available to a Ca-activated acetyl transferase (18-20). So the increase of lyso-PAF-acether observed upon hypoxic treatment would fit with such a hypothesis.

One should also mention that preliminary studies in our laboratory indicated that LAL contains an acetylase activity capable to hydrolyse PAF-acether into its lyso-derivative (in preparation). It is thus possible that PAF-acether was rapidly degraded following its secretion as it also happens in plasma (38-39).

In conclusion, we have shown that PAF-acether and its lysoderivative accumulate in lung alveoli as a response to hypoxia. PAF-acether has been described as a potent bronchoconstrictor (13) and as such could be involved in the pathology of asthma (40). It is thus tempting to speculate that PAF-acether could participate as a mediator during the well-known broncho-constriction accompanying hypoxia (10). This would justify further studies dealing both with the cellular origin and the molecular mechanisms of PAF-acether secretion during hypoxia, as well as with the possible connection and synergism with other lipid mediators such as leukotrienes and thrombo-xane A_2.

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