Biochemical Pharmacology, Vol. 37, No. 3, pp. 547–550, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

MOBILIZATION OF ARACHIDONIC ACID FROM DIACYL AND ETHER-LINKED PHOSPHOLIPIDS IN FMLP STIMULATED ALVEOLAR MACROPHAGES

J. MASLIAH,*† M. BACHELET,‡ O. COLARD,* G. BEREZIAT* and B. B. VARGAFTIG‡

*Laboratoire de Biochimie, C.H.U.-Saint-Antoine, 27, rue Chaligny 75571, Paris Cédex 12, and ‡Unité associée Institut Pasteur/INSERM 285, 28, rue du Docteur Roux 75724, Paris Cédex 15, France

(*Received* 14 *May* 1987; *accepted* 3 *August* 1987)

Abstract—Exposure of [1^{14}C] AA labeled guinea-pig alveolar macrophages to FMLP for 15 min induced an extensive mobilization of AA from phospholipids. PC and PI mainly contributed to the AA release, and labeled PE remained unchanged. Analysis of ether-linked phospholipids showed a significant breakdown of labeled diacyl and alkyl-acyl PC and an increase in labeled alkenyl-acyl PE.

When administered intravenously to guinea-pigs, the chemotactic peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP)§ triggers leukopenia and a platelet-independent bronchoconstriction [1]. Alveolar macrophages (AM) are also activated by FMLP [2], and therefore could play a role in bronchoconstriction.

N-formyl peptides induce a release of arachidonic acid (AA) from guinea-pig peritoneal macrophages [3] and from neutrophils [4] as well as an increased production of AA metabolites by macrophages [3]. The phosphatidyl inositol diphosphate breakdown is detected within the first minutes of cell stimulation, and is rapidly reversed [5]; AA release slowly increases up to 10–15 min [3, 6]. So far, the participation of each lipid subclass to the AA mobilization during FMLP stimulation has not been clearly established.

This led us to study the FMLP-induced AA release and to demonstrate that it originates mainly from PI and PC in guinea-pig AM.

MATERIALS AND METHODS

Guinea-pig AM were collected and prelabelled for 1 hr with 0.5 μCi of [1^{14}C] AA (60 Ci/mol, Amersham) as previously described [7]. The cells were then incubated with FMLP (Sigma) for 15 min. After incubation, the medium was removed and counted by liquid scintillation. The cells were then washed 3 times with saline, and scraped.

† To whom correspondence should be addressed.

§ Abbreviations used: PI, diacyl glycerophosphoinositol; PC, 1 radyl-2-acyl-glycerophosphocholine; PE, 1 radyl-2 acyl-glycerophosphoethanolamine; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; AA, arachidonic acid; TG, triacyl glycerol; PS, diacyl glycerophosphoserine; AM, alveolar macrophage; FFA, free fatty acid.

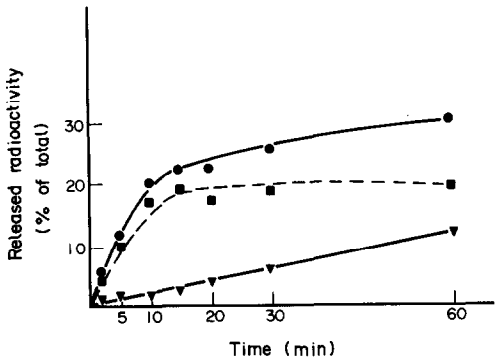

Fig. 1. Time course of AA release: data represent the percentage of radioactivity released into the culture medium by control (▼) and FMLP-stimulated (●) alveolar macrophages. Macrophages were prelabeled with [^{14}C] AA for 1 hr prior to the stimulation. At the end of the incubation, the medium was collected and the released radioactivity was measured by liquid scintillation. The results are expressed as:

$$\frac{\text{cpm released in medium} \times 100}{\text{cpm in whole cell extract} + \text{cpm in medium}}.$$

Broken lines represent the difference between FMLP and control cells.

Table 1. Effect of FMLP (15 min) on the release of radioactivity in medium by alveolar macrophages prelabeled with [1^{14}C] AA for 1 hr

	Control	10^{-12} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M
Radioactivity in medium (% of total)	2.5	2	2	2.5	22.5	26	25.5

For % determination, see Fig. 1. A single experiment (one of three) is shown.

Table 2. ^{14}C AA distribution in the lipid fractions from FMLP-treated and control cells expressed in % of total radioactivity

	PI	Total PC	Diacyl PC	Alkyl acyl PC	Alkenyl acyl PC	Unidentified	Total PE	Diacyl PE	Alkyl acyl PE	Alkenyl acyl PE	FFA	TG	Radioactivity in medium
Control cells	18.9 ± 1	34.5 ± 2	15.8 ± 1.8	15.1 ± 0.7	3.6 ± 0.7	5.8 ± 0.5	14.4 ± 0.9	3.1 ± 0.1	2.6 ± 0.2	8.6 ± 0.9	9.4 ± 1.3	7.9 ± 0.6	4.1 ± 0.9
FMLP-treated cells	11.2 ± 1.2	24.5 ± 2	10.8 ± 1.2	11.2 ± 0.6	2.6 ± 0.4	5.6 ± 0.7	15.2 ± 1	2.7 ± 0.15	2.7 ± 0.25	9.6 ± 0.9	14.8 ± 2.3	10.8 ± 0.6	15.5 ± 1.4
Difference in % of total radioactivity	−8.7***	−9.1***	−4.9**	−3.9***	−1	−0.2	+0.8	−0.4	+0.3	+1.1***	+5.5*	+2.9***	+11.4***
Difference in % of the lipid class	−38	−27	−31	−26	−29	−3.8	+5.6	−11.6	+9.5	+12.5	+58	+36	+270

Cells where prelabeled 1 hr before exposure 15 min to FMLP (10^{-6} M) or to its vehicle. Values represent the mean ± SEM of 6 experiments.
*$P < 0.02$, **$P < 0.01$, ***$P < 0.001$.
Incorporation in PS was only 3 ± 1% and did not change upon FMLP stimulation.

Total lipids were extracted by the method of Bligh and Dyer [8] and were resolved by TLC: the plates were developed in chloroform/methanol/water (65:25:4, v/v) and then in petroleum ether/diethylether/glacial acetic acid (90:30:1, v/v). The separation of the subclasses of diacyl and ether linked PC and PE was achieved according to Blank *et al.* [9]. The different lipid classes and subclasses were visualized by autoradiography, scraped into scintillation vials and counted by liquid scintillation. Student's paired *t*-test was used for statistical calculations.

RESULTS AND DISCUSSION

Figure 1 shows that 10^{-6} M FMLP induced a large increase in the release of radioactivity, which reached a plateau after 10–15 min. A similar time course has been observed for guinea-pig peritoneal macrophages [3], rabbit peritoneal leukocytes [6], and guinea-pig AM stimulated by PAF-acether [7].

As shown in Table 1, 10^{-9} M FMLP had a negligible effect whereas 10^{-8} M was very effective; maximal effect was observed at 10^{-7} and 10^{-6} M. These results are in good agreement with the equilibrium constant for the receptor occupancy (1.45×10^{-8} M) [10], and with the concentration which induces the release of thromboxane B2, contracts a guinea-pig lung strip [1], or stimulates the production of superoxide anion by guinea-pig AM [10].

The stimulation of AM by FMLP for 15 min induced a large and significant decrease of [$1^{14}C$] AA incorporated in PI and in PC (Table 2). In this respect, our results differ from those reported on guinea-pig peritoneal macrophages (release from PI only) [3], rabbit peritoneal leukocytes (release from PC only) [6], and guinea-pig peritoneal neutrophils (release from PI, PC and PE) [4].

Our experiments did not allow discrimination between a phospholipase A2 or a phospholipase C pathway for the release of AA from PI. However, in further experiments *in vitro*, we showed that FMLP did not stimulate the hydrolysis of PI by phospholipase A2 [11]. In contrast, the release from PC may result from the activation of a phospholipase A2 [11, 12].

The release of radiolabeled material to the medium by FMLP after 15 min accounted only in part for the decrease in the radioactivity of PC and PI. There was also a significant increase in the labeling of intracellular free AA and TG (Table 2). Such a transfer has been shown in resting [13] or calcium ionophore-stimulated AM [14]. This hypothesis is consistent with the well-known increase of diglycerides under the effect of FMLP [3, 4, 15].

Approximately 6% of the radioactivity was found in an unidentified phospholipidic fraction, which may be the lyso(bis)-phosphatidic acid, present in large amounts in AM [16]. The [$1^{14}C$] AA incorporated in the unidentified fraction did not change during FMLP stimulation (Table 2) nor during PAF-acether stimulation of guinea-pig AM [7].

The participation of each subclass for the total release of ^{14}C AA from PC was nearly proportional to the labeling of each species: a 31% decrease in

Table 3. Evaluation of the participation of each subclass of 2 arachidonyl phospholipid for the release of AA during the stimulation by FMLP

	% of total arachidonyl molecular species (a)	% of ^{14}C AA incorporated in 1 hr (b)	% of release in ^{14}C AA (c)	Participation of each molecular specie (d) = $\frac{(a) \times (c)}{100}$
1 Acyl 2 arachidonyl PC	9.9	22.4	−31	−3.1
1 Alkyl 2 arachidonyl PC	26.7	21.4	−26	−6.9
1 Alkenyl 2 arachidonyl PC	4.2	5.0	−29	−1.2
1 Acyl 2 arachidonyl PE	6.3	4.3	−11.6	−0.7
1 Alkyl 2 arachidonyl PE	5.2	3.7	+ 9.5	—
1 Alkenyl 2 arachidonyl PE	25.1	12.2	+12.5	—
1 Acyl 2 arachidonyl PI	21.5	27.0	−38	−8.2

(a) Values calculated from literature [22, 23]; 100% is the total arachidonyl molecular species.
(b) Values calculated from Table 2; 100% is the total ^{14}C AA incorporated in PC + PE + PI + PS.
(c) Results reported in Table 2; 100% is the initial labeling of each subclass.
(d) Evaluates the participation of each subclass to the release of AA induced by FMLP (a × c).

diacyl PC, a 26% decrease in alkyl-acyl-PC and a 29% decrease in alkenyl-aceyl-PC (Table 2). When AM were labeled in the same conditions, various challenges including PAF-acether [7] and calcium ionophore [17], also induced a decrease in alkyl-acyl and diacyl-PC in addition to PI.

Taking into account the repartition of AA into diacyl, alkyl-acyl, alkenyl-acyl-PC and diacyl-PI, the distribution of [1^{14}C] AA in AM labeled for 1 hr, and the release of [1^{14}C] AA expressed as the % decrease of the initial radioactivity of each subclass, we evaluated their participation to the release of radioactive material (Table 3). In agreement with the findings of Nakagawa *et al.* for zymosan-stimulated rabbit AM [17], diacyl-PI and alkyl-arachidonyl-PC are clearly the most important donors.

A non-significant increase in the labeling of total PE upon FMLP stimulation (Table 2) was found, which resulted from two opposite effects: a non-significant decrease of the radioactivity in diacyl-PE and a significant increase in alkenyl-acyl-PE (Table 2).

The transfer of AA into alkenyl-acyl-PE also occurred when AM were stimulated by PAF-acether [7]. Such a transfer has been described in stimulated AM [18] and platelets [19], which requires the presence of a 1 alkenyl-2 lyso-PE acceptor [20]. This mechanism implies a hydrolysis of 1 alkenyl-2 acyl-PE before the transfer occurs. Since AA represents more than 50% of the fatty acids in the Sn 2 position in this subclass [21], it should be released during the hydrolysis of alkenyl-acyl-PE. After a 1 hr labeling of AM by AA, alkenyl-arachidonyl-PE exhibited a very low specific activity (Table 3). Therefore in our experiments, the specific activity of alkenyl-arachidonyl-PE is much lower than in ^{14}C AA donors (Table 3) especially diacyl-PC. The net result of this transfer mechanism might explain the increase in the alkenyl-acyl-PE.

CONCLUSIONS

Prelabelled guinea-pig AM stimulated by FMLP showed a very marked increase in the mobilization of AA from phospholipids. This increase resulted from a significant breakdown of PI, diacyl PC and alkyl-acyl-PC. Concomitantly ^{14}C AA was increased in alkenyl-acyl-PE, which may result from the release of poorly labeled AA from alkenyl-acyl-PE and from a transfer of highly labeled AA from all PC subclasses (mainly alkyl-acyl-PC) and PI. The large release of AA from alkyl-acyl-PC increases the availability of 1 alkyl-2 lysoPC (lyso PAF-acether) which, in AM, is the precursor of PAF-acether [21]. This mechanism could lead to an increased production of PAF-acether, and may contribute to the amplification of the stimulation by FMLP.

REFERENCES

1. M. A. Boukili, M. Bureau, V. Lagente, J. Lefort, A. Lellouch-Tubiana, E. Malanchère and B. B. Vargaftig, *Br. J. Pharmac.* **89**, 349 (1986).
2. J. H. Dauber and R. P. Daniele, *Am. Rev. Resp. Dis.* **117**, 673 (1978).
3. Y. Homma, T. Hashimoto, Y. Nagai and T. Takenawa, *Biochem. J.* **229**, 643 (1985).
4. T. Takenawa, J. Ishitoya, Y. Homma, M. Kato and Y. Nagai, *Biochem. Pharmac.* **34**, 1931 (1985).
5. A. Holian and D. F. Stickle, *J. Cell. Physiol.* **123**, 39 (1985).
6. F. Hirata, B. A. Corcoran, K. Venkatasubramanian, E. Schiffmann and J. Axelrod. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2640 (1979).
7. M. Bachelet, J. Masliah, B. B. Vargaftig, G. Bérézíat and O. Colard, *Biochim. biophys. Acta* **878**, 177 (1986).
8. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
9. M. L. Blank, M. Robinson, V. Fitzgerald and F. Snyder, *J. Chromatog.* **298**, 473 (1984).
10. I. Spilberg, J. Mehta, C. Daughaday and L. Simchowitz, *J. Lab. Clin. Med.* **97**, 602 (1981).
11. J. Masliah, M. Bachelet, O. Colard, B. B. Vargaftig and G. Bérézíat. Submitted.
12. B. J. Bormann, C. K. Huang, W. M. Mackin and E. L. Becker, *Proc. natn. Acad. Sci. U.S.A.* **81**, 767 (1984).
13. J. G. Nijssen, R. S. Oosting, F. P. Nÿkamp and H. Van Den Bosch, *Lipids* **21**, 623 (1986).
14. F. R. Cochran, J. R. Connor, V. L. Roddick and B. M. Waite, *Biochem. biophys. Res. Commun.* **130**, 800 (1985).

15. M. Volpi, R. Yassin, W. Tao, T. F. P. Molski, P. H. Naccache and R. I. Sha'afi, *Proc. natn. Acad. Sci. U.S.A.* **81**, 5966 (1984).
16. S. Huterer and J. Wherret, *J. Lipid. Res.* **20**, 966 (1979).
17. Y. Nakagawa, K. Kurihara, T. Sugiura and K. Waku, *Biochim. biophys. Acta* **876**, 601 (1986).
18. T. Sugiura, O. Katayama, J. Fujui, Y. Nakagawa and K. Waku, *FEBS Lett.* **165**, 273 (1984).
19. O. Colard, M. Breton and G. Bereziat, *Biochem. J.* **222**, 657 (1984).
20. R. M. Kramer and D. Deykin, *J. biol. Chem.* **258**, 13806 (1983).
21. D. H. Albert and F. Snyder, *J. biol. Chem.* **258**, 97 (1983).
22. Y. Nakagawa, T. Sugiura and K. Waku, *Biochim. biophys. Acta* **833**, 323 (1985).
23. T. Sugiura, M. Nakajima, N. Sekiguchi, Y. Nakagawa and K. Waku, *Lipids* **8**, 125 (1983).