Vol. 93, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 13, 1980 Pages 42-49

PHOSPHOLIPID METHYLATION AFFECTS IMMUNOGLOBULIN E-MEDIATED HISTAMINE AND ARACHIDONIC ACID RELEASE IN RAT LEUKEMIC BASOPHILS

Fulton T. Crews*, Yutaka Morita†, Fusao Hirata, Julius Axelrod and Reuben P. Siraganian†

Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20205 and †Clinical Immunology Section, Laboratory of Microbiology and Immunology, National Institute of Dental Research, Bethesda, Maryland 20205

Received January 21,1980

ABSTRACT

Antigenic stimulation of rat basophilic leukemia cells sensitized with immunoglobulin E causes the release of histamine as well as arachidonic acid and its metabolites. The release of these substances is preceded by an increase in phospholipid methylation. Inhibition of phospholipid methylation is correlated to the inhibition of histamine release. Inhibition of methylation also reduces arachidonate release. Phospholipid methylation appears to be associated with both histamine secretion and the release of arachidonate and its metabolites.

INTRODUCTION

Basophils secrete histamine upon stimulation of the immunoglobulin E (IgE)-receptor complex by an antigen (1). The release process is exocytotic and temperature, calcium and energy dependent (2). Two stages appear to be involved in histamine release. The membrane is first activated by the antigen; this is followed by an exocytotic secretion of histamine. The membrane event is initiated by an antigen stimulated lateral movement and bridging of the IgE-receptor complex (3, 4). Recent work in our laboratory has shown that phospholipid methylation affects many membrane events including lipid translocation (5), membrane fluidity changes (6), coupling of the beta-adrenergic receptor with adenylate cyclase (7) and lectin induced lymphocyte mitogenesis (8). These findings prompted a study on the relationship between phospholipid methylation and histamine release from rat basophilic leukemia cells (9,10). We now report that changes in

*Research Associate in the Pharmacology-Toxicology Program of the National Institute of General Medical Sciences.

ABBREVIATIONS

IgE - immunoglobulin E ; EDTA - ethylenediamine tetraacetic acid

0006-291X/80/050042-08$01.00/0

phospholipid methylation are associated with the IgE mediated secretion of histamine and release of arachidonic acid and its metabolites.

METHODS

Preparation of Rat Leukemic Basophils: The 2H3 clone of rat basophilic leukemia cells was grown as a monolayer (11). The cells were grown in 35 mm diameter wells in Eagles minimum essential medium with Earles' balanced salt solution, 15% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 ug/ml streptomycin and 100 ug/ml gentamycin (11). The media was changed and replaced with Eagles minimum essential media containing 0.1% bovine serum albumin 30 hours after cultures were initiated with 10^6 cells detached with trypsin-EDTA. Experiments were carried out approximately 18 hours after the replacement of the media.

Histamine Release: Following stimulation with antigen the cells in a 35 mm diameter well were sensitized with 50 ul of a mouse ascitic fluid containing ovalbumin specific IgE (12). Histamine release was initiated by the addition of 10 ug/ml of ovalbumin. Histamine released into the media as well as that remaining in the cells was assayed by the automated fluorometric technique (13).

Phospholipid Methylation: Cells were incubated at 37°C with 100 uM [^{3}H-methyl]-L-methionine. The reaction was stopped by removing the media, and adding 1 ml of 10% trichloroacetic acid. Cells were detached from the wells with a rubber policeman and the wells were washed with an additional 1 ml 10% trichloroacetic acid. After centrifugation at 27,000 x g for 10 minutes, 3 ml of chloroform/methanol (2/1, v/v) was added to the pellets and the phospholipids were extracted (14). Some lysophospholipids are lost with this extraction procedure. Lysophosphatidylcholine formation was determined by extraction with n-butanol (15). Phospholipids were identified by thin layer chromatography (16).

Nucleotide Methylation: Cells were solubilized in 1 ml of 1% sodium dodecyl sulfate. Nucleotides were then extracted into 3 ml of 98% phenol. The aqueous phase was discarded and nucleotides were then precipitated by adding 5 ml of ethanol. Solutions were stored overnight at -20°C. The precipitate was pelleted by centrifugation, dissolved in 1% sodium dodecyl sulfate and counted by liquid scintillation spectrometry.

[^{14}C]-Arachidonic Acid Release: The cells were labelled with [^{14}C]-arachidonic acid (8 uM, 55.5 mCi/mM) 30 minutes after sensitization with IgE. Cells were washed thoroughly two times with Eagles minimum essential medium containing 0.1% bovine serum albumin to remove free arachidonic acid. Release of arachidonate and its metabolites was determined by taking aliquots from the media. To determine the distribution of [^{14}C]-arachidonic acid incorporation, phospholipids were separated by two dimensional thin layer chromatography with two different systems. A. Chloroform-methanol-water (65:25:4) followed by 1-butanol-acetic acid-water (60:20:20). B. Chloroform-methanol-28% aqeous ammonia (65:35:5) followed by chloroform-acetone-methanol-acetic acid-water (5:2:1:1:0.5).

RESULTS AND DISCUSSION

To examine whether rat basophilic leukemia cells could methylate phospholipids, cells were incubated with [^{3}H-methyl]-L-methionine. After 60 minutes

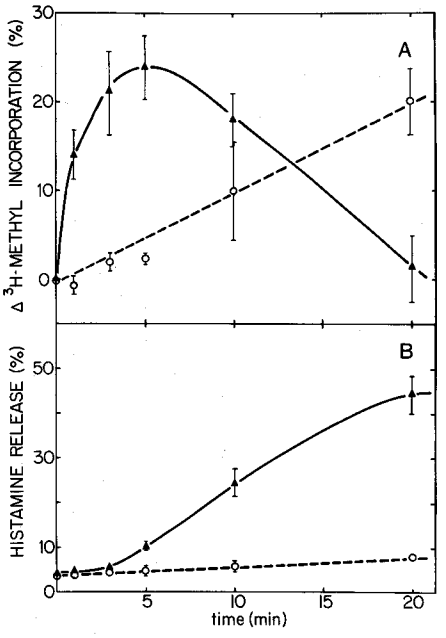

Fig. 1. Increased phospholipid methylation and histamine release. Rat basophilic leukemia cells were preincubated in the presence of 100 uM [^{3}H-methyl] methionine (440 dpm/pmol) for 90 minutes in Eagles minimum essential medium with 0.1% bovine serum albumin. During the first 60 minutes ovalbumin specific IgE was added to sensitize the cells. The cells were washed and after 30 additional minutes incubation with [^{3}H-methyl] methionine, ovalbumin (10 ug/ml) was added. Bovine serum albumin was used for controls. At each indicated time an aliquot of the supernatant was taken and along with the cells was assayed for histamine and methylated phospholipids. (Upper graph A) Percent change from the zero time value in the [^{3}H-methyl] incorporation into phospholipids at various time intervals. The zero time value varied from experiment to experiment, ranging from 11.2 to 21.9 pmol/10^6 cells of [^{3}H-methyl] incorporation into phospholipids. (▲——▲) Cells stimulated with ovalbumin; (O--O) controls. (Lower Graph B) Percent of the total histamine content released. (▲——▲) Cells stimulated with ovalbumin; (O- -O) controls. Each point represents the mean of duplicate determination in four separate experiments. Vertical bars represent the standard error of the mean.

the ^{3}H-methylated phospholipids were extracted into chloroform-methanol and separated by thin layer chromatography. The [^{3}H]-methylated phospholipids were distributed in phosphatidylcholine (61%), phosphatidyl-N,N-dimethylethanolamine (24%) and phosphatidyl-N-monomethylethanolamine (15%).

The effect of stimulation with antigen on phospholipid methylation and its relationship to histamine release was examined in rat basophilic leukemia cells preincubated with both ovalbumin specific IgE and [^{3}H-methyl]-L-methionine. Sensitization with IgE did not affect phospholipid methylation. The addition of the antigen, ovalbumin, caused a rapid increase in [^{3}H-methyl] incorporation into phospholipids (Fig. 1A). Within one minute after stimula-

tion with antigen, there was a significant ($p < 0.01$) increase in the incorporation of [^{3}H-methyl] group into phospholipids. The increase was greatest at approximately three to five minutes after stimulation. After five minutes, the [^{3}H-methyl] groups present in phospholipids began to decline. The incorporation of [^{3}H-methyl] group in the unstimulated cells continued to rise at a steady rate. Twenty minutes after the addition of antigen the amount of [^{3}H]-methylated phospholipids in the stimulated cells was decreased ($p < 0.01$). IgE-mediated histamine release became apparent three to five minutes after the addition of antigen (Fig. 1B). Secretion of histamine was complete after about 20 minutes. In cells stimulated with antigen the increase in [^{3}H-methyl] incorporation preceded histamine release whereas the decline in methylated phospholipids paralleled the release of histamine. These observations suggested a relationship between phospholipid methylation and the secretion of histamine.

Recent reports have shown that incubation of cells with 3-deazaadenosine and homocysteine thiolactone leads to the accumulation of S-adenosylhomocysteine and 3-deazaadenosylhomocysteine which then results in the inhibition of S-adenosylmethionine requiring methyltransferases (17,18). 3-Deazaadenosine and homocysteine thiolactone were added during the IgE sensitization period and the effect on phospholipid methylation and histamine release was examined. Increasing concentrations of 3-deazaadenosine in the presence of homocysteine thiolactone produced a concentration dependent inhibition of both the release of histamine and the incorporation of [^{3}H-methyl] group into phospholipids (Fig. 2). At each concentration of 3-deazaadenosine the inhibition of phospholipid methylation was almost identical to the inhibition of histamine release. This correspondence of dose-reponse curves suggests that both processes are inhibited by the drug acting at a common site (19), i.e., phospholipid methyltransferases. The addition of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine (3×10^{-4}M), another methyltransferase inhibitor (20), also blocked both phospholipid methylation (58%) and histamine release (59%). To determine if the inhibition of other methylation processes corresponded to the inhibition of his-

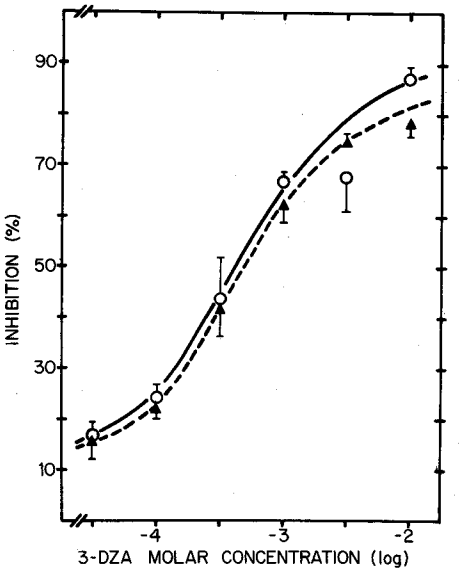

Fig. 2. Percent inhibition of histamine release and [^{3}H-methyl] incorporation into phospholipids. Various concentrations of 3-deazaadenosine and homocysteine thiolactone (2 x 10^{-4}M) were added during the 90 minutes preincubation. The incorporation of [^{3}H-methyl] into phospholipids was measured five minutes after stimulation with ovalbumin. The release of histamine was measured 20 minutes after the addition of ovalbumin. (▲ - - ▲) [^{3}H-methyl] incorporation into phospholipids. (O——O) Histamine release. Each point represents the mean of triplicate determinations. Vertical bars represent the standard error of the mean.

tamine release, nucleotide methylation was measured. 3-Deazaadenosine (10^{-3}M) and homocysteine thiolactone (2 x 10^{-4}M) inhibited nucleotide methylation by 27%, while histamine release was inhibited 59%. These observations suggest that phospholipid methylation is one of the required methylations for histamine release.

The correspondence of the antigen stimulated histamine release with the decline of the [^{3}H-methyl] labelled phospholipid suggested that the secretion of histamine is linked to the further metabolism of [^{3}H]-methylated phospholipids. [^{3}H-methyl] labelled lysophosphatidylcholine was found following antigen stimulation. This suggested that a metabolic route might be the cleavage of phosphatidylcholine by phospholipase A_2. Since arachidonic acid is incorporated to a considerable extent into phosphatidylcholine formed through the methylation pathway (21) cells were pre-incubated with [^{14}C]-arachidonic acid. The extent of incorporation of this fatty acid into phospho-

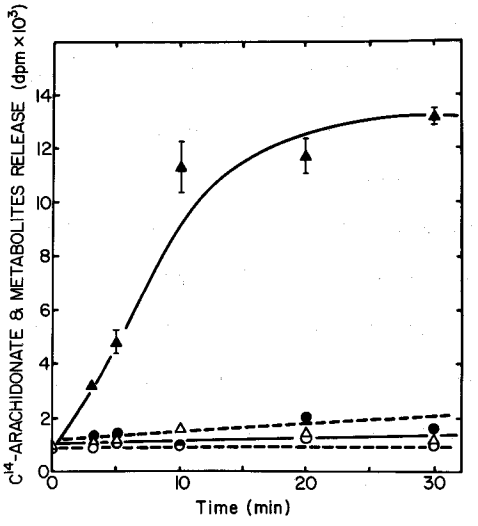

Fig. 3. Release of [^{14}C]-arachidonic acid and metabolites and its inhibition by 3-deazaadenosine. Cells were sensitized with IgE as described in text. [^{14}C]-Arachidonic acid (8 uM, 55.5 mCi/mM) was added for 30 minutes after sensitization with IgE. Cells were then washed thoroughly two times with Eagles minimum essential medium containing 0.1% bovine serum albumin to remove free arachidonic acid. Aliquots of the media were taken to determine [^{14}C]-arachidonate release. (▲——▲) Cells stimulated with ovalbumin; (△——△) unstimulated controls; (●- -●) cells stimulated with ovalbumin in the presence of 3-deazaadenosine (10^{-3}M) and homocysteine thiolactone (2 x 10^{-4}); (O--O) unstimulated controls in the presence of 3-deazaadenosine (10^{-3}M) and homocysteine thiolactone (2 x 10^{-4}). Each point represents the mean of triplicate determinations. Vertical bars represent the standard error of the mean.

lipids of rat basophilic leukemia cells was determined. Phosphatidylcholine contained 69% of the [^{14}C]-arachidonic acid incorporated into phospholipids. The addition of antigen to the cells resulted in a marked release of [^{14}C]-arachidonic acid and its metabolites into the media (Fig. 3) and this paralleled the release of histamine (Fig. 1B). To determine whether the arachidonic acid and its metabolites were related to the phospholipid methylation pathway, the effect of the methyltransferase inhibitor 3-deazaadenosine (10^{-3}M) was examined. 3-Deazaadenosine (10^{-3}M) blocked the release of arachidonic acid and its metabolites (Fig. 3) as well as histamine release.

Our results demonstrate that IgE stimulated release of histamine is closely associated with phospholipid methylation and release of arachidonic acid from these lipids. The antigen stimulated increase in [^{3}H-methyl] incorporation into phospholipids precedes histamine release. Methylation probably

occurs in a highly localized domain in the membrane where receptors and phospholipid methyltransferases are clustered (22). Phospholipid methylation has been found to decrease membrane microviscosity (6) which could facilitate lateral mobility and aggregation of IgE receptors. Concanavalin A, a lectin which aggregates membrane glycoproteins (23,24), stimulates histamine release and the decarboxylation of phosphatidylserine in rat mast cells to form phosphatidylethanolamine which is then methylated to form phosphatidylcholine. Phosphatidylcholine is then further metabolized to lysolecithin (25). These observations and the findings presented here suggest that during histamine release a small pool of phospholipids is rapidly metabolized as follows:

Phosphatidylethanolamine $\xrightarrow{+CH_3}$ phosphatidyl-N-monomethylethanolamine $\xrightarrow{2\ CH_3}$ phosphatidylcholine $\longrightarrow$ arachidonic acid + lysophosphatidylcholine. The exocytotic phase of stimulus-secretion coupling takes place as the histamine-containing granule membrane fuses with the plasma membrane. The activation of phospholipase A_2 during secretion generates arachidonic acid, a prostaglandin precursor, and lysolecithin. Lysolecithin, a known fusogen (26,27), may facilitate fusion of the granule and plasma membranes. Thus, phospholipid methyltransferase and phospholipase A_2 activation may be events modulating exocytosis.

REFERENCES

1. Ishizaka, T. and Ishizaka, K. (1975) Prog. Allergy 19, 60-121.
2. Kazimierczak, W. and Diamant, B. (1978) Prog. Allergy 24, 295-365.
3. Siraganian, R.P., Hook, W.A., Levine, B.B. (1975) Immunochemistry 12, 149-157.
4. Ishizaka, K., Ishizaka, T. and Lee, E.H. (1970) Immunochemistry 7, 687-702.
5. Hirata, F. and Axelrod, J. (1978) Proc. Natl. Acad. Sci. USA 75, 2348-2352.
6. Hirata, F. and Axelrod, J. (1978) Nature 275, 219-220.
7. Hirata, F., Strittmatter, W.J. and Axelrod, J. (1979) Proc. Natl. Acad. Sci. USA 76, 368-372.
8. Hirata, F., Toyoshima, S., Axelrod, J. and Waxdal, M.J. (1979) Proc. Natl. Acad. Sci. USA, in press.
9. Taurog, J.D., Mandozo, G.R., Hook, W.A., Siraganian, R.P. and Metzger, H. (1977) J. Immunol. 119, 1757-1761.
10. Siraganian, R.P. and Metzger, H. (1978) J. Immunol. 121, 2584-2585.
11. Barsumian, E.D., Isersky, C. and Siraganian, R.P. (1979) Fed. Proc. 38, 1019.
12. Siraganian, R.P. and Hazard, K.A. (1979) J. Immunol. 122, 1719-1725.

13. Siraganian, R.P. (1974) Anal. Biochem. 57, 383-389.
14. Hirata, F., Viveros, O.H., Diliberto, Jr., E.J. and Axelrod, J. (1978) Proc. Natl. Acad. Sci. USA 75, 1718-1721.
15. Bjerve, K.S., Daae, L.N.W. and Bremer, J. (1974) Anal. Biochem. 58, 238-245.
16. Crews, F.T., Hirata, F. and Axelrod, J. (1979) J. Neurochem., in press.
17. Zimmerman, T.P., Wolberg, G. and Duncan, G.S. (1978) Proc. Natl. Acad. Sci. USA 75, 6220-6224.
18. Richards, H.H., Chiang, P.I. and Cantoni, G.L. (1978) J. Biol. Chem. 253, 4476-4480.
19. Ariens, E.J. (1971) in Medicinal Chemistry, A Series of Monographs, Vol. 1, Drug Design, De Stevens, G. Ed., pp. 6-76, Academic Press, New York.
20. Chiang, P.K., Cantoni, G.L., Baker, I.P., Shannon, W.M., Thomas, H.J. and Montgomery, J.A. (1978) Biochem. Biophys. Res. Commun. 82, 417-423.
21. Balint, J.A., Beeler, D.A., Treble, D.H. and Spitzer, H.L. (1967) J. Lipid Res. 8, 486-493.
22. Strittmatter, W.J., Hirata, F., Axelrod, J., Mallorga, P., Tallman, J.F. and Henneberry, R.C. (1979) Nature 828, 857-859.
23. Siraganian, R.P. (1976) in Mitogens in Immunology, Oppenheim, J.J. and Rosenstreich, D.L., Eds., pp. 69-84, Academic Press, New York.
24. Keller, R. (1973) Clin. Exp. Immunol. 13, 139-147.
25. Hirata, F., Axelrod, J. and Crews, F. (1979) Proc. Natl. Acad. Sci. USA 76, 4813-4816.
26. Poole, A.R., Howell, J.I. and Lucy, J.A. (1970) Nature 227, 810-813.
27. Croce, C.M., Sawicki, W., Kritchevsky, D. and Koprowski, H. (1971) Exp. Cell Res. 67, 427-435.