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DISTRIBUTION OF PHOSPHATIDYLETHANOLAMINE ARACHIDONIC ACID IN PLATELET MEMBRANES *

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Summary

Arachidonic acid (20 : 4) and other fatty acids and aldehydes in phosphatidylethanolamine (PE) present on the platelet surface was determined. Surface-exposed PE was isolated by using 2,4,6-trinitrobenzenesulfonate, a nonpenetrating probe (Schick, P.K., Kurica, K.B. and Chacko, G.K. (1976) J. Clin. Invest. 57, 1221—1226). PE contains 50% total platelet arachidonic acid. Approx. 16% platelet PE is present on the platelet surface. The study showed that the fatty acid and aldehyde composition of PE on the platelet surface is virtually identical to that in PE present inside the platelet. Therefore, 8 nmol arachidonic acid are present in PE in the outer layer of the plasma membrane in 10^9 platelets.

Asymmetry of phospholipids has been demonstrated in the plasma membrane of human platelets [1,2]. There are five major fatty acids and several aldehydes in platelet diacylglycerophospholipids and plasmalogens [3]. The distribution of phospholipid fatty acids and aldehydes in the platelet plasma membrane lipid bilayers and in intracellular membranes is not known. Phosphatidylethanolamine (PE) represents 27% of total platelet phospholipids. PE contains a disproportionate amount of arachidonic acid, about 49% of the total

* The study was presented in part at the 7th International Congress on Thrombosis and Haemostasis (London, U.K., July, 1979). [9].

Abbreviations: PE, phosphatidylethanolamine; TNBS, 2,4,6-trinitrobenzenesulfonate; TNP, trinitrophenyl.

arachidonic acid present in platelet phospholipids, as well as several aldehydes since about 55% platelet PE is composed of plasmalogens [4]. This study was designed to determine whether the content of arachidonic acid and other fatty acids and aldehydes in the small portion of PE on the platelet surface is different from that in PE in the inner layer of the plasma membrane and in intracellular membranes. The data obtained about PE arachidonic acid, which represents one half of total platelet arachidonic acid, was used to calculate the distribution of arachidonic acid in PE in the outer and inner layers of the plasma membrane and in intracellular membranes. Analysis of the location of arachidonic acid in platelet membranes is important since platelet phospholipids are considered to be the source of free arachidonic acid and thromboxane A_2 [5–8].

Washed platelet suspensions were prepared from freshly-collected citrated human blood as previously described [1]. Contamination by erythrocytes and leukocytes was not significant. Intact platelets were incubated with 2,4,6-trinitrobenzenesulfonate (TNBS) which reacts with PE only on the platelet surface and not with PE in the inner plasma membrane lipid layer and in intracellular membranes. Details of the labeling procedure have been described [1]. Lipids and trinitrophenyl (TNP)-lipid derivatives were extracted, separated by thin-layer chromatography, and analyzed for lipid-P to determine percent PE that

TABLE I

FATTY ACIDS AND ALDEHYDES IN PE-TNP DERIVATIVE AND UNREACTED PE

Lipids were extracted from platelets incubated with TNBS and from control platelets. Phospholipids and TNP-lipid derivatives were separated by thin-layer chromatography and quantitated by lipid-P. Whole platelet PE, unreacted PE, and PE-TPN-derivatives were subjected to acid methanolysis to produce fatty acid methyl esters (FAME) and dimethyl acetal derivatives of aldehydes (DMA) for analysis by gas-liquid chromatography. In experiments shown in the table about 16% PE had reacted with TNBS during 1-h incubations. The composition of whole platelet PE, unreacted PE, and PE-TPN-derivatives shown in the table respresent the means ± S.D. of five experiments each done in duplicate. Other experiments done at 30 and 45 min when less PE had reacted showed that composition of fatty acids and aldehydes in PE-TNBS derivative and unreacted PE was the same as shown in the table.

Fatty acids aldehydes (FAME, DMA)	In whole platelet PE	In unreacted PE	In PE-TNP derivative
16 : 0 DMA	5.6 ± 0.3	6.3 ± 1.9	6.1 ± 1.4
16 : 0	4.0 ± 0.4	4.1 ± 0.4	3.4 ± 0.6
16 : 1	1.1 ± 0.4	1.0 ± 0.3	0.9 ± 0.7
18 : 0 DMA	13.1 ± 0.3	11.7 ± 1.1	12.7 ± 0.9
18 : 1 DMA	3.8 ± 0.2	3.5 ± 0.4	4.0 ± 0.7
18 : 0	15.2 ± 1.4	14.6 ± 2.0	14.6 ± 1.3
18 : 1	6.6 ± 0.9	6.8 ± 1.3	6.3 ± 1.2
18 : 2	3.2 ± 0.8	3.7 ± 0.5	1.7 ± 0.5
18 : 3, 20 : 0	0.9 ± 0.3	0.6 ± 0.4	0.7 ± 0.5
20 : 1	0.6 ± 0.3	0.5 ± 0.1	0.5 ± 0.2
20 : 3	0.8 ± 0.2	0.9 ± 0.3	1.0 ± 0.4
20 : 4	31.7 ± 2.2	32.0 ± 1.8	35.3 ± 2.0
22 : 3, 22 : 4	5.2 ± 0.2	5.6 ± 1.3	5.4 ± 0.2
24 : 1	0.9 ± 0.2	0.8 ± 0.2	1.2 ± 0.4
22 : 5, 25 : 0	3.4 ± 0.2	3.0 ± 0.2	2.6 ± 0.1
22 : 6	2.5 ± 0.2	2.2 ± 0.4	2.7 ± 1.2
Other	1.5 ± 0.6	2.9 ± 1.0	0.9 ± 0.2

had reacted with TNBS [1].

The fatty acid and aldehyde content of PE that had reacted with TNBS as well as that of unreacted PE was determined as follows. Both unreacted PE and PE-TNP derivatives were subjected to acid methanolysis to produce dimethyl acetal derivatives of aldehydes and fatty acid methyl esters. Fatty acid methyl esters and dimethyl acetal derivatives were analyzed by gas-liquid chromatography using a Hewlett-Packard GLC, model No. 5830A, and a Supelco SP 2330 column with a multi-level temperature program.

Table I shows the results of four experiments in which platelets were incubated with TNBS for 60 min. Under these conditions, 16% PE in platelets reacted with TNBS. Except for linoleic acid (18 : 2) the fatty acid composition of PE that had reacted with TNBS was not appreciably different from that in the remainder of platelet PE. The amount of linoleic acid in platelet PE is small in comparison to the amount of other PE fatty acids and aldehydes. However, the linoleic acid content of PE-TNP lipid derivatives was significantly ($P < 0.01$) lower than the corresponding figure for unreacted PE. The significance of this finding is not clear at this time. Three aldehyde derivatives were detected, the dimethyl acetal derivatives of 16 : 0, 18 : 0, and 18 : 1, which reflect plasmalogen PE. There was no difference between the composition of aldehydes in the TNP-PE derivatives and in unreacted PE.

About 16% platelet PE is present on the platelet's surface. The study shows that the composition of arachidonic acid and other fatty acids and aldehydes in PE in the outer plasma membrane lipid bilayer, except for linoleic acid, is the same as that of PE inside the platelet. It confirms that the distribution of plasmalogen PE in platelet membranes is random as has been reported [6]. Also, the data indicate that there is a random distribution of each plasmalogen species, judged on aldehyde composition, in the outer surface of the plasma membrane as compared to the inner surface and the intracellular membranes.

This information can be used to determine that there are 8 nmol arachidonic acid in PE in the outer layer of the platelet plasma membrane in 10^9 platelets. This is based on the calculation showing that there are 52 nmol arachidonic acid in PE in 10^9 platelets * and that about 16% platelet PE is present on the cell's surface. Phosphatidylinositol and phosphatidylserine are primarily located within the platelet [1,2] and thus do not provide significant amounts of arachidonic acid in the platelet surface. About 40% platelet phosphatidylcholine is present on the platelet surface [2] but its arachidonic acid content relative to the remainder of platelet phosphatidylcholine is not known. There is evidence that there is 10 nmol arachidonic acid on the platelet's exterior [10]. Therefore, arachidonic acid in surface-exposed PE represents the majority of arachidonic acid in the platelet plasma membrane outer layer.

The study shows that despite the asymmetrical distribution of PE in the platelet plasma membrane the species of fatty acids and aldehydes in surface exposed PE do not differ from that in PE located inside the platelet.

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* There are 600 nmol fatty acids and aldehydes in 10^9 human platelets based on the analysis of lipid phosphorus (9.3 μg lipid-P). Since PE is 27% of total platelet phospholipids and 31.7% of PE fatty acids represents arachidonic acid, then there are 52 nmol arachidonic acid in PE in 10^9 platelets.

References

1 Schick, P.K., Kurica, K.B. and Chacko, G.K. (1975) J. Clin. Invest. 57, 1221—1226
2 Chap, H.J., Zwaal, R.F.A. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 467, 146—164
3 Marcus, A.J., Ullman, H.L. and Safier, L.B. (1969) J. Lipid Res. 10, 108—114
4 Cohen, P. and Derksen, A. (1969) Br. J. Haematol. 17, 359—371
5 Bills, T.K., Smith, J.B. and Silver, M.J. (1976) Biochim. Biophys. Acta 424, 303—314
6 Rittenhouse-Simmons, S., Russell, F.A. and Deykin, D. (1977) Biochim. Biophys. Acta 488, 370—380
7 Lapetina, E.G., Chandrabose, K.A. and Cuatrecasas, P. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 818—822
8 Broekman, M.J., Ward, J.W. and Marcus, A.J. (1980) J. Clin. Invest. 66, 275—283
9 Schick, P.K., Schick, B.P., Brandeis, G. and Mills, D.C.B. (1979) Thromb. Haem. 42, 217 (Abstr.)
10 Perret, B., Chap, H.J. and Douste-Blazy, L. (1979) Biochim. Biophys. Acta 556, 434—446