COMPOSITION OF PHOSPHOLIPIDS AND PHOSPHOLIPID FATTY ACIDS IN RAT MAST CELLS

Kjell STRANDBERG and Susanne WESTERBERG

From the Department of Pharmacology, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

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Summary

The composition of phospholipids and phospholipid fatty acids in isolated rat serous fluid mast cells was analyzed by thin layer chromatography, gas–liquid chromatography and mass spectrometry. The phospholipids constituted about 50% of the mast cell lipids and phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylcholine, sphingomyelin and lysophosphatidylcholine were identified. The phosphatidylethanolamine fraction contained aldehydes and the highest proportion of unsaturated fatty acids. Sphingomyelin contained predominantly saturated fatty acids whereas the ratio unsaturated fatty acids: saturated fatty acids for the other phospholipids was more close to 1.

Introduction

Biologically active acidic lipids, e.g. slow reacting substance (SRS)[1,2] and prostaglandins[3,4,5] appear on histamine release in a variety of animal tissues. The prostaglandins are biosynthetized from polyunsaturated C 20-acids[6,7] split off from the 2-position of phospholipids[8,9] by a hydrolytic enzyme, probably of phospholipase A nature[10]. The chemical identity of SRS remains, however, to be disclosed. As the prostaglandins, SRS is an unsaturated hydroxy acid[11]. It has been suggested that the formation of SRS is a consequence of the activation of a mast cell phospholipase[2]. If such an enzyme were involved in the histamine release process it might be possible to demonstrate the appearance of split products from the hydrolysis during the release.

The present study was undertaken as part of the approach to test this hypothesis. In this paper we report the composition of phospholipids and phospholipid fatty acids in isolated rat mast cells.

Materials and Methods

Preparation of mast cells

Sprague-Dawley rats were fed on a diet of rat pellets nr 213 (Anticimex, Sollentuna, Sweden) and water. Mast cells from peritoneal and pleural cavities of male rats weighing 350–450 g were isolated by gradient centrifugation on Ficoll (AB Pharmacia, Uppsala, Sweden)[12]. The cells were washed three times with an isotonic saline solution containing 10% (v/v) of Sörensens phosphate buffer (Na_2HPO_4 + KH_2PO_4, 67 mM), pH 7.0 and human serum albumin 1 mg/ml (AB Kabi, Stockholm, Sweden). They were suspended in saline solution, counted in a Bürker chamber and then used immediately or stored frozen at −20°. Before lipid extraction the mast cell suspension ($1.5–3.0 \times 10^6$ mast cells/ml) was diluted with 0.5 vol of water and sonicated for 1 min at 8 μ using a MSE ultrasonicator. The number of mast cells isolated, as calculated per rat, was $1.9 \pm 0.3 \times 10^6$ (Mean ± SD, n = 16).

Lipid extraction

The sonicated mast cells, suspended in 0.6% NaCl, were extracted with chloroform : methanol

(2:1), containing 2 μg tocopherol/ml (Merck, Darmstadt, Germany) as an anti-oxidant[13]. The filtered extract (20 ml/ml of mast cell suspension) was washed with 0.2 vol of 0.6% NaCl in a separatory funnel. The phases were allowed to separate at 4 °C overnight and for 1 hr at room temperature. The lower phase was evaporated to dryness and dried to constant weight in a vacuum desiccator.

Determination of cholesterol and lipid phosphorus
Cholesterol was determined in duplicates (100–250 μg) of the lipid extract[14]. Lipid phosphorus was determined by the method of BARTLETT[15].

Column chromatography
The lipid extract (0.5–1.0 mg) was dissolved in chloroform and applied on a 0.5 g silicic acid (100 mesh, Mallinckrodt, St Louis, USA) column prepared in the same solvent. The non-polar lipids were eluted with 15 ml of chloroform and the polar lipids with 20 ml of methanol.

Thin layer chromatography (TLC)
Glass plates (0.4 × 20 × 20 cm) were coated with a 0.3 mm thick layer of Silica gel H (Merck, Darmstadt, Germany) (20 g/45 ml of 1 mM Na_2CO_3), washed by development in methanol:ether (80:20) and activated at 115 °C for 1 hr before use. Aliquots (~500 μg) of the original lipid extract and reference phospholipids were app'ied as bands using micropipettes. The chromato-plates were placed in glass jars and developed at room temperature until the solvent front had reached 16 cm. The solvent system used for phospholipid fractionation was chloroform:methanol:glacial acetic acid:water (50:30:8:4) containing 20 μg tocopherol/ml[16]. The spots were outlined by the use of iodine vapour. When the iodine had evaporated they were scraped off and transferred to tubes. The phospholipids were eluted from the Silica gel as described by SKIPSKY[16].

When phospholipids were fractionated to analyze the fatty acid composition, 1–3 mg of the lipid extract was applied on the chromatoplates. The spots were detected under UV-light after spraying the plates with 2,7-dichlorofluorescein (33.3 mg/100 ml 2 mM NaOH).

Preparation of fatty acid methylesters

(a) Phospholipids from column chromatography
The methanol fraction was evaporated to dryness. The residue was subjected to esterification in 1.0 ml of 1 M KOH in methanol under nitrogen in stoppered test tubes kept at 37 °C for 1 hr. One ml of 2 M HCl was added and the fatty acid methylesters were extracted with 10 ml of n-heptane. After washing twice with 1 ml of water, the n-heptane was evaporated to dryness under nitrogen. The residue was dissolved in 100 μl n-heptane before gas chromatographic analysis.

(b) Phospholipids from TLC
The phospholipid spots and corresponding controls of Silica gel were scraped off and transferred to 10 ml ampoules. Two ml of 14% boron trifluoride in methanol complex (Merck, Darmstadt, Germany) was added and the ampoules were flushed with nitrogen, sealed and heated in a boiling water bath for 30 or 90 min (sphingomyelin fraction)[17]. The ampoules were cooled, opened and 4 ml of n-heptane with 2 μg tocopherol/ml was added followed by 2 ml of water, or 5 M NaOH for the determinations of the dimethylacetals, added dropwise with stirring at 0 °C. After centrifugation the upper phase was collected and the extraction was repeated with 2 ml of n-heptane. The combined heptane phases were evaporated under nitrogen and redissolved in 100 μl n-heptane before gas chromatographic analysis.

Gas-liquid chromatography (GLC)-mass spectrometry
Gas-liquid chromatography was performed using a Hewlett Packard model 402 gas chromatograph equipped with a hydrogen flame ionization detector. Glass U-tube columns (4 mm × 1.8 m) with 8% EGSS-X on 100–120 mesh Gaschrom Q (Applied Science Lab. Inc., USA) or 1% SE-30 on 100–120 mesh Gaschrom Q were used. When using 8% EGSS-X, the column temperature was programmed from 125-210 °C at a rate of 5 °C/min with a 5 min isothermal delay. The 1% SE-30 column temperature was programmed from 160–235° at the same rate and delay. The temperature was kept at limit for about 10 min and the nitrogen flow rate was about 30 ml/min. The methylesters of 7

saturated and 10 unsaturated fatty acids listed below were used as reference standards. The fatty acid methylesters of the mast cell phospholipids were identified either by comparing their retention times with those of the reference compounds on the two columns or by combined gas chromatographic and mass spectrometric analysis using a LKB 9000 mass spectrometer linked to a Pye gas chromatograph equipped with an 1% SE-30 column under the same conditions as given above. The relative retention times related to heptodecanoic acid were also calculated. Quantitative measurements were made by calculating the peak areas according to the "peak height × width at half peak" method or occasionally by triangulation.

Phospholipid references
Phosphatidylethanolamine (Brain extract type V), phosphatidylserine (Brain extract type III) and sphingomyelin from bovine brain were purchased from Sigma Chemical Company, St. Louis, USA. Phosphatidylinositol, Na-salt from bovine brain, was from Koch-Light Lab. Ltd., Colnbrook, England. Phosphatidylcholine and lysophosphatidylcholine from egg were obtained from Sigma Chemical Company, St. Louis, USA.

Fatty acid standards
Myristic (14:0), palmitic (16:0), heptadecanoic (17:0), stearic (18:0), arachidic (20:0), behenic (22:0) and lignoceric (24:0) acid were obtained from Applied Science Lab. Inc., State College, P.A., USA and the methylesters were prepared with diazometan. The methylesters of palmitoleic (16:11), oleic (18:1), linolenic (18:3), cis-5-eicosaenoic (20:1), 11,14-eicosadienoic (20:2), arachidonic (20:4), erucic (22:1), 4,7,10,13,16,19-decosahexaenoic (22:6) and nervonic acid (24:1) were from the same source. Chloroform and methanol were of analytical grade. n-Heptane of analytical grade was redistilled before use.

Abbreviations: PE = phosphatidylethanolamine;
PS = phosphatidylserine;
PI = phosphatidylinositol;
PC = phosphatidylcholine;
SM = sphingomyelin;
LPC = lysophosphatidylcholine;

Results and Discussion

Lipid composition of rat mast cells
The content of total lipids, phospholipids and cholesterol in rat mast cells was determined in 8 experiments. Total lipid content was 79.1 ± 13.7 $\mu g/10^6$ mast cells. This is in agreement with previous data where the lipid content of isolated rat peritoneal mast cells was calculated to be 85 picograms per cell corresponding to 18% of the dry weight[18]. The phospholipids constituted 41.4 ± 5.3 $\mu g/10^6$ mast cells. Cholesterol and cholesterol esters amounted to 8.8 ± 2.6 $\mu g/10^6$ mast cells (mean ± S.D.).

Composition of rat mast cell phospholipids
The composition of the phospholipids was studied in 4 experiments. Table 1 shows the phospholipids of rat mast cells calculated as percentages of the total lipid phosphorus. The phospholipids separated and identified were PE (Rf = 0.83), PS (Rf = 0.68), PI (Rf = 0.62), PC (Rf = 0.43), SM (Rf = 0.25) and LPC (Rf = 0.12). PS and PI were not distinctly separated except in one experiment so they have been determined together.

Fatty acid composition of mast cell phospholipids
The distribution of the fatty acids in the phospholipid fraction is presented in Table 2. The table is based on data from 6 experiments obtained by using the 8% EGSS-X column. This column gave the best resolution of the fatty acids. The phospholipids contained relatively more unsaturated fatty acids than saturated fatty acids. Palmitic and stearic acid were the predominant saturated fatty acids.

Table 1

Composition of rat mast cell phospholipids

Phospholipid	Percentage (Mean	S.D.)
Phosphatidylethanolamine	26.7	3.3
Phosphatidylserine + phosphatidylinositol	16.1	2.0
Phosphatidylcholine	29.6	1.3
Sphingomyelin	19.8	2.2
Lysophosphatidylcholine	2.1	0.3
Front	4.7	1.3
Origin	1.0	0.7

Table 2

Fatty acid composition of mast cell phospholipids and individual phospholipid fractions

	Fatty acid distribution (%)					
Fatty acid	Total phospholipid (Mean	S.D.)	PE	PS+PI	PC	SM
16:0 DMA*	2.2	0.6				
16:0	16.1	1.4	9.8	3.7	27.3	18.0
16:1	0.9	0.4				
18:0 DMA*	2.7	0.3				
18:0	24.0	3.8	25.1	42.3	23.0	27.1
18:1	14.3	1.7	15.4	23.3	18.6	12.7
18:2	8.6	1.2	5.8	3.9	11.0	2.2
20:0, 20:1	0.9	0.2	1.6		1.5	7.3‡
20:2	1.3	0.4	1.5		1.4	
20:3	1.6	0.3	1.9	6.1†	2.0	
20:4	18.2	2.5	23.2	11.5	13.6	
22:0						5.4
22:4	4.8	0.6	8.8	5.4	1.8	
22:5, 22:6	1.7	0.4	2.2	4.0		
24:0						3.6
24:1	2.8	0.5				23.8
Unidentified			4.7			

* DMA = dimethyl acetal, tentatively identified.
† Contains some C 20:2.
‡ C 20:0 only.
Abbreviations: PE = phosphatidylethanolamine;
PS = phosphatidylserine;
PI = phosphatidylinositol;
PC = phosphatidylcholine;
SM = sphingomyelin;
LPC = lysophosphatidylcholine;

Arachidonic acid comprised nearly 20%, oleic acid about 15% and linoleic acid almost 10% of the total phospholipid fatty acids.

Fatty acid composition of individual phospholipid fractions

The distribution of fatty acids in the individual phospholipids from 4 experiments is given in Table 2. The amounts of LPC available did not permit accurate analysis. Notable are the differences in the content of palmitic, stearic, linoleic, arachidonic and polyunsaturated C22-acids. PE contained the highest amount of polyunsaturated fatty acids and had a low content of monounsaturated fatty acids. In one experiment an attempt was made to determine the dimethylacetals (DMA) of this fraction. The dimethylacetals were tentatively identified as 16:0 DMA and 18:0 DMA and constituted about 15% of the PE fatty acids. Stearic and arachidonic acid were the major fatty acids in this fraction. The PI+PS fraction contained three predominant fatty acids namely stearic, oleic and arachidonic acid. As PE, this combined fraction had a high content of C22-polyenoic acids. It also contained the highest amount of eicosatrienoic acid. PC contained the highest amount of palmitic acid found. Except for the presence of arachidonic and linoleic acid the content of polyunsaturated fatty acids in this fraction was small. SM contained lignoceric and nervonic acid which were not detected in any of the other fractions.

The ratios between saturated and unsaturated fatty acids are presented in Table 3. It can be seen that the ratio approximates 1.0 for PC while PE contains unsaturated fatty acids to a higher extent thus giving a low ratio. The PI+PS fraction gives the same ratio as the total phospholipid fraction which was in between the

Table 3

Ratios between saturated fatty acids and unsaturated fatty acids in mast cell phospholipids

Phospholipid	Saturated fatty acids (A)	Unsaturated fatty acids (B)	$\frac{A}{B}$
Phosphatidylethanolamine	35.7	59.6	0.60
Phosphatidylserine + phosphatidylinositol	46.0	54.2	0.85
Phosphatidylcholine	50.3	49.9	1.01
Sphingomyelin	61.4	38.7	1.59
Total phospholipid fraction	45.9	54.2	0.85

former two. The fatty acids of SM were essentially saturated or monounsaturated and accordingly the ratio was high compared to the other fractions.

Data are now available on the phospholipid composition of a variety of isolated cells e.g. human blood cells, rat erythrocytes, mouse fibroblasts and bovine mammary cells[19–24]. In comparison the present data on the composition of the mast cell phospholipids show similarities with that of human blood cells.

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