Phospholipid (diacyl, alkylacyl, alkenylacyl) and fatty acyl chain composition in murine mastocytoma cells

Shiro Yoshioka,* Shigeru Nakashima,* Yukio Okano,* Hiroyuki Hasegawa, Arata Ichiyama,** and Yoshinori Nozawa***

Department of Biochemistry, Gifu University School of Medicine,* Tsukasamachi-40, Gifu 500, Japan, and Department of Biochemistry, Hamamatsu Medical College,** Handa-3600, Hamamatsu 431-31, Japan

Abstract The phospholipids from murine mastocytoma FMA3 and P-815 clone cells were quantitatively analyzed, and the major glycerophospholipids were examined for their fatty acyl chain distribution. In these cells, the content of histamine was less than 1/100 of normal mouse mast cells, and FMA3 cells had 1.5-fold as much histamine content as P-815 cells. The predominant phospholipid species of both mastocytoma FMA3 and P-815 were choline-containing glycerophospholipids (48%) and ethanolamine-containing glycerophospholipids (29%). The remaining minor constituents were sphingomyelin (6%, 7%), phosphatidylinositol (7%, 5%), phosphatidylserine (2%, 5%), cardiolipin (4%, 3%), and phosphatidic acid (2%, 1% for FMA3 and P-815, respectively). The choline-containing glycerophospholipids consisted of high amounts of 1-*O*-alkyl-2-acyl type (31%, 25%) and 1,2-diacyl type (63%, 66%) and a smaller amount of 1-*O*-alk-1'-enyl-2-acyl type (7%, 8%). In contrast, ethanolamine-containing glycerophospholipids were characterized by high contents of 1-*O*-alk-1'-enyl-2-acyl type (36%, 31%) and 1,2-diacyl type (55%, 58%), and a lower level of 1-*O*-alkyl-2-acyl type (12% and 11% for FMA3 and P-815, respectively). Unlike choline-containing glycerophospholipids and sphingomyelin that were rich in palmitic acid, ethanolamine-containing glycerophospholipids, phosphatidylserine and phosphatidylinositol showed a high proportion of stearic acid in the overall fatty acid composition. The content of arachidonic acid was highest in phosphatidylinositol. Sphingomyelin had a large amount of long chain and polyunsaturated fatty acids. In both choline- and ethanolamine-containing glycerophospholipids, the predominant fatty acids in the *sn*-1-position were palmitic, stearic, and oleic acid. In the *sn*-2-position, arachidonic acid was most abundant in the 1-*O*-alkyl-2-acyl type of choline-containing glycerophospholipid and also in the 1-*O*-alk-1'-enyl-2-acyl type of ethanolamine-containing glycerophospholipid. Unexpectedly, there was a relatively high percentage distribution of saturated fatty acids in the *sn*-2-position of 1-*O*-alkyl-2-acyl-, 1-*O*-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphocholine and 1,2-diacyl-, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine. —**Yoshioka, S., S. Nakashima, Y. Okano, H. Hasegawa, A. Ichiyama, and Y. Nozawa.** Phospholipid (diacyl, alkylacyl, alkenylacyl) and fatty acyl chain composition in murine mastocytoma cells. *J. Lipid Res.* 1985. **26:** 1134–1141.

Supplementary key words mastocytoma (FMA3, P-815) • diacyl • alkylacyl • alkenylacyl • histamine

It is generally known that phospholipid metabolism is enhanced when secretory cells are exposed to various stimuli. The enhanced phosphatidylinositol (PI) metabolism, known as "PI turnover," has been reported to be a primary event coupled with the calcium mobilization and the consequent secretory reaction (1–3). In addition, the release of arachidonic acid, an obligatory precursor for biologically active compounds such as prostaglandins and leukotrienes, occurs within 30 sec in activated mast cells, either by the action of phospholipase A_2 on membrane phospholipids or by the sequential action of phospholipase C and diglyceride lipase on PI (4). The secretory granules in rat mast cell contain enzymes, chemotactic peptides, heparinproteoglycan, histamine, and serotonin (4). On the other hand, the transformed murine mast cells, mastocytomas FMA3 and P-815, are found to have much lower amounts of histamine (S. Yoshioka and Y. Nozawa, unpublished data) and enzymes. N-acetyl-β-glucosaminidase and acid phosphatase (Y. Banno, S. Yoshioka, and Y. Nozawa, unpublished observation). These two cell lines are different in 5-hydroxytryptophan decarboxylase activity, indicating a more active biosynthesis of serotonin in FMA3 than in P-815 cells (5). Both cells were observed to release much less histamine than mast cells upon stimulation with A23187 or compound 48/80. Based on the concept of stimulus-secretion coupling, one would easily expect that the rapid turnover of membrane phospholipid may not occur in the mastocytoma cells treated with these agents. Prior to the investigation of the phospholipid metabolism in murine mastocytoma cells (FMA3 and

Abbreviations: PE, ethanolamine-containing glycerophospholipids; PC, choline-containing glycerophospholipids; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; GP, *sn*-glycerol-3-phosphate; PA, phosphatidic acid; GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; DMA, dimethyl acetal.

P-815) exposed to various stimulants, the detailed analyses of phospholipid class composition and fatty acyl chain distribution were undertaken.

MATERIALS AND METHODS

Materials

Phospholipase C (*Bacillus cereus*) and phospholipase A_2 were obtained from Boehringer-Mannheim, GmbH. Silica gel H, G, and primulin dye were purchased from Sigma Chemical Company, St. Louis, MO. Vitride ($NaAlH_2[OCH_2CH_2OCH_3]_2$) was from Nakarai Chemicals Ltd., Kyoto. 1-*O*-Alkylglycerol standards were the products of Serdary Research Laboratories, London, Ontario, Canada. Fatty acid methyl ester standards were obtained from Nihon Chromato Industrial Co., Tokyo. All chemicals were of reagent grade.

Cell lines

Two cloned cell lines of murine mastocytomas, FMA3 and P-815, were employed for analysis. FMA3 is a cloned cell line obtained from the cell line FMA. The origin and biological characteristics of FMA3 line are described elsewhere (6). It was established from a solid tumor of Furths mastocytoma cells (F-line) of LAF_1 mice (7) by adapting the cells to grow in the ascites prior to transplantation to an in vitro culture. When inoculated in the abdominal cavity of LAF_1 mice, cells grow with a doubling time of 20.5 hr, reaching the maximal cell density of $\sim 10^8$ cells/mouse. On the other hand, P-815 cells, derived from the Dunn-Potters mastocytoma, were grown in the abdominal cavity of DBA/2 mice (8).

Preparation of cells

The mastocytoma cells were harvested from the peritoneal cavity of tumor-bearing mice on the 7th day after transplantation ($1–3 \times 10^6$ cells/mouse) (7). Mastocytoma cells collected from peritoneal cavities of mice LAF_1 or DBA/2 were purified using a BSA density-gradient centrifugation according to the procedure of Sullivan et al. (9). Finally, cells were suspended in a buffered salt solution (150 mM NaCl, 3.7 mM KCl, 3.0 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 1.0 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5.6 mM dextrose, 0.1% gelatin, and 10 units/ml heparin, pH 6.8) (9). All glassware was siliconized before use. Purity of cell preparations was over 90% and the viability was 97% as assessed by staining with 0.05% toluidine blue.

Assays of histamine content and enzyme activities

Histamine content was measured fluorimetrically by the modification (9) of the method of Shore, Burkhalter, and Cohn (10). Acid phosphatase activity was measured photometrically, using *p*-nitrophenyl phosphate by the method of Parish (11) and N-acetyl-β-glucosaminidase was measured by the method of Loomis (12).

TLC solvent systems

Several solvent systems were used for separation of the cellular lipids and their derivatives by TLC: system I, chloroform–methanol–ammonium hydroxide 65:35:6 (v/v); system II, chloroform–acetone–methanol–glacial acetic acid–water 3:4:1:1:0.5 (v/v); system III, chloroform–glacial acetic acid–methanol–water 75:25:5:2.2 (v/v); system IV, n-hexane–diethyl ether 4:1 (v/v).

Extraction and fractionation of lipids

Lipids were extracted from two mastocytoma cell lines by the method of Bligh and Dyer (13). Individual phospholipids were separated by two-dimensional chromatography on silica gel H plates containing 2.5% magnesium acetate, using solvent system I for the first dimension and solvent system II for the second dimension (14). After visualization with a primulin spray reagent (15), the areas corresponding to individual phospholipids were extracted from the scraped gel powder by the method of Bligh and Dyer (13).

Quantitation of phospholipid species

The quantitative analysis of the individual phospholipid species was carried out after separating them by two-dimensional chromatography as above. In this system, lyso-PI and lysophosphatidylserine (lyso-PS) were located in a single spot, but other phospholipids were well separated. For quantitative analysis of individual phospholipids, each corresponding spot was scraped off the plate and analyzed for total phosphorus content as described by Rouser, Siakotos, and Fleischer (16). The determination of relative distribution of subfractions of choline- and ethanolamine-containing glycerophospholipid was performed as described in the following section.

Fatty acid composition analysis of major phospholipids

For analysis of fatty acyl composition of the individual phospholipids, they were separated on TLC, scraped off the plate, eluted with chloroform–methanol (9:1), transmethylated with 10% BF_3/CH_3OH, and then subjected to gas–liquid chromatography (GLC) (17) as described below.

Separation and quantitation of 1,2-diacyl-, 1-*O*-alkyl-2-acyl-, and 1-*O*-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphocholine (1-*O*-alk-1'-enyl-2-acyl-GPC) and *sn*-glycero-3-phosphoethanolamine (-GPE)

Purified choline- and ethanolamine-containing fractions (1–3 mg) were treated with HCl gas for 5 min to hydrolyze the 1-*O*-alk-1'-enyl groups. The cleaved alde-

hydes, lysophospholipids, and the unreacted phospholipids were purified by preparative TLC with solvent system III. The amount of lysophospholipids determined by lipid phosphorus analysis (16) was taken as the 1-*O*-alk-1'-enyl content. The cleavage of the 1,2-diacyl and 1-*O*-alkyl-2-acyl phospholipids was negligible under this condition. The unhydrolyzed phospholipid fraction was divided into two batches for phospholipase A_2 and phospholipase C treatment. The products obtained by phospholipase C hydrolysis were acetylated by the method of Soodsma, Leroy, and Harlow (18) to yield 1,2-diacyl-3-acetylglycerol and 1-*O*-alkyl-2-acyl-3-acetylglycerol. These two species were separated by preparative TLC on silica gel G using solvent system IV according to Renkonen's method (19) and they were scraped off separately and extracted with chloroform–methanol 9:1. A relative distribution (mol %) of 1-*O*-alk-1'-enyl-2-acyl (A), 1,2-diacyl (B), and 1-*O*-alkyl-2-acyl (C) phospholipids could be calculated as follows:

$$A\ (\text{mol}\ \%) = (\text{phosphorus content of lysophospholipid derived from 1-}O\text{-alk-1'-enyl phospholipid/phosphorus content of total choline- or ethanolamine-fractions}) \times 100.$$

The proportional ratio of 1,2-diacyl and 1-*O*-alkyl-2-acyl phospholipid was then estimated by GLC analysis of their fatty acid composition using 15:0 methyl ester as an internal standard (20). Accordingly, if the ratio of 1,2-diacyl to 1-*O*-alkyl-2-acyl phospholipids was defined as X/Y, then, B and C could be obtained according to the following equations.

$$B\ (\text{mol}\ \%) = X/(X + Y) \times (1\text{-}A) \times 100$$

and

$$C\ (\text{mol}\ \%) = Y/(X + Y) \times (1\text{-}A) \times 100.$$

Determination of positional fatty acid distribution of 1,2-diacyl phospholipids

The other half of unhydrolyzed phospholipid fraction was treated with phospholipase A_2 and the resulting products were purified using preparative TLC with solvent system III. The lysophospholipids were transmethylated with 10% BF_3/CH_3OH and the fatty acyl chain composition at the *sn*-1-position was determined by GLC. The acyl chain composition at the *sn*-2-position of 1,2-diacylphospholipids was estimated from the following equation:

$$2 \times (\text{mol}\%\ \text{overall composition in each fatty acid of 1,2-diacyl-3-acetate}) - (\text{mol}\%\ \text{composition in each fatty acid of phospholipid obtained from hydrolysis by phospholipase } A_2).$$

Determination of positional fatty acid distribution of 1-*O*-alkyl-2-acyl phospholipids

1-*O*-Alkyl-2-acyl-3-acetylglycerol (1–2 mg) was reduced with Vitride to yield 1-*O*-alkylglycerol, which was purified by preparative TLC. Its isopropylidene derivative was made by a modified method of Wood (21). One ml of acetone containing 0.1 μl of 70% perchloric acid was added to the sample. After the mixture was allowed to stand at room temperature for 20 min, 1 or 2 drops of concentrated NH_4OH were added, and the product was extracted three times with n-hexane-diethyl ether 1:1 (v/v). The isopropylidene derivatives were then analyzed by GLC. Fatty acyl chain composition of *sn*-2-position was determined as described above.

Determination of fatty acyl composition of 1-*O*-alk-1'-enyl-2-acyl phospholipids

Aldehydes formed from acid treatment of plasmalogen were reduced with Vitride to the corresponding alcohols (22). The fatty alcohols were then acetylated according to Waku et al. (23) for GLC analysis, and resulting lysophospholipid was treated as above for GLC analysis of the fatty acyl chain at the *sn*-2-position.

GLC analysis of fatty acids

The fatty acid methyl esters, fatty alcohol acetates, and isopropylidene derivatives of 1-*O*-alkylglycerol were determined by GLC. Peaks of each chromatogram were assigned by comparison of their relative retention times with those of authentic standards. Analysis was carried out on a Shimazu GC-6A gas chromatograph with a glass column packed with 10% DEGS on chromosorb W 80-100 mesh (Nihon Chromato Industrial Co., Tokyo). The flow rate of carrier nitrogen gas was approximately 50 ml/min. The column temperature was maintained at 200°C, and the injector and ion detector temperatures were 250°C.

RESULTS AND DISCUSSION

There has been substantial evidence to support the concept that the provocation of cell functions is coupled with a rapid increase in phosphoinositide metabolism (24). In this context, one would expect that the rapid and transient turnover of phosphoinositides may not be elicited in cells that do not exert active responses. We have chosen murine mastocytoma cells without active secretion as a pertinent model cell to study the stimulus–response coupling in the functionally defective cells. Our previous studies have shown that when mast cells are activated by calcium ionophore A23187 and compound 48/80, PI turnover and Ca^{2+} mobilization are rapidly enhanced (1, 2, 25). Before phospholipid metabolism in the mastocytoma cells is examined, detailed analytical studies of their phospholipid and fatty acid composition are necessary.

The mouse mast cells contain a large amount of histamine (157.8 $\mu g/10^7$ cells). In contrast, the histamine content in mastocytoma cells was extremely low (2.1 and 1.4

TABLE 1. Phospholipid composition of murine mastocytomas FMA3 and P-815[a]

Phospholipid	FMA3	P-815
Phosphatidylethanolamine	29.7 ± 1.9	29.0 ± 1.5
Phosphatidylserine	2.0 ± 2.1	4.7 ± 2.1
Phosphatidylinositol	6.7 ± 0.4	5.0 ± 1.0
Phosphatidylcholine	48.9 ± 3.6	48.3 ± 2.6
Phosphatidic acid	2.0 ± 1.2	1.1 ± 1.3
Sphingomyelin	6.0 ± 4.1	7.2 ± 1.5
Cardiolipin	3.9 ± 0.6	3.3 ± 3.2

[a]The results are expressed as mol percent of total phosphorus and presented as the mean ± standard deviation of four different animals.

$\mu g/10^7$ cells in FMA3 and P-815, respectively). Moreover, the activities of released lysosomal enzymes, N-acetyl-β-glucosaminidase and acid phosphatase, were also found to be very low (data not shown).

Phospholipid class composition of murine mastocytoma FMA3 and P-815 cells

Table 1 shows the phospholipid composition of murine mastocytoma cells. The total phospholipid content was 25 $\mu g/10^6$ cells in both lines. The cell size of mastocytomas (8 μm) is somewhat smaller than normal mouse mast cells (10 μm). No major difference was found in the phospholipid composition between the two cell lines. Half of the total phospholipid is choline-containing phospholipid fraction (PC), and an ethanolamine-containing phospholipid fraction (PE) is the next most dominant component (30%). Smaller constituents are sphingomyelin (SM), PI, cardiolipin, PS, and phosphatidic acid (PA). Apparently, the mastocytoma cells display a different profile of phospholipid composition from that of the mast cells (26), which contain almost equal amounts (27%) of PE and PC and are abundant in SM (20%).

The proportional composition of ether and diacyl types of PC and PE is shown in **Table 2**. Approximately two-thirds of total PC fraction consists of 1,2-diacyl type and the remainder are 1-*O*-alkyl and 1-*O*-alk-1'-enyl types. The 1,2-diacyl type is the major component of both PE and PC. Great differences between PC and PE are seen in the proportion of 1-*O*-alk-1'-enyl and 1-*O*-alkyl types. The 1-*O*-alk-1'-enyl-2-acyl type comprises more than 30% of the total PE fraction, whereas it is much less in the PC fraction. However, PC is more abundant in the 1-*O*-alkyl type than in the 1-*O*-alk-1'-enyl type. High concentrations of ether-linked lipids have been observed in other transformed cells such as Ehrlich ascites tumor cells (27) and hepatoma 7794A and 7777 cells (28).

Fatty acyl chain composition of individual phospholipid fractions

The distribution of fatty acids in individual phospholipids is given in **Table 3** (FMA3 cells) and **Table 4** (P-815 cells). Each phospholipid displays a characteristic distribution of fatty acyl chains, but there is no conspicuous difference between these two lines. As for saturated fatty acids, the choline-containing lipids, SM and PC, show a high level of 16:0, and the acidic phospholipids, PI and PS, are rich in 18:0. The presence of a considerable amount of arachidonate in PI is in good agreement with data on human platelets (14) and neutrophils (29). It is assumed that the abundance of 20:4 may contribute to production of leukotrienes through 5-lipoxygenase in mastocytoma P-815 (30). The dimethyl acetals (DMAs) are identified as 16:0DMA and 18:0DMA, which are presented primarily in PE, but much less in PC. SM is characterized by a high content of long chain fatty acids, 24:0 and 24:1.

Positional distribution of fatty acyl chains in 1,2-diacyl-, 1-*O*-alkyl-2-acyl-, and 1-*O*-alk-1'-enyl-2-acyl-GPC

The results of FMA3 and P-815 are listed in **Table 5** and **Table 6**, respectively. The *sn*-1-position of 1,2-diacyl-GPC is composed principally of two saturated fatty acids, 16:0 and 18:0. In contrast, the *sn*-2-position is abundant in polyunsaturated fatty acids, especially 18:1 and 18:2. The 1-*O*-alkyl type has a much larger amount of 18:0 compared to the diacyl and 1-*O*-alk-1'-enyl types. The latter two lipids are rich in 16:0. The *sn*-2-position is occupied by unsaturated fatty acids, and is characterized by high proportions of 20:4 in 1-*O*-alkyl type and of 24:0 and 24:1 in the 1-*O*-alk-1'-enyl type. It is to be noted that the 20:4 content in the 1-*O*-alkyl type is much greater in P-815 than in FMA3. The level of 24:0 and 24:1 is high in the

TABLE 2. Composition of choline- and ethanolamine-containing glycerophospholipids[a]

Component	Choline-Containing Glycerophospholipids		Ethanolamine-Containing Glycerophospholipids	
	FMA3	P-815	FMA3	P-815
1,2-Diacyl	62.6 ± 9.1	65.8 ± 4.8	55.0 ± 4.0	58.1 ± 0.6
1-*O*-Alkyl-2-acyl	30.8 ± 8.7	25.0 ± 4.8	11.5 ± 3.2	10.6 ± 0.5
1-*O*-Alk-1'-enyl-2-acyl	6.7 ± 1.5	7.8 ± 1.3	35.6 ± 4.7	31.2 ± 1.5

[a]The results are presented as the mean ± standard deviation of three different animals.

TABLE 3. Fatty acid composition of mastocytoma FMA3 phospholipids and individual phospholipid fractions[a]

	Total Phospholipid	PE[b]	PC[b]	PS[b]	PI[b]	SM[b]
			% of area			
14:0	0.4 ± 0.1	1.0 ± 0.4	1.1 ± 0.6	1.0 ± 1.6		2.0 ± 1.8
16:0DMA	0.7 ± 0.2	7.6 ± 2.6	1.0 ± 0.2			
16:0	20.3 ± 0.5	10.3 ± 1.5	24.5 ± 0.8	6.4 ± 2.4	3.9 ± 0.7	34.0 ± 3.9
16:1	0.7 ± 0.2	1.3 ± 0.4	1.4 ± 0.8	1.3 ± 1.4	0.3 ± 0.1	2.9 ± 0.2
18:0DMA	0.1 ± 0.1	5.4 ± 1.5	0.6 ± 0.1			
18:0	21.2 ± 1.3	22.7 ± 0.8	18.5 ± 1.3	48.3 ± 2.1	50.9 ± 3.8	4.8 ± 0.6
18:1[c]	19.2 ± 0.8	16.0 ± 0.6	24.3 ± 2.5	17.9 ± 1.8	4.0 ± 0.3	1.6 ± 0.9
18:2	13.3 ± 1.3	10.9 ± 1.2	16.5 ± 1.3	4.6 ± 0.8	5.0 ± 0.8	2.8 ± 1.0
20:1	1.9 ± 0.3	2.2 ± 0.4	2.4 ± 0.6	1.0 ± 0.2	0.3 ± 0.1	
20:4	8.3 ± 0.7	10.7 ± 1.1	4.5 ± 1.0	5.4 ± 3.0	23.2 ± 2.7	5.3 ± 1.4
20:5	0.8 ± 0.1			1.2 ± 0.4	2.0 ± 0.5	1.7 ± 0.2
22:0	1.1 ± 0.2	1.6 ± 0.2	1.6 ± 0.3	1.3 ± 0.3	1.4 ± 0.5	3.6 ± 0.7
22:1	1.1 ± 0.4	1.5 ± 0.3	1.2 ± 0.2	2.1 ± 0.3	4.0 ± 0.9	2.4 ± 0.1
22:5	0.4 ± 0.1					8.8 ± 0.3
22:6	8.5 ± 0.1	4.9 ± 1.9	1.8 ± 0.5	5.5 ± 2.5	2.1 ± 1.0	
24:0	1.2 ± 0.2	2.5 ± 0.8	0.8 ± 0.1	2.5 ± 0.9	2.9 ± 1.1	13.8 ± 4.0
24:1	1.5 ± 0.5			1.3 ± 0.9		16.3 ± 2.9

[a]The results are presented as mean ± standard deviation of three different animals and minor components less than 1% are not included in this table.

[b]Abbreviations: PE, ethanolamine-containing glycerophospholipids; PC, choline-containing glycerophospholipids; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; DMA, dimethyl acetal.

[c]This peak contains a small amount of 18:1DMA.

TABLE 4. Fatty acid composition of P-815 phospholipids and individual phospholipid fractions[a]

	Total Phospholipid	PE	PC	PS	PI	SM
			% of area			
14:0	0.5 ± 0.1	0.8 ± 0.3	1.2 ± 0.5	1.5 ± 1.5	0.6 ± 0.6	3.9 ± 2.8
16:0DMA	1.8 ± 0.6	8.9 ± 3.3	0.5 ± 0.1			
16:0	17.4 ± 0.8	10.7 ± 1.5	25.4 ± 2.9	6.6 ± 2.1	4.1 ± 0.8	37.4 ± 4.8
16:1	0.4 ± 0.4	0.7 ± 0.2	1.7 ± 0.8	1.8 ± 1.7	0.3 ± 0.1	2.6 ± 0.1
18:0DMA	0.3 ± 0.1	6.0 ± 0.2	0.2 ± 0.1			
18:0	18.7 ± 0.4	22.5 ± 2.0	18.7 ± 0.9	50.9 ± 5.7	55.2 ± 8.1	4.8 ± 0.6
18:1[b]	18.8 ± 0.7	15.6 ± 1.4	23.8 ± 2.5	19.2 ± 3.5	4.5 ± 1.0	2.7 ± 1.1
18:2	15.6 ± 1.0	11.1 ± 1.8	16.0 ± 1.7	5.1 ± 0.8	5.8 ± 1.6	0.8 ± 0.3
20:1	0.7 ± 0.4	0.6 ± 0.2	1.2 ± 0.3			
20:4	9.2 ± 0.2	10.9 ± 2.1	4.2 ± 1.9	4.9 ± 3.5	21.1 ± 4.6	3.8 ± 1.8
20:5	0.4 ± 0.1			1.0 ± 0.2		1.3 ± 0.3
22:0	2.7 ± 0.9	1.6 ± 0.3	1.5 ± 0.2	2.5 ± 1.1	1.2 ± 0.5	1.4 ± 0.2
22:1	2.2 ± 0.4	1.8 ± 0.4			4.0 ± 1.0	1.3 ± 0.1
22:5	1.5 ± 0.1			0.9 ± 0.5		8.9 ± 0.5
22:6	5.9 ± 0.2	4.8 ± 1.4	1.6 ± 0.6	4.9 ± 2.2	1.9 ± 0.9	
24:0	1.4 ± 0.1	2.8 ± 0.4	0.9 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	14.6 ± 5.0
24:1	2.0 ± 0.3					16.4 ± 2.7

[a]The results are presented as mean ± standard deviation of three different animals and minor components less than 1% are not included in this table.

[b]This peak contains a small amount of 18:1DMA.

TABLE 5. Positioning of fatty acids in choline-containing glycerophospholipids of mastocytoma FMA3[a]

	1,2-Diacyl		1-*O*-Alkyl-2-acyl		1-*O*-Alk-1'-enyl-2-acyl	
	Position 1	Position 2	Position 1	Position 2	Position 1	Position 2
			% of area			
14:0		2.7 ± 0.3			3.6 ± 1.5	0.7 ± 0.4
16:0	47.8 ± 3.9	9.4 ± 4.1	26.7 ± 3.5	30.7 ± 3.9	46.6 ± 2.2	17.5 ± 0.4
16:1				3.2 ± 0.6		2.4 ± 0.7
18:0	33.3 ± 2.5	2.4 ± 1.8	54.1 ± 6.0	6.0 ± 0.2	31.1 ± 3.3	10.1 ± 0.5
18:1	13.6 ± 2.5	31.8 ± 2.9	19.1 ± 6.4	10.9 ± 1.6	18.7 ± 1.7	9.4 ± 1.6
18:2	5.4 ± 0.3	24.1 ± 1.9		14.6 ± 6.0	1.5 ± 0.1	5.4 ± 0.8
20:0						2.9 ± 0.9
20:1		8.6 ± 1.7		10.4 ± 7.8		0.7 ± 0.2
20:2		2.6 ± 1.6				
20:3		1.0 ± 0.5				
20:4		9.1 ± 2.2		18.3 ± 1.3		9.3 ± 0.6
20:5		1.3 ± 0.7				
22:0		2.4 ± 0.2		0.5 ± 0.2		1.4 ± 0.3
22:6		1.0 ± 0.1		3.1 ± 0.5		4.8 ± 0.2
24:0		1.9 ± 0.5		2.3 ± 0.3		16.0 ± 1.9
24:1						16.5 ± 0.7

[a]The results are presented as mean ± standard deviation of three different animals and minor components less than 1% are not included in this table.

1-*O*-alk-1'-enyl type of FMA3 but 18:1 and 18:2 are high in the same lipid of P-815.

Positional distribution of fatty acyl chain in 1,2-diacyl-, 1-*O*-alkyl-2-acyl-, and 1-*O*-alk-1'-enyl-2-acyl-GPE

Positional analysis of the 1,2-diacyl-, 1-*O*-alkyl-2-acyl-, and 1-*O*-alk-1'-enyl-2-acyl-GPEs are shown in **Table 7** and **Table 8**. The *sn*-2-position of 1-*O*-alk-1'-enyl and 1-*O*-alkyl type is characteristic of a high percentage of 20:4, particularly in P-815 cells. It is known that the *sn*-2-position of 1-*O*-alk-1'-enyl-2-acyl-GPE in human platelets is occupied exclusively by polyunsaturated fatty acids (31). Indeed, 25% of total arachidonic acid in platelets is in 1-*O*-alk-1'-enyl-2-acyl-GPE. Mastocytoma cells produce the reacting substance of anaphylaxis through 5-lipoxygenase from arachidonic acid. These cells have a high level of 1-*O*-alk-1'-enyl-2-acyl-GPE (30–35%) rich in arachidonic acid at the *sn*-2-position. Therefore, it can be considered that 1-*O*-alk-1'-enyl-2-acyl-GPE may serve as an important source of arachidonic acid which is further converted to

TABLE 6. Positioning of fatty acids in choline-containing glycerophospholipids of mastocytoma P-815[a]

	1,2-Diacyl		1-*O*-Alkyl-2-acyl		1-*O*-Alk-1'-enyl-2-acyl	
	Position 1	Position 2	Position 1	Position 2	Position 1	Position 2
			% of area			
14:0		1.6 ± 0.4			7.4 ± 2.6	2.5 ± 1.8
16:0	38.7 ± 2.5	17.3 ± 4.5	28.9 ± 3.6	24.4 ± 3.8	56.4 ± 5.1	21.2 ± 0.8
16:1				3.5 ± 0.6		
18:0	45.0 ± 2.9	3.4 ± 2.7	52.5 ± 7.5	4.3 ± 0.2	22.1 ± 4.5	13.3 ± 1.8
18:1	13.2 ± 0.2	33.1 ± 3.2	18.6 ± 7.1	7.2 ± 2.0	12.7 ± 3.7	20.1 ± 1.0
18:2	3.1 ± 0.6	26.2 ± 7.0		14.0 ± 5.6	3.3 ± 1.6	15.8 ± 1.4
20:0				0.9 ± 0.2		1.5 ± 0.1
20:1		4.0 ± 0.6		3.2 ± 1.9		
20:2		2.5 ± 0.5				
20:4		6.8 ± 0.6		31.4 ± 1.9		7.0 ± 2.5
20:5				1.7 ± 0.2		
22:0				1.9 ± 0.7		3.7 ± 1.7
22:6		2.1 ± 1.3		2.2 ± 0.8		5.4 ± 0.9
24:0		3.1 ± 0.3		3.3 ± 0.7		2.7 ± 0.8
24:1						6.9 ± 2.4

[a]The results are presented as mean ± standard deviation of three different animals and minor components less than 1% are not included in this table.

TABLE 7. Positioning of fatty acids in ethanolamine-containing glycerophospholipids of mastocytoma FMA3[a]

	1,2-Diacyl		1-*O*-Alkyl-2-acyl		1-*O*-Alk-1'-enyl-2-acyl	
	Position 1	Position 2	Position 1	Position 2	Position 1	Position 2
			% of area			
14:0					6.3 ± 1.7	0.4 ± 0.1
16:0	33.7 ± 1.9	2.3 ± 1.3	18.7 ± 5.5	23.4 ± 1.5	53.2 ± 3.0	8.1 ± 0.7
16:1	0.8 ± 0.1			2.8 ± 0.6		1.5 ± 0.7
18:0	38.1 ± 4.4	21.3 ± 9.0	65.2 ± 8.6	5.2 ± 0.4	28.6 ± 3.9	10.1 ± 1.4
18:1	18.4 ± 2.9	33.6 ± 0.5	16.1 ± 3.0	12.1 ± 2.0	11.9 ± 0.3	15.4 ± 1.2
18:2	9.0 ± 1.9	18.5 ± 2.6		11.9 ± 0.3		12.6 ± 1.0
20:1		4.2 ± 1.5		10.9 ± 1.9		0.6 ± 0.1
20:2		1.1 ± 0.5				0.6 ± 0.1
20:3		1.1 ± 0.2				1.3 ± 0.1
20:4		5.3 ± 1.4		15.8 ± 2.4		26.6 ± 1.1
20:5		0.6 ± 0.1		2.6 ± 1.2		4.4 ± 0.4
22:0		5.1 ± 0.5		6.4 ± 1.5		
22:1						1.2 ± 0.4
22:6		1.8 ± 0.9		3.6 ± 1.4		10.5 ± 0.9
24:0		3.6 ± 0.5		3.4 ± 1.0		
24:1						4.1 ± 1.5

[a]The results are presented as mean ± standard deviation of three different animals and minor components less than 1% are not included in this table.

prostaglandins and leukotrienes (30).

Mastocytoma cells used in the present study appear to be inert with respect to stimulus-secretion coupling, since they have only a very low level of histamine content. Our preliminary results have indicated that active phospholipid turnover seems not to occur upon exposure to various stimulating agents. However, much more work is needed to account for the unresponsiveness of these mastocytoma cells. It is known that treatment with n-butyric acid induces histamine production and restores responsiveness to stimulating agents such as compound 48/80, ionophore A23187, and antigen. Therefore, detailed studies on the interrelationship between phospholipid metabolism and secretory events in the n-butyric acid-treated mastocytoma cells will provide useful information for a better understanding of the role of phospholipid

TABLE 8. Positioning of fatty acids in ethanolamine-containing glycerophospholipids of mastocytoma P-815[a]

	1,2-Diacyl		1-*O*-Alkyl-2-acyl		1-*O*-Alk-1'-enyl-2-acyl	
	Position 1	Position 2	Position 1	Position 2	Position 1	Position 2
			% of area			
14:0					2.2 ± 1.1	
16:0	26.8 ± 2.2	3.5 ± 0.4	11.6 ± 1.3	22.9 ± 1.0	50.7 ± 3.4	2.3 ± 0.3
16:1				1.4 ± 0.1		
18:0	50.0 ± 4.7	21.9 ± 3.9	50.9 ± 7.2	2.3 ± 0.6	38.5 ± 3.5	1.8 ± 0.3
18:1	15.2 ± 1.4	32.3 ± 3.2	37.5 ± 6.6	13.8 ± 0.8	6.6 ± 2.5	7.2 ± 0.4
18:2	8.0 ± 0.3	13.2 ± 3.2		18.2 ± 2.3	2.2 ± 0.6	10.2 ± 1.1
20:1		0.7 ± 0.4		5.1 ± 1.0		
20:2		1.5 ± 0.2				
20:3		1.4 ± 0.2				2.3 ± 0.1
20:4		12.3 ± 1.1		25.5 ± 6.0		40.0 ± 0.6
20:5		0.2 ± 0.1				4.8 ± 1.1
22:0		3.1 ± 1.4		7.0 ± 1.1		
22:1						3.3 ± 0.5
22:5						5.4 ± 0.4
22:6		5.6 ± 2.1		2.4 ± 1.8		20.0 ± 0.5
24:0		2.6 ± 0.2		1.4 ± 1.0		

[a]The results are presented as mean ± standard deviation of three different animals and minor components less than 1% are not included in this table.

turnover in stimulus-secretion coupling, and are currently being undertaken in our laboratory.

This study was supported in part by research grants from the Ministry of Education, Culture and Science, Japan.

Manuscript received 26 November 1984.

REFERENCES

1. Ishizuka, Y., A. Imai, S. Nakashima, and Y. Nozawa. 1983. Evidence for *de novo* synthesis of phosphatidylinositol coupled with histamine release in activated rat mast cells. *Biochem. Biophys. Res. Commun.* **111:** 581–587.
2. Ishizuka, Y., and Y. Nozawa. 1983. Concerted stimulation of PI-turnover, Ca^{2+}-influx and histamine release in antigen-activated rat mast cell. *Biochem. Biophys. Res. Commun.* **117:** 710–717.
3. Tharp, M. D., R. T. Suvurungsi, and T. J. Sullivan. 1983. IgE-mediated release of histamine from human cutaneous mast cells. *J. Immunol.* **130:** 1896–1901.
4. Lewis, R. A., and K. F. Austen. 1981. Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. *Nature.* **293:** 103–108.
5. Yanagisawa, M., H. Hasegawa, A. Ichiyama, S. Hosoda, and W. Nakamura. 1984. Comparison of serotonin-producing murine mastocytomas, P-815 and FMA3. Determination of tryptophan hydroxylase, aromatic L-amino acid decarboxylase, and cellular concentration of tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Biochem. Res.* **5:** 19–28.
6. Nakamura, W., R. Fujii, T. Nakayama, and Y. Nishimoto. 1976. Characteristiques d'une lignee cellulaire de mastocytome adaptee a la culture in vitro. *C. R. Soc. Biol.* **170:** 491–495.
7. Furth, J., P. Hagen, and E. I. Hirsch. 1957. Transplantable mastocytoma in the mouse containing histamine, heparin, 5-hydroxytryptamine. *Proc. Soc. Exp. Biol. Med.* **95:** 824–828.
8. Yanagisawa, M., H. Hasegawa, and A. Ichiyama. 1982. Tryptophan hydroxylase from mouse mastocytoma P-815. Reversible activation by ethylenediaminetetraacetate. *J. Biochem.* **92:** 449–456.
9. Sullivan, T. J., K. T. Parker, W. Stenson, and C. W. Parker. 1975. Modulation of cyclic AMP in purified rat mast cells. I. Responses to pharmacologic, metabolic, and physical stimuli. *J. Immunol.* **114:** 1473–1479.
10. Shore, P. A., A. Burkhalter, and Y. H. Cohn. 1959. A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127:** 182–186.
11. Parish, R. W. 1976. A labile acid phosphatase isozyme associated with the surface of vegetative *Dictyostelium discoideum* cells. *Biochim. Biophys. Acta.* **444:** 802–809.
12. Loomis, W. F., Jr. 1969. Acetylglucosaminidase, an early enzyme in the development of *Dictyostelium discoideum*. *J. Bacteriol.* **97:** 1149–1154.
13. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–918.
14. Imai, A., K. Yano, Y. Kameyama, and Y. Nozawa. 1981. Reversible thrombin-induced modification of positional distribution of fatty acids in platelet phospholipids: involvement of deacylation-reacylation. *Biochem. Biophys. Res. Commun.* **103:** 1092–1099.
15. Wright, R.S. 1971. A reagent for the non-destructive location of steroids and some other lipophilic materials on silica gel thin-layer chromatograms. *J. Chromatogr.* **59:** 220–221.
16. Rouser, G., A. N. Siakotos, and S. Fleischer. 1966. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids.* **1:** 85–86.
17. Watanabe, T., H. Fukushima, and Y. Nozawa. 1980. Studies on thermal adaptation in *Tetrahymena* membrane lipids. Positional distribution of fatty acid in diacyl- and alkyl-acyl-phosphatidylcholines and -(2-aminoethyl)phosphonolipids from cells grown at different temperatures. *Biochim. Biophys. Acta.* **620:** 133–141.
18. Soodsma, J. F., L. C. Leroy, and R. D. Harlow. 1976. The analysis of the molecular species of fetal rabbit lung phosphatidylcholine by consecutive chromatographic techniques. *Biochim. Biophys. Acta.* **424:** 159–167.
19. Renkonen, O. 1966. Individual molecular species of phospholipids. III. Molecular species of ox-brain lecithins. *Biochim. Biophys. Acta.* **125:** 288–309.
20. Sugiura, T., Y. Masuzawa, and K. Waku. 1980. Alkenyl and alkyl ether phospholipids in pig mesenteric lymph node lymphocytes. *Lipids.* **15:** 475–478.
21. Wood, R. 1967. GLC and TLC analysis of isopropylidene derivatives of isomeric polyhydroxy acids derived from positional and geometrical isomers of unsaturated fatty acids. *Lipids.* **2:** 199–203.
22. Snyder, F., M. Blank, and R. L. Wykle. 1971. The enzymic synthesis of ethanolamine plasmalogens. *J. Biol. Chem.* **246:** 3639–3645.
23. Waku, K., H. Ito, T. Bito, and Y. Nakazawa. 1974. Fatty chain of acyl, alkenyl, and alkyl phosphoglycerides of rabbit sarcoplasmic reticulum. *J. Biochem.* **75:** 1307–1312.
24. Berridge, M. J. 1984. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* **220:** 345–360.
25. Imai, A., Y. Ishizuka, S. Nakashima, and Y. Nozawa. 1984. Differential activation of membrane phospholipid turnover by compound 48/80 and ionophore A23187 in rat mast cells. *Arch. Biochem. Biophys.* **232:** 259–268.
26. Strandberg, K., and S. Westerberg. 1976. Composition of phospholipids and phospholipid fatty acids in rat mast cells. *Mol. Cell. Biochem.* **11:** 103–107.
27. Wood, R., and F. Snyder. 1969. Tumor lipids: metabolic relationships derived from structural analyses of acyl, alkyl, and alk-l-enyl moieties of neutral glycerides and phosphoglycerides. *Arch. Biochem. Biophys.* **131:** 478–494.
28. Snyder, F., M. L. Blank, and H. P. Morris. 1969. Occurrence and nature of O-alkyl and O-alk-l-enyl moieties of glycerol in lipids of Morris transplanted hepatomas and normal rat liver. *Biochim. Biophys. Acta.* **176:** 502–510.
29. Yano, K., H. Hattori, A. Imai, and Y. Nozawa. 1983. Modification of positional distribution of fatty acids in phosphatidylinositol of rabbit neutrophils stimulated with formylmethionyl-leucyl-phenylalanine. *Biochim. Biophys. Acta.* **752:** 137–144.
30. Koshihara, Y., M. Mizumura, and S. Murota. 1982. Predominant synthesis of 5-hydroxyeicosatetraenoic acid by a cloned mastocytoma P-815 line, 2-E-6 cells. *Biochim. Biophys. Acta.* **712:** 42–47.
31. Kramer, R. M., and D. Deykin. 1983. Arachidonoyl transacylase in human platelets. Coenzyme A-independent transfer of arachidonate from phosphatidylcholine to lysoplasmenylethanolamine. *J. Biol. Chem.* **258:** 13806–13811.