Molecular species of mono-, di-, and triphosphoinositides of bovine brain

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ABSTRACT The mono-, di-, and triphosphoinositides of bovine brain were isolated by chromatography on columns of DEAE-cellulose, alumina, and silicic acid. The major molecular species in each phosphoinositide class were identified and quantitatively estimated by combined thin-layer and gas–liquid chromatography of the component diglycerides, which were released by hydrolysis with a specific brain phosphodiesterase. The diglycerides were treated with pancreatic lipase, and the positional distribution of the fatty acids was determined.

Over 27 molecular species were identified, and these accounted for about 95% of each phosphoinositide class, but the 1-stearate 2-arachidonate derivative contributed more than 40% of the total in each class. The other molecular species also were qualitatively and quantitatively similar in the three phosphoinositide classes. All the long-chain and polyunsaturated acids were confined to the 2-position and were preferentially paired with stearic acid in the 1-position. Oleic acid in the 2-position was about equally divided between species with palmitic and stearic acids in the 1-position.

These results suggest that the mono-, di-, and triphosphoinositides of the bovine brain have similar compositions and that the various molecular species may be metabolically related.

SUPPLEMENTARY KEY WORDS fatty acids · positional distribution · diglycerides · diglyceride acetates

RECENT studies have revealed that natural lecithins and cephalins are made up of characteristic molecular species (1), which are metabolized at distinctly different rates (2–4). Due to analytical difficulties it has not been possible to obtain evidence of the generality of this phenomenon in other glycerophosphatides, such as phosphatidylserine and phosphatidylinositols.

The treatment of phosphoinositides with a specific brain phosphodiesterase (5) of high activity makes it possible to isolate the diglyceride moieties, which can then be subjected to detailed chromatographic fractionation and quantitation. In the present work we have identified and quantitatively estimated the molecular species of the mono-, di-, and triphosphoinositides of bovine brain.

MATERIALS AND METHODS

The methods and general experimental conditions were similar to those previously described (6, 7), except as noted below.

Preparation of Phosphoinositides

Phosphoinositides were prepared from ox brain obtained fresh from the slaughterhouse and transported to the laboratory on dry ice. Tri- and diphosphoinositides were isolated by a modified version of the procedure of Hendrickson and Ballou (8). The inositide-rich fraction of Hendrickson and Ballou was precipitated five times from chloroform by the addition of methanol; it was then dissolved in 500 ml of chloroform-methanol 1:1 (v/v). The lipids were converted to the free acid form by vigorous shaking with 100 ml of 1 N HCl. The two phases were allowed to separate, the upper phase was discarded, and the lower chloroform phase was taken to dryness in

Abbreviations: MPI, monophosphoinositides; DPI, diphosphoinositides; TPI, triphosphoinositides; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; C_{14}–C_{22}, fatty acids with 14–22 carbon atoms; C_{32}–C_{46}, diglyceride acetates with a total number of fatty acid carbon atoms (including those of acetic acid) of 32–46. Fatty acids designated by number of carbon atoms: number of double bonds: number of carbons from the last double bond to end of chain.

a rotary evaporator. The lipid residue was dissolved in a minimal volume (about 5 ml) of chloroform, and 7 volumes of methanol were added. The solution was left for 2 hr at −15°C, and the small precipitate that formed was centrifuged down and discarded. The methanolic solution was held in an ice bath, and 0.1 N NaOH in methanol was carefully added to neutralize and precipitate the lipids as sodium salts. The precipitate was collected by centrifugation and redissolved in chloroform–methanol–water 20:9:1 (v/v/v) in the appropriate dilution for loading onto the DEAE-cellulose column. The linear gradient elution was begun with 1700 ml of chloroform–methanol–water 20:9:1 (v/v/v) in the mixing chamber and 1700 ml of 0.54 M ammonium acetate in chloroform–methanol–water in the solvent reservoir. The less steep gradient improved the separation of di- and triphosphoinositides. Hendrickson and Ballou (8) found that, in addition to the major triphosphoinositide peak which was eluted with approximately 0.5 M ammonium acetate, a minor peak containing, presumably, a mixed calcium and magnesium chelate of triphosphoinositide was rapidly eluted in approximately 0.1 M salt. No such early component was observed in the modified procedure, and it is likely that the preliminary treatment with acid, in addition to saving considerable time, was more effective in removing divalent cations from the preparation. The characterization and chromatographic purity of the lipids were as previously described (5). Triphosphoinositide appeared as a single spot on TLC. The diphosphoinositide preparation contained less than 5% of triphosphoinositide.

For the isolation of monophosphoinositide, 1 kg of ox brain was extracted with a total volume of 4 liters of chloroform–methanol 1:1 (v/v), and the mixture was centrifuged. The lipid extract was taken to dryness under reduced pressure. The residue was dissolved in 500 ml of chloroform–methanol 2:1 (v/v), and the solution was shaken with 100 ml of 0.9% NaCl; the aqueous phase was discarded. The lipids were applied to a column of alumina (1 mg of phosphorus per g of alumina) in chloroform–methanol 1:1, and eluted as described by Dawson (9). The inositide-rich fraction, which was eluted with ethanol–chloroform–water 5:2:2 (v/v/v), was taken to dryness, redissolved in chloroform–methanol 4:1 (v/v), and adsorbed on a column of silicic acid (10). The monophosphoinositide was eluted in chloroform–methanol 3:2 (v/v), and samples were examined by TLC (11), and by paper chromatography and electrophoresis of the water-soluble phosphate esters obtained by mild alkaline hydrolysis (12). The preparation was found to be contaminated with a ninhydrin-positive material with properties consistent with those of lysophosphatidylethanolamine. This was completely removed from the monophosphoinositide by separation on a column of DEAE-cellulose (Whatman DE-32), acetate form, essentially as described by Rouser, Kritchevsky, Heller, and Lieber (13). The lysophosphatidylethanolamine was eluted with chloroform–methanol 7:3 (v/v), and the monophosphoinositide with chloroform–methanol 4:1 (v/v) containing 10 ml of concentrated NH_4OH per liter. The monophosphoinositide contained phosphorus, inositol, and free fatty acids in the molar proportions 1:0.97:1.96 and traveled as a single spot on thin-layer chromatograms.

Hydrolysis of Phosphoinositides

Triphosphoinositide was hydrolyzed to a diglyceride and a water-soluble inositol triphosphate as previously described (5) by a specific phosphodiesterase present in high concentration in aqueous extracts from acetone powders of ox brain. The conditions were modified for diphosphoinositide degradation as follows: duplicate samples of diphosphoinositide (26.3 mg) were incubated in 42 ml of 0.05 M Tris-HCl buffer (pH 7.2) containing brain extract (9.5 mg of protein) and 0.025% cetyltrimethylammonium bromide at 37°C for 60 min. Since the activity of the ox brain extracts was somewhat variable with respect to monophosphoinositide, this lipid was hydrolyzed by the enzyme obtained from the supernatant fraction of a rat brain homogenate (14). Duplicate samples of monophosphoinositide (17.5 mg) were incubated in 30 ml of 0.05 M acetate buffer (pH 5.4) containing brain extract (6.2 mg of protein), 0.001 M $CaCl_2$, and 0.01 M cysteine at 37°C for 90 min. In each case, after incubation, the diglycerides were removed by extracting the incubation mixture four times with 40 ml of diethyl ether. Samples of the aqueous phase were analyzed for water-soluble phosphates (15) to determine the percentage hydrolysis of the substrates. It was calculated that each lipid was hydrolyzed to the extent of 60–70%.

Preparation and Resolution of Diglyceride Acetates

The diglycerides were purified by TLC and acetylated as previously described (6). The acetates were resolved on the basis of total number and distribution of double bonds per molecule by argentation TLC (6, 7). The diglyceride acetates corresponding to the various groups of unsaturation were separately recovered by suspending the gel scrapings in diethyl ether–methanol–acetic acid 60:40:1 (v/v/v) (0.5 g/5 ml), diluting the mixture to 25% water, and extracting the three-phase suspension with an equal volume of petroleum ether (three times). The ratios of the diglycerides among and within the various bands were determined by GLC (6, 7) using tridecanoin (50–300 μg) as an internal standard.

Analysis of Fatty Acids

The fatty acids were quantitatively estimated by GLC following methylation or transmethylation (6, 7). The identities of the unsaturated acids were confirmed by argentation TLC and hydrogenation. The positional distribution of the fatty acids in the mono-, di-, and triphosphoinositides was determined by analysis of the acids which were released when the corresponding diglycerides (7) were treated with pancreatic lipase. The acid distribution in the various diglyceride acetates recovered from the silver nitrate fractionation was determined in a similar manner. The positional placement of the fatty acids in the tri- and diphosphoinositides found by pancreatic lipase agreed well with that obtained previously (5) for the intact phosphoinositides in experiments with phospholipase A_2 of bee venom. In all cases, the reaction products as well as any unreacted starting materials were purified and recovered by TLC, prior to GLC analysis of the fatty acids.

Calculations

The structure of the original phosphoinositides was determined by proportional summation and normalization of all the analytical data. The accuracy of the final result was estimated by matching the mole percentage composition, and positional distribution of the fatty acids in the original phosphatides, against those determined for the derived acetates. In several instances the experimental data were compared with random distribution data which were derived by calculation (6). The three types of phosphoinositides were compared with respect to the positional distribution and molecular association of the fatty acids as described previously for phosphatidylcholine and phosphatidylethanolamine of egg yolk (7).

RESULTS

The yields of phosphoinositides obtained from 1 kg of ox brain were 550, 400, and 500 μmoles, respectively, for the tri-, di-, and monophosphoinositides. Wells and Dittmer (16) found concentrations of 500–800 μmoles/kg for triphosphoinositide and 650–1000 μmoles/kg for diphosphoinositides, depending on the extraction procedures used. Dawson and Eichberg (17) have reported values of 615 μmoles/kg for triphosphoinositide and 45 μmoles/kg for diphosphoinositide. These discrepancies emphasize the problem of postmortem autolysis as well as the consequences of the procedures employed to extract fresh brain. No comparable values appear to be available for the monophosphoinositide content of ox brain. Our yield is likely to be low since the isolation procedure involved three separate column steps, and narrow cuts of the column eluates were taken to reduce contamination with other fractions. Because of the large amounts of brain required to prepare the lipids in substrate quantities and the relative inefficiency of some of the methods of enrichment, the monophosphoinositides and polyphosphoinositides came from different brain samples.

In view of the rapid autolysis and interconversion of the phosphoinositides that may occur during extraction and the different methods employed for their isolation, we cannot be absolutely certain that the final preparation represented all the native molecular species.

Fatty Acid Composition

Table 1 gives the over-all composition and the positional distribution of the fatty acids of the three phosphoinositide classes. All are characterized by a high content of stearic and arachidonic acids, which is similar to the findings of others (5, 8, 18) for the di- and triphosphoinositides. The arachidonate is specifically found in position 2, while the stearate is attached to position 1 of the glyceride residue as also noted previously (5). Oleic acid is the other major unsaturated acid in these phosphatides; it is located in both positions 1 and 2. All phosphoinositides also contained significant amounts of eicosatrienoic acid, which was made up of nearly equal proportions of the 5, 8, 11 ($n-9$) and the 8, 11, 14 ($n-6$) isomers. Both acids occurred exclusively in the 2-position. The identification of the two trienoic acids in the phosphoinositides of the bovine brain coincides with the findings in the human brain (19). There are minor differences in the relative amounts of the trienoic fatty acids in the three phosphoinositides, but the importance of these discrepancies remains to be established in view of the differences in the origin of the samples.

Table 2 gives the fatty acid composition of the silver nitrate subfractions of the diglycerides derived from the mono-, di-, and triphosphoinositides. The estimated molar proportions of the acids are seen to approximate closely the ratios required for the pairing of all components according to the degree of unsaturation of the subfraction. The diglyceride moieties analyzed were free of alkenyl ethers as indicated by the absence of dimethylacetal peaks from the gas chromatograms of the fatty acids. This is supported by the nearly theoretical proportions of phosphorus, inositol, and fatty acids in these phosphatides. Appreciable quantities of plasmalogens are found in ox brain, but these appear to be largely confined to the ethanolamine phosphoglycerides (20).

Molecular Weight Distribution

Table 3 gives the molecular weight distribution of the total mono-, di-, and triphosphoinositides of the bovine brain as estimated by GLC of derived diglyceride acetates. In all phosphatide classes the major constituents

TABLE 1 Composition and Positional Distribution of Fatty Acids of the Mono-, Di-, and Triphosphoinositides of Ox Brain

Fatty Acids	Monophosphoinositides				Diphosphoinositides				Triphosphoinositides			
	Total	1-*	2-†	Reconst.‡	Total	1-*	2-†	Reconst.	Total	1-*	2-†	Reconst.
						moles %						
14:0	tr.	0.1		tr.	tr.	0.1		tr.	tr.	0.3		0.1
16:0	7.9	14.6		7.3	5.2	8.6		4,3	4.3	6.6		3.3
16:1	0.2		0.8	0.4	0.5		0.8	0.4	tr.		0.2	0.1
18:0	37.7	74.4		37.2	37.3	69.3		34.7	36.8	68.9		34.5
18:1	10.0	9.9	10.4	10.2	14.5	20.2	12.7	16.5	14.0	21.1	10.3	15.7
18:2	1.2	0.8	1.8	1.3	1.2	0.3	0.8	0.6	0.6	1.0	1.2	1.1
20:1§	0.5	0.2	1.2	0.7	1.5	1.0	1.1	1.0	1.2	1.7	1.6	1.6
20:2§	0.7		2.4	1.2	1.7	0.5	3.1	1.8	2.0	0.4	4.4	2.4
20:3($n-9$)	2.2		4.7	4.9	4.9		9.8	10.4	5.0		10.0	10.8
20:3($n-6$)	2.6		5.2		5.0		11.0		5.4		11.6	
20:4($n-6$)	33.8		67.0	33.5	22.5		48.9	24.4	26.4		51.5	25.7
22:2§	tr.				0.5		1.4	0.7	1.1		2.2	1.1
22:3	2.2		6.6	3.3	5.5		10.1	5.0	3.1		6.5	3.3
22:4	tr.				tr.		0.3	0.2	0.1		0.5	0.3
22:5($n-5$)	tr.				tr.				tr.			
22:6($n-3$)	1.0		tr.	tr.	tr.		tr.	tr.	tr.		tr.	tr.

* Free fatty acids released by pancreatic lipase.
† Fatty acids from the monoglycerides formed by action of pancreatic lipase on the diglyceride.
‡ Reconstituted composition = (1-acids + 2-acids)/2.
§ Tentative.

TABLE 2 Fatty Acid Composition of the Diglyceride Acetates of the Phosphoinositides of Ox Brain*

Fatty Acids	Monoenes			Dienes			Trienes			Tetraenes			Pentaenes			Hexaenes
	MPI	DPI	TPI	MPI	DPI	TPI	MPI	DPI	TPI	MPI	DPI	TPI	MPI	DPI	TPI	MPI
								moles %								
14:0	0.5	0.5	2.5													
16:0	27.5	23.0	23.0	19.0	1.5	3.0	4.5	2.0	2.0	4.0	2.5	2.0				tr.
16:1	3.0	2.5		1.0	2.0	0.5										
18:0	22.0	26.5	24.5	15.5	21.5	21.5	45.5	44.0	42.5	41.5	37.0	41.5				tr.
18:1	44.0	45.5	47.0	24.0	44.0	39.5		4.0	5.5	4.5	10.5	6.5	33.5	36.0	40.0	
18:2				15.0	4.0	4.5		0.5	0.5				16.5	4.0	5.5	tr.
20:1	3.0	2.0	3.0	6.0	8.0	11.0								4.0	2.5	
20:2				19.5	19.0	20.0								6.0	2.0	
20:3($n-9$)+($n-6$)							50.0	46.0	44.5	0.5	2.5	1.5				
20:4($n-6$)										45.5	39.5	43.5	33.5	36.0	40.0	tr.
22:2								3.5	5.0							
22:3										4.0	8.0	5.0	16.5	10.0	7.5	
22:4														4.0	2.5	
22:6($n-3$)																tr.

* Diglyceride acetates resolved by TLC on silver nitrate-treated plates. Each fatty acid reported as a percentage of saturation class.

TABLE 3 Molecular Weight Distribution of Mixed Diglyceride Acetates Derived from Ox Brain Phosphoinositides

Carbon Number	Monophosphoinositides			Diphosphoinositides			Triphosphoinositides		
	Original*	Reconst.†	Random‡	Original	Reconst.	Random	Original	Reconst.	Random
					moles %				
34	0.6	0.3	0.7	tr.	0.1	0.3	tr.	0.3	0.2
36	7.0	6.3	8.0	4.8	5.3	6.1	2.4	3.3	4.4
38	15.7	14.0	30.5	14.2	13.0	32.1	11.2	11.4	29.9
40	69.6	72.6	39.7	66.0	69.1	38.1	71.1	71.5	41.5
42	7.1	6.8	19.1	13.0	11.7	18.8	13.5	12.6	20.4
44			1.9	2.0	0.8	4.2	1.8	0.9	3.4
46			0.1			0.4			0.2

* As estimated by direct GLC of the total diglyceride acetates before and after hydrogenation.
† Estimates of reconstituted composition obtained by algebraic summation and normalization of data from argentation TLC.
‡ Random distribution determined as previously described (6).

are those with one C_{18} and one C_{20} (C_{40}), with two C_{18} (C_{38}), and with one C_{18} and one C_{22} (C_{42}) fatty acid per molecule. The similarities in the proportional contributions of the phosphoinositides of the three types reflect similarities in both the fatty acid composition and the pairing. The finding of large amounts of the species containing C_{18} and C_{20} acids was expected in view of the high proportion of stearic and arachidonic acids in these phosphatides. There exists, however, considerable selectivity in the over-all association of the fatty acids in the phosphatide molecules as shown by the difference between the experimental and the random values derived by calculation from the fatty acid composition. Table 3 also includes the reconstitution values obtained for the diglyceride acetates by algebraic summation and normalization of the data from argentation TLC. The close agreement between these and the original total indicates that the execution of the detailed analytical procedure was not accompanied by significant losses of the polyunsaturated acids due to autoxidation.

Table 4 gives the molecular weight distribution of the major diglyceride acetates recovered from the silver nitrate fraction for each class of phosphoinositides, along with the random values calculated on the basis of the fatty acid composition of the appropriate fractions. It is seen that only a few major diglyceride acetates or phosphatidylinositols occur in any one group of molecules having a given degree of unsaturation. This is true for all three phosphoinositide classes, and is in contrast with the random values, which usually cover a wider range of molecular weights (Table 3).

The proportions of the different unsaturation groups in the total phosphatide mixture, however, show some variation. Thus, while the monoenes make up about 10% of the mono- and diphosphoinositides, the triphosphoinositides contain only 6% of this fraction. In contrast, both tri- and diphosphoinositides contain more than twice as much trienes as do the monophosphoinositides. The triphosphoinositides contain about three times as much pentaenes as the monophosphoinositides and twice as much as the diphosphoinositides. Although the saturated fatty acids accounted for 41–46% of the total, no fully saturated species were recognized in any of the phosphoinositide classes. Calculations indicated

TABLE 4 MAJOR DIGLYCERIDE ACETATES DERIVED FROM THE PHOSPHOINOSITIDES OF OX BRAIN

Degree of Saturation*	Carbon Number	Monophosphoinositides		Diphosphoinositides		Triphosphoinositides	
		Experimental	Random	Experimental	Random	Experimental	Random
				moles %			
Saturates	32						
	34	tr.	3.0	tr.	1.5		1.1
	36		28.6 20.8		21.5 18.1		18.7 16.9
	38		68.4		77.0		80.2
Monoenes	34	3.0	0.2	1.0	0.4	5.0	
	36	53.0	17.7	49.0	13.4	45.0	9.6
	38	42.0 10.5	78.1 9.8	48.0 10.1	78.2 14.0	46.0 6.4	83.3 12.5
	40	2.0	3.9	2.0	8.0	4.0	7.1
	42				0.1		
Dienes	36	12.0	8.0	5.0	4.8	4.0	1.1
	38	48.0 6.1	70.1 2.9	51.0 8.1	56.8 5.6	48.0 11.1	56.7 4.5
	40	37.0	21.8	36.0	31.3	37.0	41.9
	42	3.0	0.1	8.0	7.0	11.0	0.3
Trienes	36		0.1		0.1		
	38	9.0	14.8	5.0	9.8	5.0	7.3
	40	91.0 9.2	60.6 6.8	88.0 19.5	59.7 14.2	85.0 22.6	74.4 14.6
	42		24.5	7.0	30.3	10.0	18.1
	44				0.1		0.2
Tetraenes	36						
	38	8.0	16.6	5.0	10.1	4.0	6.8
	40	84.0 71.8	81.8 32.4	79.0 58.3	81.1 24.1	86.0 50.3	89.1 33.4
	42	8.0	1.5	16.0	8.0	10.0	3.7
	44		0.1		0.8		0.4
Pentaenes	38		1.8		2.6		
	40	67.0	91.7	72.0	79.7	80.0	7.5
	42	33.0 2.5	6.1 7.5	8.0 3.9	13.6 8.4	11.0 9.6	67.0 1.7
	44		0.4	20.0	3.4	9.0	21.4
	46				0.7		4.1
Hexaenes	40		36.1		13.7		8.4
	42	tr.	54.3 2.7	tr.	44.7 3.9	tr.	56.5 3.8
	44		7.8		33.8		32.5
	46		1.8		7.8		2.6

* Each acetate reported as a percentage of saturation class, and each saturation class as a percentage of total acetate mixtures. Saturation classes were obtained by $AgNO_3$-TLC of glycerides.

that as much as 20% of saturated phosphatides could have occurred by a random association of the fatty acids in these glycerol esters. The random estimates for the other saturation classes were similarly quite different from the experimental values which illustrate the high degree of specificity noted for most fatty acid pairings.

DISCUSSION

On the basis of the analytical data presented above we reconstituted the original composition of the three classes of phosphoinositides. Table 5 lists the major individual monophosphoinositides as well as their mono- and diphospho derivatives. The molecular species have been specified as previously explained (6). The species with reversed fatty acid placement occur in much smaller amounts, but can be estimated from the fatty acid distributions reported in Table 1. The estimates were derived by reconstitution of the over-all molecular weight distribution (Table 3), the molecular weight distribution of the subfractions (Table 4), and the fatty acid composition of the subfractions (Table 2). On the basis of the data given in Tables 2–4 and the knowledge of the positional placement of the fatty acids, it can be seen that the monounsaturated inositides in each phosphatide class are largely made up of the palmitoyl and stearoyl oleates, which occur in nearly equal proportions, although the combined total in the triphosphoinositides is only about one-half of the total in the mono- and diphosphoinositides. In view of the relatively small total amount of palmitic compared with stearic acid in these phosphatides, the finding of equal proportions of palmitic and stearic acid species in the monoenes represents a preferential enrichment of palmitic acid in this fraction.

TABLE 5 MAJOR PHOSPHOINOSITIDES OF THE BRAIN*

Chemical Classes	Fatty Acids 1-†	Fatty Acids 2-‡	Phosphoinositides Mono	Di	Tri
			moles %		
Monoenes	14:0	18:1	0.10	0.10	0.32
	16:0	16:1	0.21		
	16:0	18:1	5.13	4.46	2.83
	18:0	16:1	0.42	0.51	0.06
	16:0	20:1	0.42	0.20	0.13
	18:0	18:1	3.97	4.66	2.83
	18:0	20:1	0.21	0.20	0.26
Dienes	16:0	18:2	0.61	0.08	0.33
	18:1	16:1	0.12	0.33	0.11
	18:1	18:1	1.22	3.42	4.34
	18:0	18:2	1.22	0.56	0.67
	16:0	20:2	0.49	0.16	0.33
	18:0	20:2	1.88	2.93	4.12
	18:1	20:1	0.36		
	20:1	20:1	0.18	0.65	1.23
Trienes	16:0	20:3	0.83	0.78	0.90
	18:1	18:2		0.20	0.23
	18:0	20:3	8.35	17.16	19.18
	18:1	22:2		1.37	2.26
Tetraenes	16:0	20:4	5.74	2.92	2.01
	18:0	20:4	59.58	43.15	41.73
	18:1	20:3	0.72	2.92	1.51
	18:1	22:3	5.74	9.33	5.03
Pentaenes	18:1	20:4	1.68	2.81	7.66
	18:2	22:3	0.83	0.31	1.05
	20:2	22:3		0.47	0.38
	20:1	22:4		0.31	0.48
Hexaenes	16:0	22:6	tr.		
	18:0	22:6	tr.		
	18:2	20:4	tr.		

* Each molecular species reported as a percentage of the total phosphatide.

† Fatty acids released by pancreatic lipase.

‡ Fatty acids from monoglycerides released by pancreatic lipase.

The major dienes in all three phosphatide classes were made up of the dioleoyl and the 1-stearoyl 2-eicosadienoyl species, which occurred in nearly equal proportions in any one phosphatide, although the total differed in each of the phosphatides. Only the monophosphoinositides contained a significant proportion of 1-stearoyl 2-linoleoyl species. The small amount of the 1-palmitoyl 2-linoleoyl species in this inositide was in keeping with the proportion of palmitic acid in the total saturated acids.

The major triene in all phosphoinositides was 1-stearoyl 2-eicosatrienoyl species, which was made up of nearly equal proportions of the two isomers. The monophosphoinositides, however, contained only one-half the amount of this triene found in the di- and triphosphoinositides.

The main tetraene in each phosphatide was the 1-stearoyl 2-arachidonoyl species. In addition, the monophosphoinositide also contained significant amounts of the 1-palmitoyl 2-arachidonoyl species as well as the 1-oleoyl 2-docosatrienoyl species. The diphosphoinositides had particularly high levels of the latter species.

The pentaenes were the most complex of all the fractions examined. The data in Table 5 give only a partial indication of the complexity, as many species had to be deleted because their proportion of the total was so minor. Nevertheless, in all three phosphatides, the 1-oleoyl 2-arachidonoyl species could be recognized as the major component; it was most abundant in the triphosphoinositides.

These data show that the phosphoinositides possess glyceride structures comparable with those identified for other natural glycerophosphatides (1, 4, 6, 7). Furthermore, the three inositides are made up of molecular species which are qualitatively very similar. This would be anticipated in view of the demonstrated origin of the di- and triphosphoinositides as products of further phosphorylation of the monophosphoinositides (21). Since the proportions of some molecular species in the different phosphatides differ significantly, it would appear that not all species of the monophosphoinositides were con-

TABLE 6 ASSOCIATION OF PALMITIC AND STEARIC ACIDS WITH UNSATURATED FATTY ACIDS IN THE PHOSPHOINOSITIDES OF OX BRAIN*

Molecular Species	Major Unsaturated Acid	Major Saturated Acid			Degree of Preference for Palmitic Acid*		
		MPI	DPI	TPI	MPI	DPI	TPI
Monoenes	18:1	16:0, 18:0	16:0, 18:0	16:0, 18:0	6.2	6.9	8.5
Dienes	20:2	18:0	18:0	18:0	1.2	0.4	0.7
Trienes	20:3	18:0	18:0	18:0	0.5	0.3	0.4
Tetraenes	20:4	18:0	18:0	18:0	0.5	0.5	0.4

* Degree of preference = $(16:0/18:0)_{class}/(16:0/18:0)_{total}$. Nonpreferential association = 1.0 ± 0.1 (assuming a relative error of ±10%). Other legends as in Table 5.

verted to the higher phosphates to the same extent. Alternatively, the difference in the quantitative composition could have resulted from a rapid postmortem degradation of the polyphosphoinositides. In the latter case, however, a differential hydrolysis would have to be implicated to account for the discrepancies.

In view of this uncertainty, we compared the molecular species in the three inositides on the basis of the nature of the major fatty acids esterified and the degree of preference shown (7). It was hoped that such a comparison might reveal similarities among the phosphatides that are no longer perceptible due to secondary changes in the composition of the molecular species. The degree of preference was calculated by setting up a characteristic ratio of proportions as shown in Table 6, which also gives the results of some of the associations thus tested. It is seen that in all three phosphoinositides, for each molecular species there is a strikingly similar pairing of the major unsaturated fatty acid with a particular saturated acid. All the phosphatides specifically combine palmitic and oleic acids in the monoenes although there are significant amounts of stearic and oleic acid pairs. In contrast, stearic acid is predominant over palmitic acid in the formation of the dienes, trienes, and tetraenes of the corresponding eicosaenoic acids.

The present findings are consistent with the concept that polyphosphoinositides originate from the corresponding monophosphoinositides, but that rates of phosphorylation of different species may be different. These conclusions require further confirmation by metabolic experiments.

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