Molecular species composition of the major phospholipids in brain and retina from rainbow trout (*Salmo gairdneri*)

Occurrence of high levels of di-($n-3$)polyunsaturated fatty acid species

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The molecular-species compositions of the diacyl classes of the major phospholipids from the brain and retina of rainbow trout (*Salmo gairdneri*) were determined. A total of 46 possible species was identified. Didocosahexaenoyl species were major components of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) from retina, comprising 14.1, 41.3 and 28.3 % of the respective totals. This species was also abundant in PE and PS from brain, accounting for 14.9 and 19.9 % of the totals respectively. Small amounts of di-polyunsaturated fatty acid species [$C_{22:6(n-3)}$ with $C_{20:5(n-3)}$, and $C_{22:6(n-3)}$ with $C_{22:5(n-3)}$] were also found in these phospholipids. Phosphatidylinositol (PI) from both tissues contained no di-polyunsaturated fatty acid species. Retinal PI contained 40.1 % $C_{18:0}$ - $C_{20:4(n-6)}$ with 14.9 % of $C_{18:0}$ - $C_{20:5(n-3)}$; brain PI contained 42.3 % of $C_{18:0}$ - $C_{20:5}$ and 10.4 % of $C_{18:0}$ - $C_{20:4}$ species. Brain PC contained a substantial amount of nervonic acid-containing species with the pair $C_{18:1}$ - $C_{24:1}/C_{24:1}$ - $C_{18:1}$ comprising 8.9 % of the total.

INTRODUCTION

Polyunsaturated fatty acids (PUFA) of the ($n-6$) series predominate in most mammalian tissues (Vergoesen, 1975), whereas ($n-3$)PUFA predominate in the tissues of most fish species (Henderson & Tocher, 1987; Sargent *et al.*, 1989). However, it has long been of interest that phospholipids from mammalian brain, retina and spermatozoa are very rich in ($n-3$)PUFA, especially $C_{22:6(n-3)}$ (docosahexaenoic acid) (Poulos *et al.*, 1973; Sastry, 1985). Dietary-deprivation studies have shown that ($n-3$)PUFA are very tenaciously retained by neural tissues (Tinoco, 1982) in that two or three generations are required to reduce significantly the ($n-3$)PUFA content of brain in rat (Bourre *et al.*, 1984) and brain and retina of rhesus monkeys (*Macaca mulata*) (Neuringer *et al.*, 1986). These studies also showed that depletion of ($n-3$)PUFA in brain and retina results in reduced visual and cognitive abilities in rhesus monkeys (Connor *et al.*, 1984; Neuringer *et al.*, 1984). Furthermore, rats on diets supplemented with $C_{18:3(n-3)}$ fatty acid showed superior learning ability (Lamprey & Walker, 1976; Coscina *et al.*, 1986). However, the precise role(s) of $C_{22:6(n-3)}$ fatty acid in neural tissues is unknown.

Fish, like mammals, also accumulate and retain $C_{22:6(n-3)}$ fatty acid in brains and retinas at high levels above those found in non-neural tissues. Trout brain and retina have $C_{22:6(n-3)}/C_{20:5(n-3)}$ ratios of approx. 4:1 and 8:1 respectively (Tocher & Harvie, 1988), whereas a ratio of 2:1 is normally found in non-neural tissues (Henderson & Tocher, 1987). $C_{22:6(n-3)}$ fatty acid accounted for 24.9 and 28.4 % of total fatty acids in trout brain and retina respectively. The distribution of specific fatty acids,

including $C_{22:6(n-3)}$, in individual phospholipids was also similar in trout and rat neural tissues (Tocher & Harvie, 1988).

Molecular-species analysis has proved a very useful tool in the study of phospholipid metabolism. Some unexpected species have been found, especially di-polyunsaturated fatty acid species in tissues such as rat erythrocytes (Robinson *et al.*, 1986), rat testes (Blank *et al.*, 1985) and bovine retina (Louie *et al.*, 1988). Methods for the detailed analysis of phospholipid molecular species from ($n-3$)PUFA-rich sources have recently been developed (Bell, 1989). The resolving power of the method is shown by the fact that 39 molecular species, including di-($n-3$)PUFA species, were identified in phospholipids from cod roe (Bell, 1989). The fatty acid composition of the phospholipids calculated from the molecular-species data was in good agreement with that found directly by g.l.c. analysis of the fatty acid methyl esters (Bell, 1989). The method is now applied to define the molecular species compositions of phosphatidylcholine (PC), (diacyl)phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) from the brain and retina of rainbow trout (*Salmo gairdneri*).

MATERIALS AND METHODS

Materials

Rainbow trout (*Salmo gairdneri*) (400–600 g body wt.), obtained from a commercial fish farm, were maintained in a freshwater aquarium at 4–6 °C on a standard diet (Ewos Ltd., Westfield, West Lothian, Scotland, U.K.).

Abbreviations used: Molecular species are abbreviated as follows: e.g. 16:0-22:6 PC is 1-palmitoyl($C_{16:0}$)-2-docosahexaenoyl($C_{22:6}$)-sn-glycero-3-phosphocholine; BHT, butylated hydroxytoluene; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; ROS, rod outer segments.

Fish were killed by decapitation and the brains and eyes removed. Brains were used immediately and eyes were frozen at -70°C until required. *Bacillus thuringiensis* type strain IAM 12077 was obtained from the National Collection of Industrial and Marine Bacteria Ltd., Aberdeen, Scotland, U.K.

Phospholipase C from *Bacillus cereus* (Sigma Type XIII), butylated hydroxytoluene (BHT), 3,5-dinitrobenzoyl chloride, dipalmitolein (di-*cis*-9-hexadecenoylglycerol), diolein (di-*cis*-9-octadecenoylglycerol), trimyristin (tritetradecanoylglycerol), triolein (tri-*cis*-9-octadecenoylglycerol), triicosenoin (tri-*cis*-11-eicosenoylglycerol), trinervonin (tri-*cis*-15-tetracosenoylglycerol), and the following PC species were obtained from Sigma Chemical Co., Poole, Dorset, U.K.: egg-yolk PC, dilauryl PC, (didodecyl PC), dimyristoyl PC (ditetradecanoyl PC), dipalmitoyl PC, (dihexadecanoyl PC), distearoyl PC, (dioctadecanoyl PC), 1-stearoyl-2-arachidonoyl PC, (1-octadecanoyl-2-*cis*-5,8,11,14-eicosatetraenoyl PC). Dieicosenoin (di-*cis*-11-eicosenoylglycerol), dinervonin (di-*cis*-15-tetracosenoylglycerol) and didocosaheptaenoin (di-*cis*-4,7,10,13,16,19-docosaheptaenoylglycerol) were obtained from Nu-Chek Prep Inc., Elysian, MN, U.S.A. Sodium methoxide was from Aldrich Chemical Co. Ltd., Gillingham, Dorset, U.K.

T.l.c. and high-performance t.l.c. plates coated with silica-gel 60 (Merck), AnalaR-grade acetic acid, propan-1-ol, propan-2-ol and pyridine were purchased from BDH Ltd., Poole, Dorset, U.K. All other solvents of h.p.l.c. grade were from Rathburn Chemicals, Walkerburn, Peeblesshire, Scotland, U.K.

Ultrasphere ODS and Ultrasphere C8 h.p.l.c. columns (25 cm $\times$ 0.46 cm; 5 μm particle size) were obtained from Altex/Beckman (Beckman Instruments U.K. Ltd., High Wycombe, Bucks., U.K.).

Extraction and purification of lipids

The brains were taken from ten trout, the meninges removed and the remaining tissue homogenized in 30 ml of chloroform/methanol (2:1, v/v) using a Polytron tissue disrupter. Total lipid was extracted essentially by the method of Folch *et al.* (1957). All solvents apart from those used for t.l.c. and h.p.l.c. contained 0.01% (w/v) BHT, and samples were stored at -20°C under N_2 between preparative procedures.

Phospholipid classes were separated by t.l.c. using methyl acetate/propan-1-ol/chloroform/methanol/aq. 0.25% KCl (25:25:25:10:9, by vol.) (Vitiello & Zanetta, 1978) and revealed under u.v. light after spraying with 0.1% (w/v) 2,7-dichlorofluorescein in methanol containing 0.01% (w/v) BHT. Phospholipids were eluted from the silica gel with three 40 ml washes of chloroform/methanol/water (5:5:1, by vol.), rotary-evaporated under vacuum at 35°C and finally dried under a stream of N_2 . PC, PS and PI were purified further by t.l.c. in chloroform/ethanol/water/triethylamine (30:35:6:35, by vol.) (Leray *et al.*, 1987) and the lipids revealed and eluted as described above. 2,7-Dichlorofluorescein was removed from lipids by extracting with a solution of 2% (w/v) KHCO_3 .

Retinas were removed from 100 frozen trout eyes. Lipids were extracted by homogenization in chloroform/methanol (2:1, v/v) and a Folch extract prepared as before. Neutral lipid was removed by preparative t.l.c. in hexane/diethyl ether/acetic acid (70:30:1, by vol.). Phospholipids were eluted from the

origin with chloroform/methanol/water (5:5:1, by vol.), dried, then separated into classes as described previously.

The purity of the final phospholipids was checked by high-performance t.l.c. in methyl acetate/propan-1-ol/chloroform/methanol/aq. 0.25% KCl (25:25:25:10:9, by vol.) and lipids detected by spraying with 3% (w/v) copper acetate in aq. 8% (v/v) phosphoric acid and charring at 160°C for 15 min. Fatty acid methyl esters were prepared by esterification in 2 ml of 1% (v/v) H_2SO_4 in methanol at 50°C under N_2 for 16 h (Tocher & Harvie, 1988). The fatty acid composition of the phospholipids was determined by g.l.c. of fatty acid methyl esters in a Packard 436 gas chromatograph fitted with a CP Wax 52CB fused-silica capillary column (50 m $\times$ 0.32 mm internal diameter) (Chrompack U.K. Ltd., London E14 9TN, U.K.) using hydrogen as carrier gas (Tocher & Harvie, 1988).

Preparation of 3,5-dinitrobenzoyl derivatives

Portions (1 mg) of PC, PE and PS were hydrolysed with 100 units of phospholipase C from *B. cereus* using a two-phase system of 1 ml of diethyl ether and 1 ml of 0.01 M-Tris/ H_2SO_4 , pH 7.4, at room temperature under N_2 (Renkonen, 1965). PI was hydrolysed with a PI-specific phospholipase C prepared from *B. thuringiensis* (Ikezawa & Taguchi, 1981). At the end of the incubations, 1,2-diacylglycerols were extracted and purified by t.l.c. in hexane/diethyl ether/acetic acid (50:50:1, by vol.) (Bell, 1989); alkenylacylglycerols obtained from PE plasmalogen were separated at this stage. Amounts of phospholipids remaining after phospholipase C digestion were less than 2% of the starting material.

Diacylglycerols were derivatized in dry pyridine with 3,5-dinitrobenzoyl chloride at 60°C for 45 min under N_2 , extracted, and washed as described by Takamura *et al.* (1986). The purity of the 1,2-diacyl-3-dinitrobenzoylglycerols was checked by high-performance t.l.c. in hexane/diethyl ether/acetic acid (70:30:1, by vol.). The diacylglycerols were converted quantitatively into their dinitrobenzoyl derivative by this procedure. Standard diacylglycerols were derivatized by the same method.

Separation of molecular species

The 1,2-diacyl-3-dinitrobenzoyl derivatives were chromatographed by h.p.l.c. at $19-21^{\circ}\text{C}$ on reverse phase columns by using a Pye-Unicam 4010 pump and three isocratic solvent systems. An ODS column was used with methanol/propan-2-ol (19:1, v/v) at a flow rate of $1.0\text{ ml}\cdot\text{min}^{-1}$, and acetonitrile/propan-2-ol (4:1, v/v) at a flow rate of $1.0\text{ ml}\cdot\text{min}^{-1}$ (Takamura *et al.*, 1986); a C8 column was used with methanol/water/acetonitrile (93:5:2, by vol.) at a flow rate of $1.2\text{ ml}\cdot\text{min}^{-1}$ (Bell, 1989). Peaks were detected at 254 nm with a Pye-Unicam 4020 detector and quantified by using a Shimadzu C-R3A recording integrator.

It is not possible to resolve all the molecular species present in individual phospholipid classes using one or even two solvent systems. Therefore the third solvent was developed by one of us (Bell, 1989) for separating molecular species of phospholipids from fish tissues containing large amounts of $\text{C}_{20:5(n-3)}$ and $\text{C}_{22:6(n-3)}$ fatty acids to complement those of Takamura *et al.* (1986). Each of these solvent systems gives species that are co-eluted, but between them they are capable of resolving all but some very similar saturated and monounsaturated

species and positional isomers. The first solvent (methanol/propan-2-ol) gave the basic molecular-species composition and the other two solvent systems resolved most species that were co-eluted.

Peaks were identified from plots of $\log[10 \times (\text{retention time relative to } 16:0-22:6)]$ versus the effective carbon number on the C-1 position of the acylglycerol as described by Patton *et al.* (1982) and Takamura *et al.* (1986). 16:0-22:6 (the abbreviations footnote explains this terminology) is a convenient reference species in phospholipids from fish tissues. These graphs were constructed for each solvent system from known standards. Molecular species containing $(n-3)$ PUFA had previously been identified by collecting peaks after chromatography of cod roe PC and preparing fatty acid methyl esters to identify the component fatty acids by g.l.c. (Bell, 1989). Each sample was chromatographed three times in each of the solvent systems and the S.D. values calculated. Where final peak areas were calculated by differences, the S.D. values for the contributing peaks were added to give the final error. For clarity, results are given to one decimal place.

Synthesis of diacylglycerols

Molecular species containing $C_{20:1}$ and $C_{24:1}$ fatty acids were synthesized from known triacylglycerols by base-catalysed transesterification followed by reaction with Grignard reagent to produce diacylglycerols. This permitted identification of such species from plots of $\log(10 \times \text{relative retention time})$ versus the effective carbon number on the C-1 position of the acylglycerol, and by direct comparison of retention times. The method could not resolve positional isomers such as 18:1-20:1 and 20:1-18:1.

Mixtures of trimyristin ($C_{14:0}$) + triecosenoin [$C_{20:1(n-9)}$], trimyristin + trinervonin [$C_{24:1(n-9)}$], triolein [$C_{18:1(n-9)}$] + triecosenoin and triolein + trinervonin were randomized as follows, by using sodium methoxide as a catalyst (Gunstone, 1967). A 10 mg portion of each triacylglycerol was dried in a 1 ml conical screw-cap vial which was warmed to 80 °C to melt the lipid. Then 1 μ l of 4% (w/v) sodium methoxide in methanol was added, the mixture stirred vigorously and gassed with N_2 to remove the methanol, then stirred for a further 30 min at 80 °C under N_2 . The contents of the vial were removed with 5 ml of hexane and extracted with 3 $\times$ 5 ml water to hydrolyse the remaining sodium methoxide. The hexane layer was dried with anhydrous Na_2SO_4 and the solvent was evaporated under a stream of N_2 . Triacylglycerols were purified from hydrolysis products and fatty acid methyl esters by t.l.c. in hexane/diethyl ether/acetic acid (90:10:1, by vol.). Yields of randomized material were low, estimated at 1–10% in different preparations.

Randomized triacylglycerols were converted into diacylglycerols by reaction with ethyl magnesium bromide as described by Christie (1982). 1,2-Diacylglycerols were purified by t.l.c. in hexane/diethyl ether/acetic acid (70:30:1, by vol.) and derivatized as described above.

RESULTS

Species containing a saturated or monoenoic fatty acid at *sn*-1 and a PUFA at *sn*-2 were the most abundant class of species in all phospholipids in both brain and retina (Tables 1 and 2). However, there were substantial differences between the molecular-species composition of

the different phospholipids in the same tissue and between the individual phospholipids in the two tissues. In brain PC and PE, 16:0-22:6 was the major single species, whereas 18:0-22:6 was the major species in PS in both brain and retina and this species also contributed significantly to PC, PE and PI in both tissues (Tables 1 and 2). The next major contributing species was 18:1-22:6, especially in PE and PS.

Di- $(n-3)$ PUFA species were found in very large amounts in retina, totalling 43.5, 29.0 and 14.6% in PE, PS and PC respectively, with 95% or more of these totals being di-22:6 (Table 2). Brain PS and PE also contained 24.1 and 17.8% respectively of di- $(n-3)$ PUFA species with over 80% of these as di- $C_{22:6}$ (Table 1), whereas PI contained no di-PUFA species in either tissue.

PI was unique in that most species in both tissues contained a saturated fatty acid on *sn*-1 and a PUFA on *sn*-2 (Tables 1 and 2). The main exceptions to this were 18:1-20:5 (6.2%) in brain and 18:1-20:4 (5.8%) in retina. Furthermore, 18:0-20:5 was the predominant species in brain PI, accounting for 42.3%, with 18:0-20:4 only accounting for 10.4%, and 16:0-20:5, 18:1-20:5, 16:0-20:4 and 18:1-20:4 all present at between 3.0 and 9.2% (Table 1). In contrast, retinal PI contained 40.1% of 18:0-20:4 and 14.9% of 18:0-20:5; 16:0-20:4 and 18:1-20:4 totalled 18.1%, with 16:0-20:5 and 18:1-20:5 only totalling 3.6%, reflecting the greater abundance of arachidonate in retina (Table 2).

PC was characterized by the highest percentages of species containing only saturated and monounsaturated fatty acids, about 20% of which was 16:0-18:1/14:0-20:1 (Tables 1 and 2). Although this pair was not resolved by using the methods employed, $C_{16:0}$ and $C_{18:1}$ fatty acids are both much more abundant than $C_{14:0}$ and $C_{20:1}$ fatty acids in both tissues, i.e. 16:0-18:1 is the major species. The pair 18:0-18:1/16:0-20:1 totalled 5.9% of brain PC but only 2.8% of retinal PC; fatty acid analysis suggests approximately equal amounts of each species in brain, but mainly 18:0-18:1 in retina, where $C_{20:1}$ fatty acid only comprised 0.8% of total fatty acids. In brain PC, di-monoene species totalled 20.1%, of which the pair 18:1-24:1/24:1-18:1 comprised 8.9%. Another six species containing $C_{24:1}$ fatty acid were found in PC from brain.

18:0-22:6 comprised a third of the PS from brain and di- $C_{22:6}$ was the next most important species. 16:0-22:6, 18:1-22:6 and 18:0-18:1/16:0-20:1 were each present at between 6.7 and 8.1%. Species containing only saturates and monoenes totalled 18.5%. In PS from retina, di-22:6 was the most abundant species, with 18:0-22:6 slightly less abundant than in brain. The two other major species were 16:0-22:6 and 18:1-22:6, these four species comprising almost 80% of the total. PE from retina was dominated by 22:6-22:6 (41.3%) with 18:1-22:6, 16:0-22:6 and 18:0-22:6 together accounting for 33.5%. Species containing only saturates and monoenes totalled 5.4%. In PE from brain, 22:6-22:6 was less abundant (14.9%) and this, together with 16:0-22:6, 18:1-22:6 and 18:0-22:6, were the major species, totalling 57.8%. Species containing only saturates and monoenes comprised 20.5%.

DISCUSSION

Molecular-species analyses of the four main diacyl phospholipids from brain and retina have not previously

Table 1. Molecular-species composition of phospholipids from the brain of the rainbow trout

The 1,2-diacyl-3-dinitrobenzoylglycerol derivatives were prepared and chromatographed by isocratic reverse-phase h.p.l.c. as described in the Materials and methods section. Peaks were detected at 254 nm with a Pye-Unicam 4020 detector and quantified using a Shimadzu C-R3A recording integrator. The first solvent (methanol/propan-2-ol) gave the basic molecular-species composition and the other two solvent systems resolved most co-eluted species. Each sample was chromatographed three times in each of the solvent systems and the s.d. calculated. Where final peak areas were calculated by differences, the s.d. values of the contributing peaks were added to give the final error. For clarity, results are given to one decimal place. Compositions are corrected to 100%; tr, <0.1%; —, not detected. The following fatty acid isomers were identified by g.l.c.: $C_{16:1(n-9)}$ and $C_{16:1(n-7)}$, $C_{18:1(n-9)}$ and $C_{18:1(n-7)}$, $C_{20:1(n-9)}$ and $C_{20:1(n-7)}$, $C_{24:1(n-9)}$ and $C_{24:1(n-7)}$, $C_{18:2(n-6)}$, $C_{20:4(n-6)}$, $C_{20:5(n-3)}$, $C_{22:5(n-3)}$ and $C_{22:6(n-3)}$. The most saturated (sat) fatty acid is assumed to be on position 1 of the acylglycerol (Gunstone, 1967). In co-eluted pairs the species in parentheses are likely to be minor components based on the fatty acid composition of the phospholipids.

Species	Phospholipid ...	Composition (mol/100 mol)			
		PC	PE	PS	PI
PUFA-PUFA					
22:6-20:5; 20:5-22:6		0.2±0.0	0.9±0.1	0.5±0.0	—
22:6-22:6		0.9±0.0	14.9±0.7	19.9±0.5	—
22:6-22:5; 22:5-22:6		—	2.0±0.0	3.7±0.2	—
	Total ...	1.1	17.8	24.1	—
Sat-PUFA					
14:0-22:6		0.2±0.1	1.1±0.0	tr	—
14:0-20:4		0.3±0.1	—	—	—
16:0-20:5		2.5±0.1	3.7±0.1	tr	9.2±0.1
16:0-22:6		29.0±1.7	18.2±0.1	7.2±0.2	6.4±0.2
16:0-20:4		0.7±0.0	0.5±0.1	0.4±0.1	5.9±0.3
16:0-22:5		1.0±0.2	1.0±0.1	0.3±0.1	0.9±0.1
18:0-20:5		1.3±0.1	4.6±0.3	1.8±0.1	42.3±0.2
18:0-22:6		2.4±0.0	14.5±0.3	33.1±0.4	8.0±0.4
18:0-20:4		0.6±0.1	1.0±0.0	0.6±0.0	10.4±0.2
18:0-22:5		0.3±0.1	0.2±0.0	2.0±0.2	0.9±0.1
	Total ...	38.3	44.8	45.4	84.0
Monoene-PUFA					
16:1-22:6		tr	0.1±0.0	tr	—
18:1-20:5		0.1±0.0	2.2±0.1	0.8±0.1	6.2±0.1
18:1-22:6		2.0±0.3	10.2±1.5	6.7±0.2	1.4±0.3
18:1-20:4		0.4±0.0	0.3±0.0	0.3±0.1	3.0±0.2
18:1-22:5		tr	1.6±0.2	3.1±0.1	0.4±0.0
20:1-20:5		0.6±0.1	0.4±0.3	0.5±0.1	—
20:1-22:6		0.9±0.0	1.0±0.1	0.7±0.4	0.1±0.1
20:1-20:4		—	tr	—	1.3±0.2
20:1-22:5		tr	0.1±0.1	0.1±0.1	0.3±0.0
24:1-20:5		2.4±0.3	0.4±0.1	—	—
24:1-22:6		1.0±0.2	0.2±0.0	—	—
24:1-20:4		0.4±0.0	—	—	—
	Total ...	7.8	16.5	12.2	12.7
Sat-sat					
14:0-16:0; 16:0-14:0		1.1±0.5	0.1±0.0	0.6±0.0	—
16:0-16:0; (14:0-18:0)		1.4±0.0	0.1±0.0	0.4±0.2	—
16:0-18:0; 18:0-16:0		0.8±0.1	—	0.4±0.1	—
	Total ...	3.3	0.2	1.4	—
Sat-monoene					
16:0-16:1; (14:0-18:1)		1.6±0.1	—	—	—
16:0-18:1; (14:0-20:1)		21.1±0.9	3.8±0.1	1.1±0.1	0.3±0.0
18:0-18:1; 16:0-20:1		5.9±0.1	3.6±0.1	8.1±0.1	0.7±0.1
18:0-20:1		0.3±0.1	—	0.2±0.0	—
16:0-24:1		0.2±0.0	—	—	—
18:0-24:1		0.2±0.1	—	—	—
	Total ...	29.3	7.4	9.4	1.0
Monoene-monoene					
16:1-18:1; 18:1-16:1		1.4±0.0	1.1±0.2	—	0.6±0.1
18:1-18:1; 16:1-20:1		3.5±0.4	7.7±0.0	3.6±0.1	1.1±0.2
18:1-20:1; 20:1-18:1		3.7±0.1	3.2±0.1	2.9±0.1	0.6±0.2
20:1-20:1		2.4±0.2	0.4±0.1	0.5±0.2	—
18:1-24:1; 24:1-18:1		8.9±0.2	0.5±0.0	0.7±0.1	—
20:1-24:1; 24:1-20:1		0.2±0.0	—	—	—
	Total ...	20.1	12.9	7.7	2.3

Table 2. Molecular species composition of phospholipids from the retina of trout

Experiments were performed and results derived as detailed in Table 1. The unknown species ran close to 24:1-22:6 in methanol/propan-2-ol (19:1, v/v), to 18:0-16:1 in acetonitrile/propan-2-ol (4:1, v/v) and to 24:1-22:5 in methanol/water/acetonitrile (93:5:2, by vol.).

Species	Phospholipid ...	Composition (mol/100 mol)			
		PC	PE	PS	PI
PUFA-PUFA					
22:6-20:5; 20:5-22:6		0.4±0.0	0.7±0.2	0.2±0.1	—
22:6-22:6		14.1±0.4	41.3±0.4	28.3±1.0	—
22:6-22:5; 22:5-22:6		0.1±0.0	1.5±0.1	0.5±0.2	—
	Total...	14.6	43.5	29.0	—
Sat-PUFA					
14:0-22:6		0.7±0.1	2.5±0.0	—	—
14:0-20:4		—	0.7±0.0	—	—
14:0-22:5		0.7±0.1	tr	—	—
16:0-20:5		4.9±0.1	2.9±0.8	1.4±0.1	1.7±0.1
16:0-22:6		26.5±0.2	10.6±0.2	13.6±0.3	12.5±0.1
16:0-20:4		1.2±0.0	1.0±0.0	0.4±0.1	12.3±0.6
16:0-22:5		0.6±0.3	1.0±0.1	0.8±0.2	0.3±0.1
16:0-18:2		1.3±0.2	—	—	—
18:0-20:5		1.2±0.3	1.7±0.0	0.9±0.2	14.9±0.5
18:0-22:6		8.8±0.2	7.2±0.4	27.3±0.5	6.9±0.2
18:0-20:4		0.6±0.1	0.9±0.1	0.3±0.1	40.1±0.3
18:0-22:5		tr	0.4±0.0	0.9±0.3	—
	Total...	46.5	28.9	45.6	88.7
Monoene-PUFA					
16:1-22:6		1.1±0.1	0.4±0.0	—	—
18:1-20:5		0.8±0.0	2.9±0.1	0.5±0.0	1.9±0.1
18:1-22:6		1.8±0.5	15.7±0.6	10.4±0.8	1.8±0.3
18:1-20:4		0.5±0.0	0.7±0.1	0.5±0.1	5.8±0.1
18:1-22:5		0.8±0.1	0.6±0.1	0.8±0.2	—
20:1-20:5		—	—	0.4±0.1	—
20:1-22:6		0.8±0.2	1.8±0.1	3.3±0.2	—
20:1-20:4		tr	—	1.8±0.2	0.4±0.1
20:1-22:5		tr	—	0.5±0.1	—
	Total...	5.8	22.1	18.2	9.9
Sat-sat					
14:0-16:0; 16:0-14:0		0.8±0.1	—	—	—
16:0-16:0; (14:0-18:0)		0.6±0.0	—	—	—
	Total...	1.4	—	—	—
Sat-monoene					
14:0-16:1		0.9±0.1	—	—	—
16:0-16:1; 14:0-18:1		2.7±0.3	—	—	—
16:0-18:1; (14:0-20:1)		19.6±0.5	1.3±0.0	1.9±0.2	—
18:0-16:1		—	tr	0.4±0.0	—
18:0-18:1; 16:0-20:1		2.8±0.0	0.8±0.1	1.8±0.2	—
	Total...	26.0	2.1	4.1	—
Monoene-monoene					
16:1-18:1; 18:1-16:1		0.6±0.1	0.4±0.0	—	—
18:1-18:1; 16:1-20:1		1.8±0.1	2.4±0.1	1.8±0.2	0.7±0.1
18:1-20:1; 20:1-18:1		0.5±0.1	0.5±0.1	0.7±0.1	—
20:1-20:1		0.1±0.0	—	—	—
18:1-24:1; 24:1-18:1		0.2±0.0	—	—	—
	Total...	3.2	3.3	2.5	0.7
Unknown		2.2±0.2	—	—	—

been reported in the detail presented here. However, molecular species of diacyl phospholipids containing (*n*−3)PUFA at both positions have been demonstrated previously in frog retina (Avelano & Bazan, 1977; Wiegand & Anderson, 1983) and bovine retina (Miljanich *et al.*, 1979; Avelano & Bazan, 1980). In rat erythrocytes, significant amounts of di-(*n*−6)PUFA and di-

(*n*−3)PUFA species were found in diacyl PE and PS (Robinson *et al.*, 1986). The fatty acid compositions of fish retinas strongly suggested the presence of di-PUFA species (Tocher & Harvie, 1988) and the present study confirms this. The total amount of di-PUFA species in trout retinal PC, PE and PS at 14.6, 43.5 and 29.0% respectively significantly exceeds the levels reported for

bovine retinal PC, PE and PS, which contained 9.5, 17.3 and 15.3 % of 'supraenes' (species containing more than six double bonds and so including monoene-22:6 species) respectively (Avelldano & Bazan, 1983). Bovine retinal PI also contained a small amount of 'supraenes' (1.5 %) (Avelldano & Bazan, 1983), but trout retina and brain PI contained no di-PUFA species, although they did contain 1.8 % and 1.5 % respectively of monoene-22:6 species.

The di-PUFA species are concentrated in the rod outer segments (ROS). In one study of bovine ROS, PC, PE and PS contained 31.1, 20.6 and 51.5 % 'supraenes' respectively (Avelldano & Bazan, 1983) and in another study di-22:6 accounted for 3.8, 27.2 and 42.9 % of frog ROS PC, PE and PS respectively (Louie *et al.*, 1988). The data reported in the present study suggest that trout retinal ROS will provide a richer source of di-PUFA species and could therefore be a very useful tissue for investigating the physical effects of di-PUFA species on membrane structure. Although not as marked as in retina, trout brain PE and PS also contained relatively high levels of di-PUFA species. However, there are little comparative data in the literature on brains from other species, although it has been reported that toad brain does not contain any di-PUFA species (Avelldano & Bazan, 1977).

The predominant di-PUFA species in trout brain and retina was di- $C_{22:6}$, as previously reported for frog retina (Wiegand & Anderson, 1983; Louie *et al.*, 1988) and implied for bovine retina (Avelldano & Bazan, 1983). Similarly, 22:5-22:6/22:6-22:5 has also been reported previously, with 22:5($n-3+n-6$)-22:6($n-3$) accounting for 10 % of species in PE from frog ROS (Wiegand & Anderson, 1983). Frog ROS PE also contained 22:4($n-6$)-22:6($n-3$) (10 %) and 20:4($n-6$)-22:6($n-3$) (5 %) (Wiegand & Anderson, 1983), but no di-PUFA species containing ($n-6$)PUFA were identified in trout tissues.

The very different contents of the 18:0-20:4($n-6$) and 18:0-20:5($n-3$) species in brain and retinal PI was predictable from earlier fatty-acid-composition data (Tocher & Harvie, 1988). The major species in trout retina PI was 18:0-20:4 (40.1 %), which is the normal situation in mammals and fish, e.g. mono-, di- and tri-phosphoinositides from bovine brain (over 40 % in each class) (Holub *et al.*, 1970), rat erythrocyte PI (44.1 %) (Robinson *et al.*, 1986) and cod roe PI (36.7 %) (Bell, 1989). Therefore, the high percentage (42.3 %) of 18:0-20:5($n-3$) in trout brain PI is very unusual, and indeed is the first report of an animal PI which is not mainly 18:0-20:4($n-6$) other than lung surfactant PI, which comprises mainly saturated/monoene-containing species (Rustow *et al.*, 1988). This may be of considerable relevance to eicosanoid metabolism in brain.

A novel molecular species found in trout was the 24:1-18:1/18:1-24:1 pair present at almost 9 % in brain PC. The identity of this species was confirmed by synthesis and comparison of retention times. Several other $C_{24:1}$ -fatty acid-containing species were also resolved. The presence of $C_{24:1}$ fatty acid in phospholipids from trout brain was previously noted by Tocher & Harvie (1988). A previous study also reported the presence of almost 6 % $C_{24:1}$ fatty acid in PC from carp brain (Natarajan *et al.*, 1985). In trout retina only 0.2 % $C_{24:1}$ fatty acid was found in PC and PE, and this was reflected in the virtual absence of $C_{24:1}$ -fatty acid-containing species in the trout retina. There are no previous reports in the

literature of any molecular species of phospholipids containing $C_{24:1}$ fatty acid, either in mammals or fish.

The study of trout brain and retina phospholipids has provided further evidence for an important and possibly essential role for docosahexaenoic acid and di-22:6 molecular species in neural tissues. However, the properties that di-22:6 phospholipids confer on membrane structure and the precise nature of their role(s) in the specialized functions of these tissues remain to be elucidated. The levels of the di-22:6 species found here suggest that neural tissues from trout, or other fish species, will be a useful system for study. The mechanisms for controlling the molecular species composition of different phospholipid classes in different tissues also requires investigation. Furthermore, the unique composition of PI in brain suggests that this tissue will be particularly interesting with respect to eicosanoid metabolism.

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