

Characterization of the total lipid and fatty acid composition of rat olfactory mucosa

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Abstract Phospholipid accounted for 81% (by weight) of the total lipid of rat olfactory mucosa. Phosphatidylcholine (46% of total phospholipids) and phosphatidylethanolamine (26%) were the predominant phospholipids. Phosphatidylinositol (8%), sphingomyelin (6%), and phosphatidylserine (7%) were the next most abundant phospholipids, with cardiolipin (4%) and phosphatidic acid (1%) present in lesser amounts. Only trace amounts of the polyphosphoinositides, phosphatidylinositol monophosphate, and phosphatidylinositol bisphosphate were detected. Sterol was the major neutral lipid present (83% of the total neutral lipid mass) with lesser amounts of triacylglycerols (7%), steryl esters (6%), free fatty acids (4%), and diacylglycerols (1%). Monoacylglycerols were detected only in trace amounts. The sterol to phospholipid ratio was 0.39:1. Most of the phospholipids of the olfactory mucosa showed a high polyunsaturated fatty acid content, with the arachidonic acid (20:4) and docosahexaenoic acid (22:6) residues predominating. The fatty acids in sphingomyelin, however, were almost totally saturated and included the 24:0 and 24:1 residues, which were not detected in other phospholipids. Polyunsaturated fatty acids accounted for less than 25% of the total fatty acid of any individual neutral lipid and comprised largely linoleic and arachidonic acids. The results are discussed in relation to the putative role of lipids in olfactory signal transduction. — Russell, Y., P. Evans, and G. H. Dodd. Characterization of the total lipid and fatty acid composition of rat olfactory mucosa. *J. Lipid Res.* 1989. 30: 877–884.

Supplementary key words densitometry • epithelium • gas-chromatography-mass spectrometry • neutral lipid • phospholipid • polyphosphoinositides • signal transduction • thin-layer chromatography

The mechanism of the initial event in mammalian olfaction, the interaction of odorants with the sensory membranes, remains obscure (reviewed in reference 1). The majority of odorants are volatile hydrophobic substances that have the general ability to fluidize phospholipid bilayers (1–3). The lipophilicity of odorants and evidence indicating that specific receptor proteins are not required in odorant transduction (3, 4) has given rise to suggestions that lipids play an important part in odor recognition (5–9). Lipids have a putative role in the transduction processes of both vision (10, 11) and taste (12, 13), and it might be that olfactory signal transduction is regulated by the lipids of the olfactory mucosa. Phospholipid turnover has been implicated in transduction processes in

neurotransmitter, hormone, and growth factor reception in other tissues (reviewed in references 14 and 15).

Investigations into the interaction of odorants with lipid membranes have been carried out (see, for example, 7–9). However, there is a lack of basic knowledge of the type, concentration, and metabolism of lipids within the olfactory mucosa. It is, perhaps, conceivable that unique lipids may be present in the olfactory system that could be involved in odorant reception and/or transduction. To the authors' knowledge only a single analysis of the lipids of an olfactory mucosa has been reported (16). The analysis of the lipids of bovine olfactory mucosa revealed a high phospholipid content and identified phosphatidylcholine, phosphatidylethanolamine, and sterol as the major lipids present (16). The present study reports the results from a detailed analysis of the lipids of rat olfactory mucosa, including fatty acid composition. No unusual lipids were identified. However, the tissue was found to have significant polyunsaturated fatty acid content which has similarly been observed for gustatory (17) and photoreceptor (18) sensory epithelia.

MATERIALS AND METHODS

Extraction of lipids

Male Wistar rats, 200–225g in weight, were killed by stunning and cervical dislocation prior to decapitation.

Abbreviations: BHT, 2, 6-di-tert-butyl-4-methylphenol; CL, cardiolipin; DAG, diacylglycerol; EGTA, ethylene glycol bis-(β -aminoethyl ether) N, N, N',N'-tetraacetic acid; FA, fatty acid; FAME(s), fatty acid methyl ester(s); PA, phosphatidic acid; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PI-P, phosphatidylinositol monophosphate; PI-P₂, phosphatidylinositol bisphosphate; SD, standard deviation; SE, steryl ester; SEM, standard error of the mean; SM, sphingomyelin; TAG, triacylglycerol; TLC, thin-layer chromatography; Tris, 2-amino-2(hydroxymethyl)-propane-1, 3-diol.

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The severed head was cut in sagittal section and the ethmoturbinates were excised. The tissue was immediately placed in 5 ml ice-cold 10 mM Tris-HCl buffer (pH 7.6) containing 155 mM NaCl, 5.6 mM KCl, and 1 mM EGTA. The tissue was washed with two further 5-ml portions of the above buffer (to remove superficial blood and debris) and then with two 5-ml portions of ice-cold 10 mM Tris-HCl buffer (pH 7.6) containing 155 mM NaCl, 5.6 mM KCl, and 2.1 mM CaCl_2 . The washed tissue was then placed in 1 ml of the second buffer. Quantitative extraction of total lipid was carried out following the method of Folch, Lees, and Sloane Stanley (19) in the presence of 0.01% (w/v) BHT as antioxidant. Tissue dissociation was achieved by sonication in ice-cold solvent for 6×30 sec, with 30 sec cooling between each sonication step (20) at the medium power setting of a 100 W disintegrator (Measuring and Scientific Equipment, U.K.) and using a probe of 10-mm tip diameter. A second method of lipid extraction was necessary to achieve quantitative extraction of the more polar polyphosphoinositides (21). In this procedure lipids were extracted by the addition of 10 ml ice-cold chloroform-methanol 2:1 (v/v) containing 0.01% (w/v) BHT and sonicated as described above. Hydrochloric acid (0.8 ml, 1 M HCl) was added to the sonicated solution and mixed thoroughly prior to centrifugation at 200 g for 5 min (Mistral 2L). The lower organic phase was removed by aspiration and washed with 2 ml 0.3 M HCl. The organic phase was filtered through phase-separating paper (Whatman LabSales Ltd., Maidstone, Kent, U. K.) and the solvent was evaporated in a stream of nitrogen. Lipid extracts were desiccated *in vacuo* in the presence of phosphorus pentoxide and self-indicating silica gel prior to further analysis.

Separation and quantification of lipids

Phosphatidylcholine, PE, PS, CL, and SM were separated on thin-layer silica gel 60 plates (20 × 20 cm; E. Merck, Darmstadt, West Germany). All TLC was carried out at 20°C. Lipid extract (300–500 μg) was loaded and the plates were developed using chloroform-methanol-water-0.88 sp.gr.ammonia 130:70:8:0.5 (v/v/v/v) in the first dimension and chloroform-acetone-methanol-glacial acetic acid-water 10:4:2:2:1 (v/v/v/v/v) in the second dimension (22). Phosphatidic acid, PI, PI-P, and PI-P₂ were separated on silica gel 60 plates impregnated with potassium oxalate. The plates were impregnated with potassium oxalate by prior chromatography in methanol-water 2:3 (v/v) containing 1% (w/v) potassium oxalate (23). The plates were subsequently activated by drying at 110°C for 15 min. Two dimensional chromatography was carried out using chloroform-methanol-3.3 M ammonia 43:38:12 (v/v/v) in the first dimension (24) and chloroform-acetone-methanol-acetic acid-water 10:4:2:2:1 (v/v/v/v/v) in the second dimension. Individual phospholipids were identified by reference to authentic stan-

dards (Sigma Chemical Company, Poole, U.K.). Additional confirmation of the identity of the lipids was obtained using a ninhydrin spray reagent (25) and a glycolipid spray reagent (26). Lipid bands were visualized by exposing plates to iodine vapor, except when fatty acids were to be analyzed. Where the mass of lipid was too low to be detected by exposure to iodine vapor, lipid was radiolabeled by incubating the ethmoturbinates in 1 ml 10 mM Tris-HCl (pH 7.6) containing 155 mM NaCl, 5.6 mM KCl and 2.1 mM CaCl_2 in the presence of 10 μCi [³²P]orthophosphate at 30°C for 1 h. Reactions were quenched by the addition of 10 ml ice-cold incubation buffer and washed with two further 10-ml portions of incubation buffer prior to extraction of the lipids. In these experiments TLC was carried out in one dimension (27, 28). Radioactive zones were detected by exposure to Kodak Diagnostic X-Omat X-Ray Film, for 48–72 h depending on the intensity of labeling. Individual lipid zones were scraped from the TLC plate and the phospholipids were eluted from the silica with two 5-ml portions of chloroform-methanol-water 5: 5: 1 (v/v/v). The solvent was evaporated in a stream of nitrogen and the sample was desiccated prior to further analysis.

Phospholipids were estimated by measurement of total phosphate essentially according to the procedure of Bartlett (29) as described by Christie (30). Digestion of the lipid was, however, carried out by the addition of 0.4 ml 72% (w/v) perchloric acid to the solvent-free lipid and heating at 175°C for 15 h in a forced-draught oven (31).

Neutral lipids were separated by chromatography on silica gel 60 plates (20 × 20 cm) by developing in one dimension with hexane-diethyl ether-glacial acetic acid 80: 20: 1 (v/v/v). Prior to loading the lipid samples the silica gel 60 plates were cleaned by developing in one dimension with methanol-diethyl ether 1:1 (v/v) (30). Neutral lipids were estimated by densitometry (32).

Preparation of fatty acid methyl esters

Lipids were transmethylated according to a modified method of Drenthe and Daemen (33). Lipid bands on silica gel 60 plates were sprayed lightly with a solution of 0.1% (w/v) BHT in methanol prior to visualization by spraying with water (26). The phosphoinositides were labeled with [³²P]orthophosphoric acid as described earlier and autoradiography was carried out after spraying the plates with 1% (w/v) BHT in methanol. Phospholipids were eluted from the silica gel with two 5-ml portions of chloroform-methanol-water 5: 5: 1 (v/v/v). Neutral lipids were eluted with two 5-ml portions of hexane-diethyl ether 1:1 (v/v). The solvent was evaporated in a stream of nitrogen and 10 μg heneicosanoic acid (21:0, internal standard) was added prior to the addition of 40 μl chloroform-methanol 1:1 (v/v) 200 μl boron trifluoride-methanol complex (about 14% boron trifluoride, BDH Chemicals, Poole, Dorset, U. K.). The sample

was flushed with nitrogen, sealed in a vial fitted with a Teflon-lined cap, and heated at 100°C for 1 h. After the sample had cooled, the FAME were extracted with 400 µl pentane ('Distol' grade, Fisons Scientific Apparatus, Loughborough, U. K.).

The pentane extract was concentrated by evaporating the solvent in a stream of nitrogen and redissolving in hexane ('Distol' grade, Fisons Scientific Apparatus). The sample (2 µl) was injected into a gas chromatograph (Hewlett-Packard Model No. 5890A) equipped with an Ultra 2 capillary column (25 m × 0.2 mm × 0.11 µm, cross-linked 5% phenylmethyl silicone, Hewlett-Packard part No. 19091B-002). The chromatograph was operated with a column flow of 1 ml helium/min, a split ratio of 10, and an injection temperature of 230°C. The oven temperature was programmed to rise from 100°C to 200°C at 10°C/min, rest at 200°C for 6 min, then rise to the final temperature of 220°C at 10°C/min. The total run time was 30 min. Compounds eluting from the capillary column passed into a mass spectrometer (Hewlett-Packard mass selective detector, model 5970B), which produced mass spectra by scanning the mass of ion fragments in the range 40–400 a. m. u. Each FAME present in the extract was identified by comparison of its retention time and mass spectrum with those of authentic FAME. Quantification of FAME was achieved by producing calibration graphs of FAME ion abundance/21:0Me ion abundance versus pmol FAME injected/pmol 21:0Me injected, ion abundance values being obtained by integration of the peak for each FAME.

RESULTS

Extraction of lipids

Excision of both sets of ethmoturbinates from five rats yielded tissue of mean wet weight of 142 (± SD = 13.2) mg per individual. This weight included the cartilaginous supporting tissue. After washing and freeze-drying, the dry weight of the tissue was 28.4 (± 2.4)% giving a moisture content of 81.3 (± 1.6)%. A sonication protocol (21) was adopted to disrupt the tissue because successful homogenization of the tissue in chloroform-methanol 2:1 (v/v) was prevented by the cartilage. Sonication resulted in partial disintegration of the cartilage which partitioned into the organic phase and accounted for about 46% of the organic extract. Total lipid accounted for 0.9 (± 0.1)% of the tissue wet weight and 4.5 (± 0.4)% of the tissue dry weight.

Thin-layer chromatography lipids

Good resolution of the phospholipids was given by two-dimensional chromatography using 400–500 µg of lipid extract. The phospholipids, PC, PE, PS, PI, SM, and CL, were each present in sufficient quantities to allow detection by iodine staining. It was, however, necessary to

radiolabel the lipids to enable autoradiographic detection of the low amounts of PA, PI-P, and PI-P₂. In these experiments the inositol lipids, PI, PI-P, and PI-P₂, (in addition to PC and PA) were radiolabeled and bands of radioactivity coincided with the mass of those lipids detected by iodine staining. Negligible radioactivity was observed in the PE, PS, SM, and CL bands. Lysophospholipids were not detected on exposure of the plate to iodine vapor or on labeling the phospholipids with [³²P]orthophosphate. This study did not include analyses to demonstrate the presence of plasmalogens or alkylacylglycerophospholipids.

Neutral and phospholipid composition

The compositions of total neutral lipids and phospholipids in the rat olfactory mucosa are given in **Tables 1a and 1b**. Phospholipids accounted for 81% (SD = 10.5, *n* = 6) and sterol for 16% of the total lipid, with DAG, FA, TAG, and SE making up the remainder. Only trace amounts (< 0.1 wt % of the total neutral lipid) of MAG were evident. Phosphatidylcholine was the predominant phospholipid (46%) with PE (26%) as the second most

TABLE 1a. Phospholipid composition of rat olfactory mucosa

	Weight % Total Phospholipid	Weight % Total Lipid
PC	46.42 ± 0.89	37.53 ± 0.72 (5)
PE	26.47 ± 0.80	21.40 ± 0.65 (5)
PI	7.96 ± 0.46	6.44 ± 0.37 (5)
PS	6.96 ± 0.27	5.63 ± 0.22 (5)
SM	6.18 ± 0.41	5.00 ± 0.33 (5)
CL	3.97 ± 0.76	3.21 ± 0.61 (5)
PA ^a	1.28 ± 0.10	1.03 ± 0.08 (4)
Unknowns ^a	0.55 ± 0.16	0.45 ± 0.13 (4)
PI-P ^a	0.16 ± 0.02	0.13 ± 0.02 (4)
PI-P ₂ ^a	0.05 ± 0.01	0.04 ± 0.01 (4)

TABLE 1b. Neutral lipid composition of rat olfactory mucosa

	Weight % Total Neutral Lipid	Weight % Total Lipid
Sterol	82.65 ± 4.89	15.83 ± 0.93 (6)
Triacylglycerol	7.09 ± 0.64	1.36 ± 0.12 (5)
Steryl ester	5.63 ± 0.30	1.08 ± 0.06 (6)
Fatty acid ^a	3.88 ± 0.38	0.74 ± 0.07 (5)
Diacylglycerol	0.73 ± 0.23	0.14 ± 0.05 (3)
Monoacylglycerol	Tr	Tr

Results are expressed in terms of weight % relative abundance within the phospholipid (Table 1a) and neutral lipid (Table 1b) detected in the sample and weight % relative abundance of the total lipid ± SEM of determinations from the number of animals given in parentheses (). The number of determinations carried out for each animal was 2–4 except as noted. Phospholipid mass = [(µg phosphorus × 100)/(% phosphorus content)], assuming an average mol wt of 775. The phosphorus content of the phospholipids was assumed to be 4% (37), except for PI-P (6.6%) and PI-P₂ (9.0%); Tr, trace (< 0.05% of total lipid).

^aOnly one determination per animal.

abundant phospholipid. Phosphatidylinositol, PS, SM, and CL were present in lesser amounts, with PA, PI-P, and PI-P₂ present only in trace amounts. Unknowns accounted for 0.5% of the total lipid and were not glycolipids or lipids with amino groups. The sterol to phospholipid molar ratio was 0.39:1 assuming a mean molecular weight of 775 for phospholipid. The sterol was assumed to be predominantly cholesterol as is the case in the mature CNS (34) and mammalian tissue in general (35). A cholesterol to phospholipid molar ratio in the region of 0.7–1.1 and high SM content would have been indicative of a plasma membrane-like profile (36).

Fatty acid composition

The fatty acid composition of the total lipid of rat olfactory mucosa is given in **Table 2** and **Table 3**. Free fatty acids comprised largely myristate (14:0), palmitate (16:0), and stearate (18:0), and accounted for less than 1% of the total lipid mass. The fatty acid composition of the free fatty acid pool did not resemble that of either the glycerolip-

ids or the phospholipids, indicating that significant lipase activity or activation of lipase in the extraction procedures did not occur. Dimethylacetals were not detected on fatty acid methyl ester analysis of any of the lipid classes investigated. The phospholipids contained considerable amounts of polyunsaturated fatty acids, in particular docosahexaenoate (22:6, n-3) and arachidonate (20:4, n-6). The 22:5(n-6) and 22:5(n-3) fatty acid residues were present in trace amounts or as 1–2% of the total fatty acids in the majority of phospholipids, but were not detected in either PC or SM. Arachidonate was present as >10% of the total fatty acid of all the phospholipids in the rat olfactory mucosa except SM, in which only trace amounts were detected. The PE/CL and PI fractions contained the greatest amounts of arachidonate. Linoleate (18:2, n-6) constituted >1% of the total fatty acid in all lipid classes, but the 18:3 (n-6) residue, γ -linolenate, was not detected throughout the analyses.

The major fatty acids present in PI, PI-P, and PI-P₂ were palmitate, stearate, and arachidonate. Arachidonate

TABLE 2. Fatty acid composition (mol %) of the phospholipids of rat olfactory mucosa

Fatty Acid ^a	Total Phospholipid	SM	PS	PC	PE/CL	PI	PI-P*	PI-P ₂ *	PtdA*
14:0	Tr	1.5 ± 0.1	1.0 ± 0.1	Tr	Tr	1.0 ± 0.2	4.8 ± 0.3	4.6 ± 0.4	1.5 ± 0.2
15:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
16:0	25.2 ± 0.3	46.2 ± 1.3	17.9 ± 0.8	35.6 ± 0.6	10.3 ± 0.2	17.0 ± 0.4	27.1 ± 1.0	26.3 ± 0.5	24.7 ± 0.6
16:1(n-7)	Tr	-	-	Tr	Tr	Tr	Tr	Tr	Tr
17:0	Tr	Tr	-	Tr	Tr	Tr	Tr	Tr	Tr
18:0	16.3 ± 0.1	19.3 ± 0.9	29.8 ± 1.3	14.2 ± 0.3	12.3 ± 0.1	30.2 ± 0.3	17.3 ± 0.6	16.3 ± 0.5	14.1 ± 0.4
<i>cis</i> 18:1(n-9)	11.5 ± 0.3	5.3 ± 0.7	13.9 ± 0.4	13.9 ± 0.2	9.3 ± 0.4	4.1 ± 0.5	12.4 ± 0.3	12.1 ± 0.2	20.2 ± 0.7
<i>trans</i> 18:1(n-9)	2.9 ± 0.1	Tr	2.1 ± 0.8	3.8 ± 0.1	2.2 ± 0.3	1.3 ± 0.2	2.6 ± 0.2	2.8 ± 0.1	3.5 ± 0.2
18:2(n-6)	3.7 ± 0.1	1.7 ± 0.5	1.9 ± 0.3	3.7 ± 0.3	2.9 ± 0.2	1.4 ± 0.2	5.2 ± 0.8	4.4 ± 0.1	4.8 ± 0.1
20:0	Tr	6.0 ± 0.6	Tr	-	-	-	Tr	Tr	Tr
20:3(n-6)	Tr	-	Tr	Tr	Tr	Tr	1.1 ± 0.2	Tr	Tr
20:4(n-6)	26.0 ± 0.3	Tr	10.7 ± 0.3	22.4 ± 0.6	34.5 ± 1.1	29.2 ± 0.7	18.4 ± 1.3	20.4 ± 0.4	21.9 ± 1.1
20:5(n-3)	1.9 ± 0.2	-	Tr	1.3 ± 0.1	2.0 ± 0.1	3.3 ± 0.2	2.4 ± 0.6	3.0 ± 1.0	Tr
22:0	Tr	5.5 ± 0.2	-	-	-	-	-	-	-
22:4(n-6)	3.1 ± 0.2	-	5.1 ± 0.4	1.6 ± 0.2	5.9 ± 0.2	1.9 ± 0.1	2.2 ± 0.3	2.2 ± 0.2	2.0 ± 0.2
22:5(n-6)	1.1 ± 0.1	-	2.4 ± 0.2	-	2.0 ± 0.1	1.1 ± 0.1	Tr	Tr	Tr
22:5(n-3)	Tr	-	1.2 ± 0.2	-	1.5 ± 0.2	Tr	Tr	Tr	Tr
22:6(n-3)	8.2 ± 0.1	-	14.2 ± 1.2	3.6 ± 0.1	16.9 ± 0.6	9.4 ± 0.8	6.5 ± 0.1	7.8 ± 0.4	7.3 ± 0.8
24:0	Tr	9.8 ± 0.5	-	-	-	-	-	-	-
24:1	-	4.6 ± 0.3	-	-	-	-	-	-	-
Σ Saturated ^b	41.5 ± 0.1	88.3 ± 0.8	48.7 ± 0.6	49.8 ± 0.9	22.6 ± 0.2	48.2 ± 0.8	49.2 ± 1.7	47.2 ± 0.4	40.3 ± 1.4
Σ Monosat. ^c	14.4 ± 0.3	9.9 ± 0.4	15.9 ± 0.5	17.7 ± 0.2	11.5 ± 0.7	5.4 ± 0.7	15.0 ± 0.4	14.9 ± 0.2	23.7 ± 0.8
Σ n-6	33.9 ± 0.4	1.7 ± 0.5	20.0 ± 0.9	27.7 ± 0.9	45.3 ± 0.9	33.6 ± 0.5	26.9 ± 0.9	27.0 ± 0.1	28.7 ± 1.2
Σ n-3	10.1 ± 0.1	0	15.3 ± 1.2	4.9 ± 0.1	20.4 ± 0.4	12.7 ± 1.0	8.9 ± 0.5	10.8 ± 0.6	7.3 ± 0.8
Sat./unsat. ^d	0.7 ± 0.1	7.6 ± 0.6	1.0 ± 0.1	1.0 ± 0.1	0.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
n-6/n-3	3.4 ± 0.1	0	1.4 ± 0.2	5.7 ± 0.1	2.3 ± 0.1	2.6 ± 0.2	3.0 ± 0.1	2.5 ± 0.1	3.9 ± 0.5
UI ^e	202.4 ± 1.2	13.3 ± 1.3	185.4 ± 5.6	149.2 ± 3.5	309.8 ± 2.4	211.0 ± 7.5	162.1 ± 5.6	175.9 ± 1.8	172.7 ± 3.7

Values are the mean ± SEM of 2–4 determinations from each of three rats, except where marked with an asterisk, when only one determination was possible. The separation of PE and CL was not achieved using the TLC procedure described in Materials and Methods, hence the fatty acid composition of the pooled lipids is given; Tr, trace (< 1.0% total fatty acid); -, not detected.

^aCarbon chain length: number of double bonds.

^bSum of saturated fatty acids.

^cSum of monounsaturated fatty acids.

^dRatio of saturated to unsaturated fatty acids.

^eUnsaturation index = Σ [(percentage of each unsaturated fatty acid) (number of double bonds therein)].

TABLE 3. Fatty acid composition (mol %) of the neutral lipids of rat olfactory mucosa

Fatty Acid ^a	DAG*	FA*	TAG*	SE*
14:0	5.5 ± 1.2	43.1 ± 0.8	4.7 ± 0.8	10.6 ± 1.4
15:0	Tr	Tr	Tr	Tr
16:0	31.4 ± 2.0	30.7 ± 0.4	38.9 ± 0.6	37.5 ± 2.0
16:1(n-7)	Tr	Tr	3.7 ± 1.6	Tr
17:0	Tr	Tr	Tr	-
18:0	24.0 ± 0.6	8.6 ± 0.6	9.2 ± 0.8	17.8 ± 0.4
<i>cis</i> 18:1(n-9)	14.3 ± 1.8	13.5 ± 0.2	25.2 ± 0.7	24.9 ± 1.7
<i>trans</i> 18:1(n-9)	2.2 ± 0.3	1.4 ± 0.1	5.0 ± 0.6	Tr
18:2(n-6)	8.5 ± 2.1	2.8 ± 0.2	13.4 ± 0.2	9.3 ± 0.2
20:0	-	-	-	-
20:3(n-6)	Tr	-	Tr	-
20:4(n-6)	14.0 ± 5.0	-	Tr	Tr
20:5(n-3)	-	-	Tr	-
22:0	-	-	-	-
22:4(n-6)	Tr	-	Tr	-
22:5(n-6)	-	-	Tr	-
22:5(n-6)	-	-	Tr	-
22:6(n-3)	-	-	Tr	-
24:0	-	-	-	-
24:1	-	-	-	-
Σ Saturated ^b	61.0 ± 2.7	82.3 ± 0.3	52.8 ± 0.5	65.9 ± 1.5
Σ Monoenoic ^c	16.6 ± 1.5	14.9 ± 0.1	33.9 ± 0.3	24.9 ± 1.7
Σ n-6	22.5 ± 4.0	2.8 ± 0.2	13.4 ± 0.2	9.2 ± 0.2
Σ n-3	0	0	0	0
Sat./unsat. ^d	1.6 ± 0.2	4.7 ± 0.1	1.1 ± 0.1	2.0 ± 0.1
n-6/n-3	0	0	0	0
UI ^e	89.6 ± 16.5	20.5 ± 0.4	60.7 ± 0.6	43.4 ± 1.3

Values are the mean ± SEM of 2-4 determinations from each of three rats, except where marked with an asterisk when only one determination was possible. Tr, trace (< 1.0% total fatty acid); -, not detected.

^aCarbon chain length:number of double bonds.

^bSum of saturated fatty acids.

^cSum of monounsaturated fatty acids.

^dRatio of saturated to unsaturated fatty acids.

^eUnsaturated index = Σ [(percentage of each unsaturated fatty acid) (number of double bonds therein)].

was more abundant in PI than in the polyphosphoinositides. The polyphosphoinositides also contained significantly greater amounts of oleic acid (*cis*18:1, n-9) than did PI, although in general they contained more C16 and less C18 fatty acids. The fatty acid content of PA was very similar to that of the polyphosphoinositides and this is consistent with the biosynthesis of inositol lipids proceeding via PA (14).

Saturated fatty acids (mainly palmitate and stearate) accounted for less than 50% of the total fatty acid content of the phospholipids other than SM, and for between 50-66% of the total fatty acid content of the neutral lipid classes. In contrast over 88% of the fatty acid of SM was saturated. The fatty acid composition of SM was also unusual in its high content (> 25%) of arachidate (20:0), behenate (22:0), lignocerate (24:0), and nervonate (24:1), which were absent or detected only in trace amounts in the other phospholipids and neutral lipids. However, it must be noted that SM isolated from other sources has also been found to contain a preponderance of long-chain (> C20), saturated and monounsaturated fatty acids (reviewed in ref. 38).

The major fatty acids present in the neutral lipid classes were myristate, palmitate, stearate, oleate, and linoleate. Arachidonate accounted for 14% of the total fatty acid present in DAG, but was only present in trace amounts in TAG and SE fractions. Other fatty acids of chain length greater than C20 were, if detected, only present in trace amounts in DAG, TAG, and SE and were not detected as the free fatty acids. The fatty acid compositions of DAG and the polyphosphoinositides of rat olfactory mucosa were similar, with palmitate, stearate, oleate, and arachidonate predominating. Results from the analysis of lipids from other mammalian tissues have shown that the fatty acid content of the DAG generally resembles the fatty acid composition of the inositol lipids within the same tissue (39-41).

DISCUSSION

Olfactory mucosal lipids and odorant reception

The lipids of the olfactory mucosa have long been postulated to be involved in the reception and transduction

of odorants (5, 6). Indeed, olfactory stimulation has been suggested to be induced by conformational change of the receptor-bearing membranes as a consequence of the adsorption of odorants to the lipid layer of the membrane of the olfactory neuron (6, 7). Recent results indicate that lipids do play an important part in olfaction (8, 9).

The present study is the first comprehensive analysis of the lipids of the olfactory mucosa. The phospholipid to neutral lipid ratio of 8:2 obtained was comparable to that of 7:3 obtained previously for bovine olfactory mucosa (16). High phospholipid:neutral lipid ratios of 7:3 to 9:1 are usually observed in neuronal tissues (34). The low amounts of olfactory tissue obtained from rats and the technical difficulties involved in obtaining olfactory tissue from the rat without the concomitant excision of nonolfactory tissue meant that analysis of the total lipid content of the whole mucosa was carried out. Koyama, Sawada, and Kurihara (16), in their analysis of bovine olfactory mucosa, presented the analysis of the lipids of subcellular fractions. However, no significant differences between the various areas or subcellular fractions were found.

It has been shown that the lipid composition of liposomes greatly affects the magnitude and direction (depolarization or hyperpolarization) of membrane potential changes in response to odorants (8, 9). Responses of PC-, PE-, SM-, and cholesterol-containing liposomes to various odorants were studied (9). These are major lipids in both bovine (16) and rat olfactory mucosa (present study). In contrast to PC, PE, and cholesterol, the addition of SM to azolectin liposomes sensitized the liposomes to odorants, i.e., the response to odorants was enhanced at low odorant concentrations (16). On the basis of these results it was suggested that the lipid composition of the receptor membranes of each olfactory neuron may differ from cell to cell furnishing individual olfactory neurons with different sensitivities to various odorants (9). However, analytical techniques currently available only allow the accurate determination of pmol amounts of lipid and verification of this hypothesis is not likely in the foreseeable future.

Role of inositol lipids in the olfactory mucosa

It is widely accepted that inositol lipids are involved in signal transduction events (14, 42) rather than their presence being an absolute requirement for the maintenance of membrane structure and stability. The presence of PI and the polyphosphoinositides, PI-P and PI-P₂ which are produced by the sequential phosphorylation of PI by specific kinases, suggests that the so-called PI cycle may operate within rat olfactory mucosa. This has been confirmed by recent unpublished results (Wood, M. A. and Y. Russell). In this cycle PI acts as the parent molecule for the generation of the two second messengers DAG and inositol-1,4,5-trisphosphate by the action of the enzyme phosphoinositidase C on PI-P₂ (14, 43). Only trace quan-

ties of polyphosphoinositides have been detected in all tissues studied to date (44, 45). The difference between the fatty acid content of the total PI and that of the total polyphosphoinositides observed in the present study is consistent with the hypothesis that only a fraction of the PI of the cell is actively metabolized to the corresponding polyphosphoinositides (46). It has been suggested that the turnover of polyphosphoinositides could operate as a signal transduction mechanism in vertebrate olfaction given that the odorant-sensitive adenylate cyclase is only modulated by certain odorant types (47).

Membrane function and polyunsaturated fatty acid content

The high polyunsaturated fatty acid content of the rat olfactory mucosa would suggest that the membranes of the olfactory mucosa are in a highly fluid state. Lipid fluidity is dependent on the nature of the phospholipid head group and its conformation, and the nature of the phospholipid acyl chains (48), with a decrease in chain length or an increase in the degree of unsaturation leading to an increase in fluidity. The high (>30%) docosahexaenoate content of the retinal rod cells is known to confer a high degree of fluidity on the membranes of these cells (18). A highly fluid receptor membrane would enable transducing enzymes to work optimally over a wide range of temperatures. Indeed, the odorant-modulated adenylate cyclase has been observed to operate relatively independently of its microenvironment (49). However, although fatty acid composition does provide some indication of membrane fluidity, certain factors, such as the composition of individual lipid classes and the presence of free cholesterol, do counterbalance this effect and verification of the above speculation awaits direct measurement of the fluidity of the membranes of rat olfactory mucosa.

The enzyme phospholipase A₂, which cleaves fatty acids from the C2 position of the glycerol backbone of phospholipids, has been suggested to be receptor activated in a fashion similar to the receptor-activated adenylate cyclase. Arachidonate is the precursor of eicosanoids, such as prostaglandins and leukotrienes, and is generally found in the C2 position on the glycerol backbone of phospholipids. Evidence has been presented implicating eicosanoids as second messengers (50). It is possible that arachidonic acid could play an important role, as the precursor of eicosanoids, in the olfactory mucosa, in particular in the biosynthesis of hydroperoxyeicosatetraenoic acids which are synthesized both by the lipoxygenase pathway and directly by the action of P450 oxygenase (51) which is highly active in the rat olfactory mucosa (52, 53).

Presence of *trans* fatty acids in rat olfactory mucosa

In mammalian tissues, unsaturated fatty acids are present predominantly as the *cis* isomer (35). In the rat olfactory mucosa the *trans* 18:1 fatty acid, elaidic acid, was

present as 1–5% of the total fatty acid in the majority of the lipids. The greatest amount of *trans* 18:1 was found in TAG, in which it accounted for approximately 5 mol % of the total fatty acid. The *trans* 18:1 was also present in total phospholipid and neutral lipid fractions of the brain and liver of the male Wistar rats used in the present study (Russell, Y., and P. Evans, unpublished results). It is well established that the fatty acid composition of lipids in man and animals reflects the dietary intake of major fatty acids in tissues such as the liver, adipose tissue, platelets, and erythrocytes (54, 55). Recent results have also demonstrated dietary modulation of phospholipids, in particular PI, in rat retina (56). The presence of *trans* 18:1 in rat olfactory epithelium was attributed to hydrogenated fats in the diet of the rats (35). Change in fatty acid composition of the lipids of olfactory mucosa with variations in dietary fatty acids has not been investigated to date.

Analysis of the lipid composition of rat olfactory mucosa did not reveal the presence of any unusual lipids, although the analysis was not facilitated by the small quantities of lipid available. Lipids that have previously been postulated to have important roles in signal transduction in other tissues were detected and investigations into the role(s) of inositol lipids in rat olfactory mucosa are currently underway. ■

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