Altered brain lipid composition in cyclooxygenase-2 knockout mouse

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Abstract Cyclooxygenase (COX)-2 plays an important role in brain arachidonic acid (20:4n-6) metabolism, and its expression is upregulated in animal models of neuroinflammation and excitotoxicity. Our hypothesis was that brain lipid composition would be altered in COX-2 knockout (COX-2$^{-/-}$) compared with wild-type (COX-2$^{+/+}$) mice, reflecting the important role of COX-2 in brain lipid metabolism. Concentrations of different lipids were measured in high-energy microwaved brain from COX-2$^{-/-}$ and COX-2$^{+/+}$ mice. Compared with the COX-2$^{+/+}$ mouse brain, the brain of the COX-2$^{-/-}$ mouse had a statistically significant 15% increase in phosphatidylserine (PtdSer) and significant 37, 27, and 32% reductions in triacylglycerol and cholesterol concentrations and in the cholesterol-to-phospholipid ratio, respectively. The normalized concentration of palmitic acid (16:0) was increased in PtdSer, as was the brain concentration of unesterified arachidic acid (20:0). A lifetime absence of COX-2 produces multiple changes in brain lipid composition. These changes may be related to reported changes in fatty acid kinetics and in resistance to neuroinflammation and excitotoxicity in the COX-2$^{-/-}$ mouse.—Ma, K., R. Langenbach, S. I. Rapoport, and M. Basselin. **Altered brain lipid composition in cyclooxygenase-2 knockout mouse. *J. Lipid Res.* 2007.** 48: **848–854.**

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Cyclooxygenase (COX)-2 catalyzes the conversion of the second messenger, arachidonic acid (AA; 20:4n-6), to prostaglandins and thromboxanes (1, 2). It participates in neuroreceptor-initiated signaling involving the activation of cytosolic phospholipase A_2 (cPLA$_2$), which releases AA from membrane phospholipids (3–5). Brain COX-2 expression is upregulated in animal models of excitotoxicity, neuroinflammation, and ischemia (6–9), and COX-2 has been considered a target of nonsteroidal anti-inflammatory drugs in the treatment of clinical neuroinflammation (10).

COX-2 knockout (COX-2$^{-/-}$) mice have been created to elucidate the roles of COX-2 in brain and other organs (11). Compared with its wild-type (COX-2$^{+/+}$) littermate, the brain of the COX-2$^{-/-}$ mouse shows altered neurotransmitter-initiated signaling involving AA and its metabolites and increased resistance to ischemia, stroke, and *N*-methyl-D-aspartate-induced excitotoxicity (5, 9, 12). It also demonstrates upregulated expression of a number of enzymes involved in AA metabolism (13), including COX-1, cPLA$_2$, and secretory phospholipase A_2 (sPLA$_2$), as well as a reduced concentration of prostaglandin E_2, a COX-2-mediated product of AA (14, 15).

To further address the role of COX-2 in brain lipid metabolism, in this study we examined brain lipid composition in COX-2$^{-/-}$ and COX-2$^{+/+}$ mice. We used high-energy microwaving to prepare the brain for analysis to prevent ischemia-induced changes in concentrations of brain unesterified fatty acids, acyl-CoAs, anandamide, and eicosanoids (16–20). We measured brain concentrations of unesterified fatty acids and "stable" lipids [phospholipids, total cholesterol (cholesterol plus cholesteryl ester), and triacylglycerol] as well as concentrations of fatty acids esterified in these stable lipids.

MATERIALS AND METHODS

Chemicals

1,2-Diheptadecanoyl-*sn*-glycero-3-phosphocholine (di-17:0-PC) and heptadecanoic acid (17:0), used as internal standards, were obtained from Sigma-Aldrich (St. Louis, MO). Silica gel TLC plates were purchased from EMD Chemicals (Gibbstown, NJ). All solvents were HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ) or EMD Chemicals. Nembutal® (50 mg/ml) was obtained from Abbott Laboratories (Chicago, IL). K603-100 kits were purchased from BioVision Research

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Abbreviations: AA, arachidonic acid; CerPCho, sphingomyelin; ChoGpl, choline glycerophospholipid; COX, cyclooxygenase; cPLA$_2$, cytosolic phospholipase A_2; EtnGpl, ethanolamine glycerophospholipid; FAME, fatty acid methyl ester; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; sPLA$_2$, secretory phospholipase A_2.

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Products (Mountain View, CA), and TR0100 kits were obtained from Sigma-Aldrich.

Animals

This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication 86-23) under a protocol approved by the Animal Care and Use Committee of the National Institute of Child Health and Human Development. Three month old male COX-$2^{-/-}$ and COX-$2^{+/+}$ mice from a C57BL/6J×129/Ola genetic background (11) (Taconic Farms, Germantown, NY) were provided free access to standard rodent chow (NIH-31; Zeigler, Gardners, PA) and water. They were euthanized by an overdose of Nembutal® (100 mg/kg, ip) and then subjected to head-focused microwave irradiation (5.5 kW, 1.2 s; Cober Electronics, Stamford, CT) to stop brain metabolism (16–20). The brain was removed, frozen on dry ice, and stored at −80°C until analyzed.

Brain lipid extraction and quantification

Total lipids from frozen microwaved whole brain were extracted using a partition system of chloroform-methanol-0.5 M KCl (2:1:0.75, v/v) (21). The lipids were separated by TLC on silica gel-60 plates. Phospholipid classes were separated using the solvent system chloroform-methanol-glacial acetic acid-water (60:50:1:4, v/v). This method separates ethanolamine glycerophospholipid (EtnGpl), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), choline glycerophospholipid (ChoGpl), and sphingomyelin (CerPCho). Cholesteryl esters, triacylglycerol, and unesterified fatty acids were separated using the solvent system heptane-diethylether-glacial acetic acid (60:40:3, v/v) (22). Each band was scraped from the TLC plate and converted to fatty acid methyl esters (FAMEs) with 1% H_2SO_4 in anhydrous methanol for 3 h at 70°C (23).

Individual FAMEs were separated by gas-liquid chromatography, and fatty acid concentrations were calculated by proportional comparison of gas-liquid chromatography peak areas with the areas of 17:0 and di-17:0-PC internal standards (24). Phospholipid content was determined by assaying the lipid phosphorus content of the TLC scrapings (25). Briefly, phospholipid bands were scraped into a glass tube, 0.5 ml of water and 0.65 ml of perchloric acid (70%) were added, and the contents were digested at 180°C for 1 h. Samples were cooled to room temperature, 0.5 ml of ascorbic acid (10%, w/v), 0.5 ml of ammonium molybdate (2.5%, w/v), and 3.0 ml of water were added, and the reaction mixture was mixed. It then was boiled for 5 min and cooled, and the absorbance was measured at 797 nm. Phospholipid concentrations were determined using standard curves. Total cholesterol (cholesterol plus cholesteryl ester) and triacylglycerol concentrations were determined with the commercial kits K603-100 (Biovision) and TR0100 (Sigma-Aldrich), respectively.

Statistical analysis

Statistical analysis was performed by unpaired, two-tailed *t*-tests using GraphPad Prism version 4.0b for Macintosh (GraphPad Software, San Diego, CA; www.graphpad.com). Results are expressed as means ± SD (n = 8). Significance was taken as $P \leq 0.05$.

RESULTS

Brain weight

There was no statistically significant difference in mean brain weight between COX-$2^{-/-}$ and COX-$2^{+/+}$ mice, 0.42 ± 0.05 and 0.39 ± 0.05 g, respectively (n = 8).

Unesterified plasma fatty acids

Although not part of this study, we reported previously that there was no statistically significant difference in the mean concentrations of plasma palmitic (16:0), palmitoleic (16:1n-7), stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6), α-linolenic (18:3n-3), arachidonic (20:4n-6), or docosahexaenoic (22:6n-3) acid between unanesthetized COX-$2^{-/-}$ and COX-$2^{+/+}$ mice (5).

Brain phospholipid, triacylglycerol, and cholesterol concentrations

The brain concentration of total phospholipid (nmol/g wet weight) did not differ significantly between COX-$2^{-/-}$ and COX-$2^{+/+}$ mice (**Table 1**). Of individual phospholipids measured, the brain concentration of PtdSer was increased significantly by 15% in the COX-$2^{-/-}$ mouse, whereas concentrations of EtnGpl, ChoGpl, PtdIns, and CerPCho did not differ significantly between groups (Table 1). Mean brain concentrations of triacylglycerol and cholesterol were decreased significantly by 37% and 27%, respectively (Table 1), and the cholesterol-to-phospholipid ratio was reduced by 32%.

Unesterified brain fatty acids

Of the unesterified fatty acids examined (**Table 2**), only the brain concentration of arachidic acid (20:0) was altered significantly (+58%) in COX-$2^{-/-}$ compared with COX-$2^{+/+}$ mice.

Esterified fatty acids in brain cholesteryl ester, triacylglycerol, and phospholipids

The concentration of esterified palmitic acid (16:0) in cholesteryl ester (nmol/g wet weight brain) was significantly less (−40%) in COX-$2^{-/-}$ than in COX-$2^{+/+}$ mice, whereas concentrations of other fatty acids were unchanged. Similarly, calculated concentrations of esterified

TABLE 1. Brain lipid concentrations in COX-$2^{+/+}$ and COX-$2^{-/-}$ mice

Lipid	COX-$2^{+/+}$	COX-$2^{-/-}$
	μmol/g brain	
EtnGpl	24.3 ± 2.4	26.6 ± 3.6
PtdIns	2.4 ± 0.4	2.2 ± 0.4
PtdSer	9.4 ± 0.4	10.8 ± 1.2[a]
ChoGpl	23.4 ± 3.3	24.1 ± 3.2
CerPCho	4.3 ± 1.0	4.5 ± 0.8
Total phospholipid	63.9 ± 7.6	68.9 ± 5.4
Triglyceride	0.51 ± 0.17	0.32 ± 0.12[b]
Total cholesterol	51.8 ± 8.1	38.0 ± 11.8[b]
	(cholesterol plus cholesteryl ester)	
Total cholesterol/total phospholipid	0.810 ± 0.095	0.551 ± 0.125[c]

CerPCho, sphingomyelin; ChoGpl, choline glycerophospholipid; COX, cyclooxygenase; EtnGpl, ethanolamine glycerophospholipid; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine. Values shown are means ± SD (n = 8).

[a] $P < 0.01$ versus COX-$2^{+/+}$ mean.

[b] $P < 0.05$ versus COX-$2^{+/+}$ mean.

[c] $P < 0.001$ versus COX-$2^{+/+}$ mean.

TABLE 2. Esterified fatty acid concentrations in brain lipids of COX-$2^{+/+}$ and COX-$2^{-/-}$ mice

Fatty Acid	COX-$2^{+/+}$	COX-$2^{-/-}$	COX-$2^{+/+}$	COX-$2^{-/-}$
	Cholesteryl Ester		Triacylglycerol	
16:0	40.0 ± 15.4	23.6 ± 11.4[a]	234 ± 88	149 ± 31[a]
16:1n-7	nd	nd	17.0 ± 8.1	2.6 ± 1.3[b]
18:0	54.1 ± 21.0	60.2 ± 37.1	146 ± 16	131 ± 17
18:1n-9	31.1 ± 13.5	26.5 ± 11.2	203 ± 98	88 ± 21[c]
18:2n-6	17.2 ± 7.7	9.9 ± 6.0	173 ± 95	56 ± 28[c]
18:3n-3	nd	nd	nd	nd
20:0	nd	nd	8.0 ± 5.0	3.7 ± 1.2[a]
20:4n-6	19.7 ± 8.0	16.8 ± 7.0	26.0 ± 9.0	18.3 ± 6.5[a]
22:6n-3	29.5 ± 14.9	26.1 ± 9.1	83 ± 35	57 ± 18
	Unesterified Fatty Acid		Phospholipid	
16:0	32.5 ± 6.0	27.3 ± 10.0	24,052 ± 3,551	23,394 ± 1717
16:1	nd	nd	460 ± 120	395 ± 146
18:0	51.5 ± 4.0	52.8 ± 14.8	24,632 ± 2,782	25,143 ± 2,461
18:1n-9	16.7 ± 4.8	13.4 ± 7.1	22,220 ± 5,077	21,342 ± 1,638
18:2n-6	15.5 ± 8.9	18.8 ± 11.1	712 ± 112	810 ± 111
18:3n-3	nd	nd	nd	nd
20:0	0.98 ± 0.37	1.55 ± 0.42[a]	481 ± 112	496 ± 105
20:4n-6	5.1 ± 2.2	3.6 ± 2.3	9,718 ± 1,060	9,611 ± 940
22:6n-3	8.4 ± 4.6	7.2 ± 4.3	18,425 ± 2,406	17,711 ± 1,456

All values shown are means ± SD (nmol/g brain; n = 8). nd, not detected.
[a] $P < 0.05$ versus COX-$2^{+/+}$ mean.
[b] $P < 0.001$ versus COX-$2^{+/+}$ mean.
[c] $P < 0.01$ versus COX-$2^{+/+}$ mean.

palmitic (16:0), palmitoleic (16:1n-7), oleic (18:1n-9), linoleic (18:2n-6), arachidic (20:0), and arachidonic (20:4n-6) acids were significantly less in brain triacylglycerol (Table 2).

In the COX-$2^{+/+}$ brain, ChoGpl contained mainly saturated palmitate and stearate, monounsaturated oleic acid, polyunsaturated AA, and docosahexaenoic acid. PtdIns was rich in stearate and AA, whereas PtdSer was enriched in stearate, oleate, and docosahexaenoate (**Table 3**). CerPCho was rich in stearate, lignoceric acid (24:0), and nervonic acid (24:1n-9). Although the esterified fatty acid concentration in total phospholipid did not differ significantly between groups (Table 2), esterified 16:0, 18:1n-9, and 20:4n-6 concentrations (nmol/g wet weight brain) were increased in PtdSer.

Esterified fatty acids normalized to stable lipid concentration

When esterified fatty acid concentrations in the stable lipids were normalized to the concentration of the stable lipid in which they were found and calculated (nmol fatty acid/μmol lipid) (**Table 4**), the only significant effect of the COX-$2^{-/-}$ condition was a 62% increase of esterified palmitate (16:0) in PtdSer.

DISCUSSION

Brain lipid composition was altered in several ways in the COX-$2^{-/-}$ compared with the COX-$2^{+/+}$ mouse. There was a statistically significant 15% increase in the brain concentration of PtdSer and 37% and 27% decreased concentrations of triacylglycerol and cholesterol, respectively. Esterified fatty acid concentrations (nmol/g wet weight brain) generally reflected the concentrations of the stable lipids in which they were found, with the exception of palmitate (16:0), which was disproportionately increased in PtdSer. In addition, unesterified arachidic acid (20:0) was increased by 58%. In another study, we reported no difference in plasma concentrations of unesterified fatty acids between COX-$2^{-/-}$ and COX-$2^{+/+}$ mice (5). Along with evidence that the activities of several enzymes in the AA cascade, $cPLA_2$, $sPLA_2$, and COX-1 (13), are increased in the brain of the COX-$2^{-/-}$ mouse (15) and that neuroreceptor-initiated signaling involving AA and its metabolites is altered (5), our findings indicate that a lifelong absence of COX-2 produces important changes in brain lipid composition and AA metabolism.

Our measured brain stable lipid and esterified fatty acid concentrations in the COX-$2^{+/+}$ mice are comparable to previously published values for nonmicrowaved (% fatty acid composition) and microwaved (nmol/g wet weight brain) mouse brain (26–28), whereas our unesterified fatty acid concentrations are similar to reported values in the microwaved mouse brain (26, 29). Concerning the sum of the FAMEs derived from fatty acids for glycerophospholipids in COX-$2^{+/+}$ and COX-$2^{-/-}$ mice (Table 4), we obtained values of 2.04 and 1.92 for ChoGpl, 2.5 and 2.6 for PtdIns, 1.46 and 1.36 for EtnGpl, and 1.37 and 1.54 for PtdSer. These values are in the expected ranges, in that FAMEs derived from plasmanyl and plasmenyl components of EtnGpl and ChoGpl were not included in the FAME tables (30); nor were FAMEs of other fatty acids that we did not list (31). For triacylglycerol, the ratio was much <3, likely because our concentration estimate, with its large standard deviation, was too high (0.51 μmol/g compared with 0.12–0.3 μmol/g in the literature) (32).

TABLE 3. Esterified fatty acid concentrations in brain phospholipid classes of COX-2$^{+/+}$ and COX-2$^{-/-}$ mice

Fatty Acid	COX-2$^{+/+}$	COX-2$^{-/-}$	COX-2$^{+/+}$	COX-2$^{-/-}$
	EtnGpl		PtdIns	
16:0	2,309 ± 335	2,427 ± 420	285 ± 118	268 ± 47
16:1	123 ± 46	134 ± 41	nd	nd
18:0	9,816 ± 1,531	9,883 ± 1,407	2,323 ± 542	2,211 ± 473
18:1n-9	6,674 ± 793	7,193 ± 1,576	767 ± 127	705 ± 229
18:2n-6	199 ± 78	260 ± 72	32 ± 21	27 ± 10
18:3n-3	nd	nd	nd	nd
20:0	238 ± 94	231 ± 59	72 ± 55	40 ± 20
20:4n-6	4,972 ± 840	5,005 ± 598	2,125 ± 793	2,021 ± 619
22:6n-3	10,681 ± 1,800	10,622 ± 1,570	280 ± 135	301 ± 107
	PtdSer		ChoGpl	
16:0	159 ± 58	286 ± 76[a]	18,990 ± 3,410	20,749 ± 4,394
16:1	nd	nd	nd	nd
18:0	6,812 ± 1,374	7,282 ± 917	8,041 ± 1,284	8,078 ± 1,369
18:1n-9	2,604 ± 230	3,390 ± 357[b]	12,537 ± 2,582	10,931 ± 1,680
18:2n-6	49 ± 33	52 ± 34	386 ± 106	384 ± 66
18:3n-3	nd	nd	nd	nd
20:0	96 ± 32	105 ± 22	147 ± 45	128 ± 29
20:4n-6	323 ± 60	404 ± 77[c]	2,488 ± 541	2,317 ± 328
22:6n-3	4,100 ± 865	4,503 ± 1,264	2,641 ± 581	2,481 ± 343
	CerPCho			
16:0	125.2 ± 71.9	100.0 ± 54.4		
16:1	2.6 ± 1.0	3.8 ± 3.0		
18:0	3,522 ± 429	3,108 ± 580		
18:1n-9	107 ± 75	77.8 ± 30.4		
18:2n-6	10.5 ± 1.8	16.6 ± 8.2		
18:3n-3	nd	nd		
20:0	160 ± 50	141 ± 39		
20:4n-6	181 ± 129	130 ± 55		
24:0	243 ± 201	191 ± 92		
24:1n-9	651 ± 189	656 ± 274		
22:6n-3	30.4 ± 19.7	20.4 ± 11.2		

All values shown are means ± SD (nmol/g brain; n = 8). nd, not detected.
[a] $P < 0.01$ versus COX-2$^{+/+}$ mean.
[b] $P < 0.001$ versus COX-2$^{+/+}$ mean.
[c] $P < 0.05$ versus COX-2$^{+/+}$ mean.

Although abnormal behavior has not been noted in the COX-2$^{-/-}$ mouse (33), its brain compared with that of the COX-2$^{+/+}$ mouse shows increased resistance to injury produced by ischemia or kainate or *N*-methyl-D-aspartic acid microinjection (5, 9, 12, 34). Some of the abnormalities in lipid composition that we report in this study may contribute to this resistance. For example, the decreased brain triacylglycerol concentration in the COX-2$^{-/-}$ mouse may increase its resistance to ischemia/reperfusion damage (9, 12), because triacylglycerol can be a source of nutrient fatty acids for brain phospholipid biosynthesis (35–37) and its concentration is increased in cerebral ischemia (38, 39).

The total cholesterol concentration (cholesterol plus cholesteryl ester) was decreased by 27% in the COX-2$^{-/-}$ brain, thereby reducing the total cholesterol-phospholipid ratio by 32%. The reduction in this ratio would be expected to increase membrane fluidity (40). The reduction was not related to a reduced brain CerPCho concentration (Tables 1, 3), which can alter cholesterol concentration (41, 42), but may have been related to reduced COX-2-derived metabolites such as prostacyclin, because prostacyclin was reported to enhance neutral and acid cholesteryl ester hydrolase activity in aortic smooth muscle cells (43). The nonnormalized concentration of palmitic acid was decreased significantly in cholesteryl ester.

In nervous tissue, PtdSer is synthesized from preexisting phosphatidylcholine (PtdCho) or phosphatidylethanolamine (PtdEtn) by exchanging choline or ethanolamine with L-serine in a reaction catalyzed by PtdSer synthase I/II (44, 45). However, the increased brain concentration of PtdSer in the COX-2$^{-/-}$ mouse was not accompanied by a significant change in ChoGpl or EtnGpl concentration, although changes in the PtdCho and PtdEtn components of ChoGpl or EtnGpl were not measured, and inhibition of PtdSer decarboxylation to PtdEtn cannot be ruled out (46, 47). On the other hand, increased brain PtdSer concentration may have been related to increased activity of $sPLA_2$, for which PtdSer and PtdEtn are preferred substrates (15, 48). The increased unesterified arachidate (20:0) suggests disturbed saturated fatty acid synthesis or oxidation, as does the disproportionate increase of normalized esterified palmitate (16:0) (+80%) in PtdSer.

Increased brain PtdSer in the COX-2$^{-/-}$ mouse may influence multiple processes, as PtdSer modulates cell signaling and apoptosis (45, 49) and can regulate the translocation and activity of protein kinase C, which mediates exocytosis, signal transduction, and cell division (50–52). PtdSer also can modulate the binding of glutamate to its receptors (53), the expression of acetylcholine receptors

TABLE 4. Brain fatty acid concentration per μmol of phospholipid class, triacylglycerol, or cholesteryl ester class in COX-$2^{+/+}$ and COX-$2^{-/-}$ mice

Fatty Acid	COX-$2^{+/+}$	COX-$2^{-/-}$	COX-$2^{+/+}$	COX-$2^{-/-}$
	EtnGpl		PtdIns	
16:0	0.0997 ± 0.0187	0.0922 ± 0.0183	0.1172 ± 0.0504	0.1246 ± 0.0360
16:1	0.0051 ± 0.0023	0.0050 ± 0.0015	nd	nd
18:0	0.4073 ± 0.0802	0.3738 ± 0.0498	0.9878 ± 0.3306	1.0222 ± 0.3118
18:1n-9	0.2769 ± 0.0483	0.2726 ± 0.0610	0.3248 ± 0.0944	0.3282 ± 0.1354
18:2n-6	0.0083 ± 0.0038	0.0098 ± 0.0027	0.0160 ± 0.0098	0.0180 ± 0.0130
18:3n-3	nd	nd	nd	nd
20:0	0.0096 ± 0.0037	0.0088 ± 0.0023	0.0304 ± 0.0024	0.0260 ± 0.0062
20:4n-6	0.2058 ± 0.0390	0.1898 ± 0.0252	0.9100 ± 0.4164	0.9472 ± 0.3914
22:6n-3	0.4423 ± 0.0846	0.4031 ± 0.0671	0.1034 ± 0.0338	0.1418 ± 0.0624
	PtdSer		ChoGpl	
16:0	0.0170 ± 0.0070	0.0276 ± 0.0109[a]	0.8600 ± 0.2230	0.8828 ± 0.1711
16:1	nd	nd	nd	nd
18:0	0.6061 ± 0.2479	0.7001 ± 0.2300	0.3627 ± 0.0813	0.3443 ± 0.0554
18:1n-9	0.2636 ± 0.0229	0.3257 ± 0.0996	0.5604 ± 0.1150	0.4658 ± 0.0648
18:2n-6	0.0053 ± 0.0039	0.0049 ± 0.0036	0.0176 ± 0.0057	0.0164 ± 0.0023
18:3n-3	nd	nd	nd	nd
20:0	0.0101 ± 0.0032	0.0102 ± 0.0041	0.0067 ± 0.0021	0.0055 ± 0.0020
20:4n-6	0.0343 ± 0.0062	0.0385 ± 0.0129	0.1127 ± 0.0328	0.0991 ± 0.0157
22:6n-3	0.4355 ± 0.0897	0.4330 ± 0.1901	0.1187 ± 0.0340	0.1066 ± 0.0204
	Triacylglycerol			
16:0	0.4937 ± 0.2395	0.4730 ± 0.2170		
16:1	0.0467 ± 0.0400	0.0523 ± 0.0321		
18:0	0.2973 ± 0.0851	0.3675 ± 0.1092		
18:1n-9	0.4323 ± 0.2334	0.3392 ± 0.2166		
18:2n-6	0.3685 ± 0.2145	0.2710 ± 0.1484		
18:3n-3	nd	nd		
20:0	0.0298 ± 0.0130	0.0457 ± 0.0193		
20:4n-6	0.0559 ± 0.0278	0.0619 ± 0.0261		
22:6n-3	0.2816 ± 0.1431	0.1964 ± 0.0724		

All values shown are means ± SD (nmol/μmol; n = 8). nd, not detected.
[a] $P < 0.05$ versus COX-$2^{+/+}$ mean.

(54), acetylcholine (55) and dopamine release (56), and Ca^{2+} and γ-aminobutyrate uptake by neurons (57, 58).

Increased brain PtdSer also has been reported in $cPLA_2$ knockout mice (26) as well as in postmortem brain from patients with Alzheimer disease (59), schizophrenia (60), allergic encephalomyelitis (61), and Down syndrome (62). Brain PtdSer is decreased by ischemia (63, 64), whereas PtdSer pretreatment is reported to reduce ischemic brain damage (65). Thus, the PtdSer concentration seems to be a sensitive marker of brain metabolic dysfunction.

As far as we know, ours is the first study to quantify brain concentrations of a number of lipid classes in the COX-$2^{-/-}$ mouse. The reduced level of PtdSer suggests that it might now be worthwhile to measure individual PtdSer species containing different acyl groups in the *sn*-1 and *sn*-2 positions of PtdSer. With regard to EtnGpl and ChoGpl, they are composed of PtdEtn and ethanolamine plasmalogen at a ratio of 11.1 μmol/g to 7.4 μmol/g and of PtdCho and choline plasmalogen at a ratio of 22.6 μmol/g to 1.1 μmol/g, respectively (29), but as the brain concentrations of EtnGpl and ChoGpl did not differ significantly between the COX-$2^{-/-}$ and COX-$2^{+/+}$ mice, we have no reason to believe that the concentrations of their phosphatidyl and plasmalogen components would differ significantly between the groups, although this can be tested. Species of PtdIns also might be examined in the future (66), despite the absence of a significant difference in its concentration between the two mouse groups.

In conclusion, a lifelong absence of COX-2 results in multiple changes in brain lipid composition, including an increase in brain PtdSer and a reduction in brain cholesterol. These changes correspond to changes in enzymes and metabolites of the AA cascade (14, 15). Our findings provide new information on how COX-2 influences brain lipid composition and may be related to changes in brain structure and function and in susceptibility to ischemia and neurotoxicity in the COX-$2^{-/-}$ mouse. For comparison, it would be of interest to examine the effects of chronically administered COX inhibitors (10) on brain lipid composition and parameters of the AA cascade.

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