

Elongation, desaturation, and esterification of essential fatty acids by fetal rat brain in vivo

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Abstract Tracer amounts of either [$1\text{-}^{14}\text{C}$]linolenic (18:3n-3, LNA), or [$1\text{-}^{14}\text{C}$]linoleic (18:2n-6, LA) acids were intracranially injected into 19- to 20-day-old rat fetuses, and the time course of the in vivo formation and esterification of their long chain polyenoic metabolites was determined for up to 20 h. A rapid disappearance of free LNA and LA, with apparent half-lives of 60 and 40 min, respectively, was noticed. One hour after LNA injection, 32.3% and 14.3% of the total brain radioactivity was found in the neutral glyceride (NG) and phospholipid (PL) fractions, respectively. After 20 h, PL radioactivity attained a level of 75%. Phosphatidylcholine (PC), diacylglycerol (DG), and triacylglycerol (TG) constituted 40%, 23%, and 9% of the total brain label at 1 h, and 35%, 10%, and 14% at 20 h, respectively. Ethanolamine-containing PL (including plasmalogen) radioactivity accounted for about 10% up to 6 h and increased nearly 3-fold at 20 h, primarily due to an increase in the amount of labeled docosapentaenoic and docosahexaenoic acids (DHA), the elongation-desaturation products of LNA. A similar pattern of incorporation into NG and PL fraction was observed after the administration of [$1\text{-}^{14}\text{C}$]LA. After 1 h, PC, DG, and TG species constituted 23%, 10%, and 23% of the total brain radioactivity, whereas after 20 h it accounted for 44%, 6%, and 10%, respectively. Although radioactivity in the ethanolamine PL also increased substantially from 4% at 1 h to 29% at 20 h, the main labeled fatty acid in this fraction was LA. Labeled arachidonic acid (AA) constituted 42.7% of the total radioactivity in phosphatidylinositol (PI) at 20 h. At this time, it comprised 12.5% and 14% of the total radioactivity in PC and ethanolamine PL, respectively, suggesting a high degree of esterification selectivity. Comparison of the total amounts of LA and LNA and their corresponding labeled AA and DHA metabolites in brain and liver after 3 and 6 h indicated that the contribution of liver metabolism to the elongation-desaturation under these conditions was negligible. One hour after intracerebral injection of [^3H]DHA (22:6n-3) or [^3H]AA (20:4n-6), 29.2% and 12% of total radioactivity, respectively, was found in the ethanolamine PL while 20% and 40% was incorporated in PC, respectively. PI labeling by [^3H]AA was 6- to 8-fold higher than that seen in the presence of DHA. A high percent of radioactivity (26.9% and 18.2%) was found in DG and TG species. ■ The present results indicate that the near-term fetal rat brain has the capacity to take up, convert, and selectively esterify essential fatty acids and their long-chain polyenoic derivatives into phospholipids.—Green, P., and E. Yavin. Elongation, desaturation, and esterification of essential fatty acids by fetal rat brain in vivo. *J. Lipid Res.* 1993. 34: 2099-2107.

Supplementary key words essential fatty acids • fetal brain development • docosahexaenoic acid • arachidonic acid • elongation-desaturation

Docosahexaenoic (DHA, 22:6n-3) and arachidonic (AA, 20:4n-6) acids constitute the major long-chain polyunsaturated fatty acids (PUFA) of brain tissue (1, 2). DHA and AA are generated by elongation-desaturation of linolenic acid (LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6), respectively, both of which are essential fatty acids (EFA) normally provided through the diet. PUFA serve as integral membrane components and are believed to modulate physical parameters such as membrane fluidity (3, 4). In addition, PUFA may participate as nonesterified compounds or after conversion to bioactive eicosanoids (5) in complex cellular processes such as signal transduction and growth regulation (6).

The bulk of long-chain PUFA is probably transported to the brain from the liver, the principal organ responsible for the elongation-desaturation of EFA (7, 8). Several laboratories have demonstrated that brain is capable of forming its own PUFA by elongation-desaturation of EFA. This was shown both in vivo, in the weanling (9, 10) and the perinatal rat (11, 12), and in vitro, in cell culture (13, 14) and in brain tissue homogenates (15). Moore, Yoder, and Spector have recently localized the elongation-desaturation pathways of EFA to the cerebrovascular endothelium (16) and to astrocytes (17). Cortical and cerebellar neurons from the postnatal rat were not able to synthesize long chain PUFA, although they could esterify them into phospholipids after uptake from the medium (17). Selective esterification of long-chain PUFA into adult rat brain phospholipids was demonstrated both in vivo (18, 19) and in vitro in subcellular fractions (20-22).

Neuronal membranes contain fairly large amounts of PUFA (1, 2). The developmental stage during which PUFA are acquired and the metabolic routes for their ac-

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; DG, diacylglycerol; EFA, essential fatty acids; FFA, free fatty acids; HPLC, high pressure liquid chromatography; LNA, linolenic acid; LA, linoleic acid; NG, neutral glycerides; PC, phosphatidylcholine; PC-pl, choline plasmalogen; PE, phosphatidylethanolamine; PE-pl, ethanolamine plasmalogen; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, long-chain polyunsaturated fatty acids; PL, phospholipid; TLC, thin-layer chromatography; TG, triacylglycerol.

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quisition are questions of great interest. It is notable that the last several days of intrauterine life in the rat (days 17 to 21) are characterized by a rapid increase in brain weight (12). At the end of this period, neurons comprise the bulk of the cell population and have ceased dividing. At this time, the brain enters a phase of active growth, primarily comprising astroglia and oligodendroglia proliferation and differentiation (23). As the period prior to birth is identified primarily with the generation of neuronal-type plasma membranes, a detailed examination of the ability of the fetal brain to convert EFA to PUFA warranted investigation.

This report provides evidence that tracer amounts of labeled LNA and LA injected intraventricularly into the fetal brain are rapidly converted and selectively esterified as PUFA into various lipid fractions. A preliminary account of this work has recently appeared (24).

MATERIALS AND METHODS

Surgery and isotope administration

Pregnant Wistar rats (250–300 g body weight) at 19–20 days of gestation (term, 21 days) were anesthetized by intramuscular injection of 50 mg ketamine (Parke Davis, UK) and 7 mg xylazine HCl (Bayer, Germany) per kg body weight (0.2 ml/300 g body weight of a 1:1 mixture of 2% xylazine HCl-Rompun and 0.1 g/ml Ketamine). An abdominal midline incision was performed and the two uterine horns were exposed and kept moist throughout the surgery. The following labeled fatty acids were injected intracerebrally into the embryos as detailed elsewhere (25): [^{14}C]linolenic acid (53.0 Ci/mmol), [^{14}C]linoleic acid (50.0 Ci/mmol), and [^3H]arachidonic acid (240 Ci/mmol) were all purchased from New England Nuclear, Boston, MA. [^3H]docosahexaenoic acid (23.0 Ci/mmol) was a generous gift from Dr. N. G. Bazan of Louisiana State University. One to two $\mu\text{Ci}/1\ \mu\text{l}$ ethanol was injected, using custom-made syringes (33-gauge, 0.375" length from Hamilton, Reno, NV). After the uteri were returned to the abdominal cavity, the incision was closed with proper surgical sutures. Most of the rats were awake 2 h after surgery. At designated time intervals, the fetuses were delivered and the brains of viable fetuses were immediately processed as detailed in the next section.

Lipid extraction

Whole brains were homogenized in 2 ml hexane–isopropanol 3:2 (by volume), using a Polytron homogenizer, according to Hajra and Radin (26). The organic layer containing the lipid extract was separated from the residual tissue by low speed centrifugation.

Separation and isolation of lipid species

Nonpolar and polar lipids were separated by thin-layer chromatography (TLC) on preactivated silica gel G plates (Merck, Darmstadt, FRG). The solvent system used to separate mono-, di-, and triacylglycerols from free fatty acids and cholesterol consisted of a freshly prepared mixture of hexane–ether–acetic acid 40:60:2.3 (by vol) (27). Standard lipid mixtures (Sigma Chemical Co., St. Louis, MO) were run for identification purpose. The major phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS), as well as the choline and ethanolamine plasmalogens (PC-pl and PE-pl, respectively), were separated in two directions using a mixture of chloroform–methanol–methylamine (40%) 130:70:30 (by vol) for the first direction and chloroform–acetone–methanol–acetic acid–water 100:40:20:30:10 (by vol) for the second direction, with exposure to HCl fumes between the two runs (28). Spots were visualized by exposing the plates to iodine vapors for experiments in which direct counting was performed, or by spraying with water for analysis by HPLC. For direct radioactivity determination, identified lipid spots were scraped off the glass plate and counted in a Packard β -counter at 55% and > 95% efficiency for [^3H] and [^{14}C] radioisotopes, respectively, after adding 10 ml of scintillation fluid (Lumac, Landgraaf, Netherlands).

High pressure liquid chromatography (HPLC) analysis of fatty acids

Individual lipid spots separated on either unidirectional or bidirectional TLC were visualized by spraying with water and identified by cochromatography with known standards. The spots were scraped off the glass plate and release of fatty acids and their esterification with phenacyl bromide was performed essentially as described elsewhere (29). Analysis of phenacyl esters of fatty acids was carried out on a Gilson HPLC System (Dual pumps model 302, dynamic mixer model 811 and a manometric module, model 802B) controlled by the 704 HPLC System Manager, equipped with a UV detector, (model 116) (Gilson Medical Electronics (France) S.A.). Samples were injected into a 20- μl sample loop. Separations were performed on reverse phase columns (Ultrasphere-ODS, 250X4.6 I.D., Altex) coupled with an RP-18 guard column packed with ODS, C-18 (Brownlee labs, Inc.), and the eluted phenacyl esters were detected at 240 nm. Elution was performed using a linear gradient of solvents as follows: solvent A contained 40.5% acetonitrile, 40.0% methanol, and 19.5% water; solvent B was composed of 92% methanol and 8% water. The gradient started with 0% solvent B and increased to 83% during 27 min. This proportion was maintained up to 35 min and then in-

creased to 88.90% at 50 min. Solvent B was then further increased to 100% at 50.5 min and maintained so up to 60 min. Decrease to 0% solvent B was achieved at 60.5 min and maintained to the end of the run, at 65 min. The elution of phenacyl esters of 12:0–22:6 fatty acids was completed within this run, at a flow rate of 1.5 ml/min; 12:0 and 17:0 served as internal standards. The eluate was collected, a fraction each 0.5 min, and the radioactivity counted was measured after addition of 4 ml scintillation fluid (see above). The amount of each detected labeled fatty acid phenacyl ester was expressed as the percent of the sum of the counts under the respective peak from the total radioactivity eluted.

RESULTS

Uptake and esterification of [1-¹⁴C]linolenic and [1-¹⁴C]linoleic acids

Radioactively labeled linolenic and linoleic acids were administered by the intracranial route, and at designated times the total radioactivity in the brain was compared to that accumulated in the liver. As seen in Fig. 1, 3 h after [1-¹⁴C]LNA administration, the total-organ radioactivity was approximately 3-fold higher in the brain as compared to liver. At this time, nearly 25% of the injected radioactivity was found in the brain. After 20 h, there was twice as much radioactivity present in the brain as in the liver, in spite of a significant loss of radioactivity in both tissues. As the values are expressed per organ and as the liver is 3-fold larger than the brain, the radioactivity in the brain on a weight basis is even greater than that apparent in Fig. 1. Injected labeled linoleic acid followed a similar time course. Up to 6 h, brain radioactivity accounted for about 25% of the total injected isotope, while the liver accumulated less than 10% of the initial injected radioac-

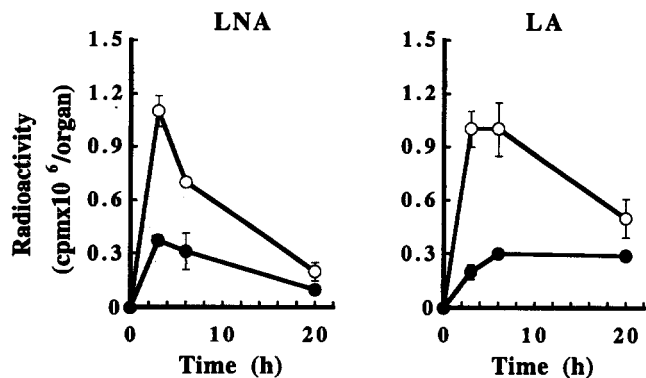


Fig. 1. Incorporation of radioactivity into brain (open circle) and liver (closed circle) at various time points after intracranial labeled linolenic (LNA) and linoleic (LA) acids administration. Values represent total organ radioactivity, mean \pm SE of 3–4 fetuses.

tivity. At 20 h, labeled LA and its metabolic products were higher than the comparable metabolites derived from LNA.

The distribution of radioactivity into the three major lipid subgroups, i.e., phospholipids (PL), neutral glycerides (NG), and free fatty acids (FFA), at various time points after intracranial injection of [1-¹⁴C]LNA is depicted in Fig. 2 (panel A). The labeled FFA disappeared rapidly, with a half-life of approximately 60 min, while the radioactivity in the esterified lipid pools increased steadily with time. The labeling of NG at early times was faster than that of PL (32.3% and 14.3% of total, respectively, at 1 h). By 6 h, nearly 60% of the label was present in the PL. At 20 h, more than 75% of the total labeled fatty acids was esterified in PL. The distribution of label into individual lipid fractions is shown in Fig. 2 (panels B–D). Incorporation of radioactivity in PC was maximal after 6 h and decreased to approximately 33% at 20 h, while that of PE gradually increased over the 20-h period, reaching almost 20% of total brain label. At that

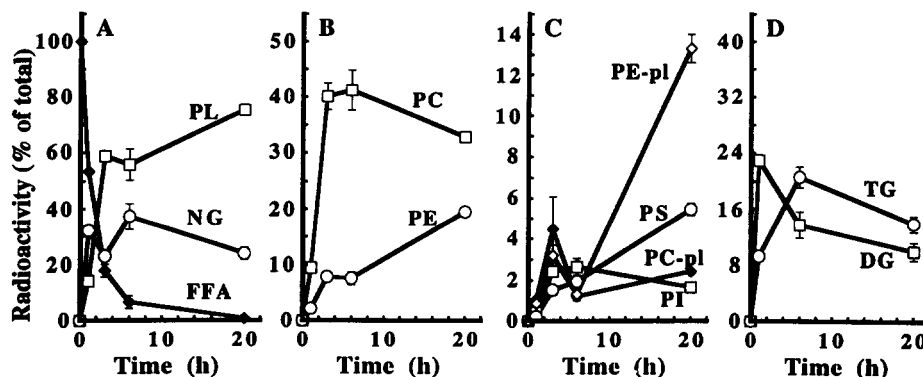


Fig. 2. Incorporation of radioactivity into the free fatty acid (FFA), total phospholipid (PL), and neutral glycerides (NG) fractions (A); individual phospholipid (B–C); and NG (D) fractions after intracranial administration of 1–2 μ Ci [1-¹⁴C]linolenic acid in 1 μ l ethanol. Values are expressed as percent of the total radioactivity of the whole brain lipid extract and are means of total 6–8 fetal brains from two pregnant rats \pm SE. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC-pl, choline plasmalogen; PE-pl, ethanolamine plasmalogen; DG, diacylglycerols; TG, triacylglycerols.

time, radioactivity in PI and PC-pl (Fig. 2, panel C), was 1.7% and 2.4% of total, respectively. The radioactivity in PE-pl and PS increased steadily over time, reaching values of 13.3% and 5.5%, respectively, at 20 h (Fig. 2, panel C). A substantial proportion of radioactivity was esterified in diacylglycerols (DG) and triacylglycerols (TG) (Fig. 2, panel D). These results emphasize that in the fetal rat brain, PC, DG, and TG are the initial esterification sites for the n-3 family of fatty acids.

The unique pattern of LNA incorporation into the fetal brain prompted us to examine the uptake and esterification of [$1-^{14}\text{C}$]LA after intracerebral injection under the same conditions. As shown in Fig. 3 (panel A), the decline of [$1-^{14}\text{C}$]LA was also rapid, with a half-life of approximately 40 min. Three hours after injection, nearly 50% of the label was found in the NG fraction. A gradual decrease of the label in the NG within the 20-h period (to 14.7% of the total), was accompanied by a concomitant increase in the labeled PL fraction (83.7% of the total).

Similar to LNA esterification, PC was the major acceptor of radioactivity after labeled LA administration, with 23.6% and 44% of total brain label at 1 h and 20 h, respectively (Fig. 3, panel B). More than 20% and 8% of the total radioactivity was found in PE and PE-pl lipids, respectively, at 20 h (Fig. 3, panels B and C). Labeling of PI and PS increased slowly over time, reaching values of 3.6% and 1.9%, respectively, at 20 h (Fig. 3, panel C). Similar to LNA, esterification of LA into NG took place predominantly in the DG and TG fractions at early time points (Fig. 3, panel D) and was reduced significantly at 20 h (5% and 8.8% of the total, respectively).

Elongation-desaturation of EFA

The time-dependent redistribution of label in the various PL species, in conjunction with the decrease of radioactivity in the NG fraction and the rapid dissipation of the unesterified precursor, suggested that EFA may be subject to elongation-desaturation. To examine this possi-

bility, HPLC analysis of the derivatized fatty acids in selected PL fractions was performed. Fig. 4 depicts the relative distribution of radioactive fatty acids in several PL species after administration of [$1-^{14}\text{C}$]LNA (upper panel) and [$1-^{14}\text{C}$]LA (lower panel).

A time-dependent esterification of elongated-desaturated labeled linolenic acid metabolites was noticed in the three major PL species (Fig. 4, upper panel). Labeled LNA was rapidly incorporated initially into all PL fractions, comprising at 6 h about 80%, 60%, and 18% of the total counts in PC, PE, and PE-pl, respectively. At 20 h, it constituted only 36.6%, 21.7%, and 8.2% of total radioactivity in the three PL species, respectively. The intermediary metabolites of the n-3 series, i.e., 20:5n-3 and possibly 22:5n-3, increased transiently and did not remain high at 20 h. In particular, 22:5n-3 accounted at 1 h for as much as 61.6% and 83% of total radioactivity in PE and PE-pl, respectively. DHA increased up to approximately 40% of the total radioactivity in PE and PE-pl and little over 12% in PC at 20 h (Fig. 4). A small fraction of the radioactivity was recycled as 16:0 and 18:1 after β -oxidation.

[$1-^{14}\text{C}$]LA was also subject to elongation-desaturation by the fetal brain, as evident from the HPLC analysis of several PL species (Fig. 4, lower panel). The majority of label in PC and PE was present in the form of labeled LA. Labeled AA remained relatively low at 20 h, 12.5% and 14% of total radioactivity in PC and PE, respectively. A significant time-dependent redistribution of radioactivity was noticed in PI. Thus, 1 h after [$1-^{14}\text{C}$]LA administration, it accounted for 78.8% of total radioactivity in this fraction, while at 20 h it declined to 10% of the total. The disappearance of LA was accompanied by an increase in AA, which attained the level of about 40% at 20 h. The intermediary, 20:3n-6, increased slightly in all the fractions at 3-6 h after precursor injection, but decreased at 20 h, concomitantly with the increase in 20:4. The labeled β -oxidation-recycling products, 16:0 and 18:1, also ap-

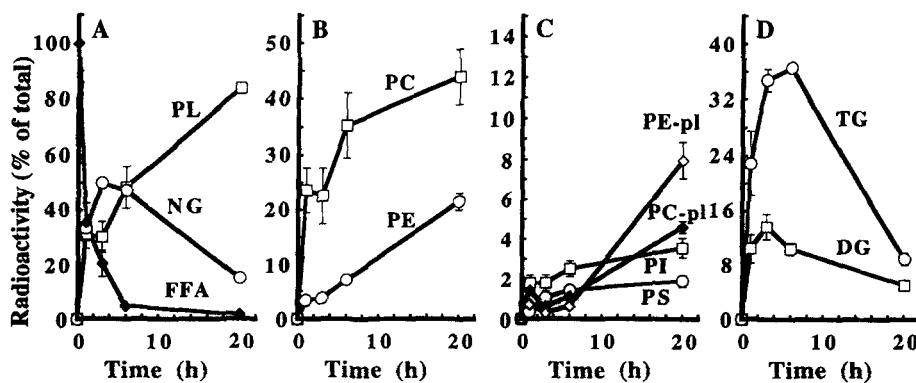


Fig. 3. Incorporation of radioactivity into the various lipid fractions after intracranial [$1-^{14}\text{C}$]linoleic acid administration. Amounts and procedures as described in legend to Fig. 2.

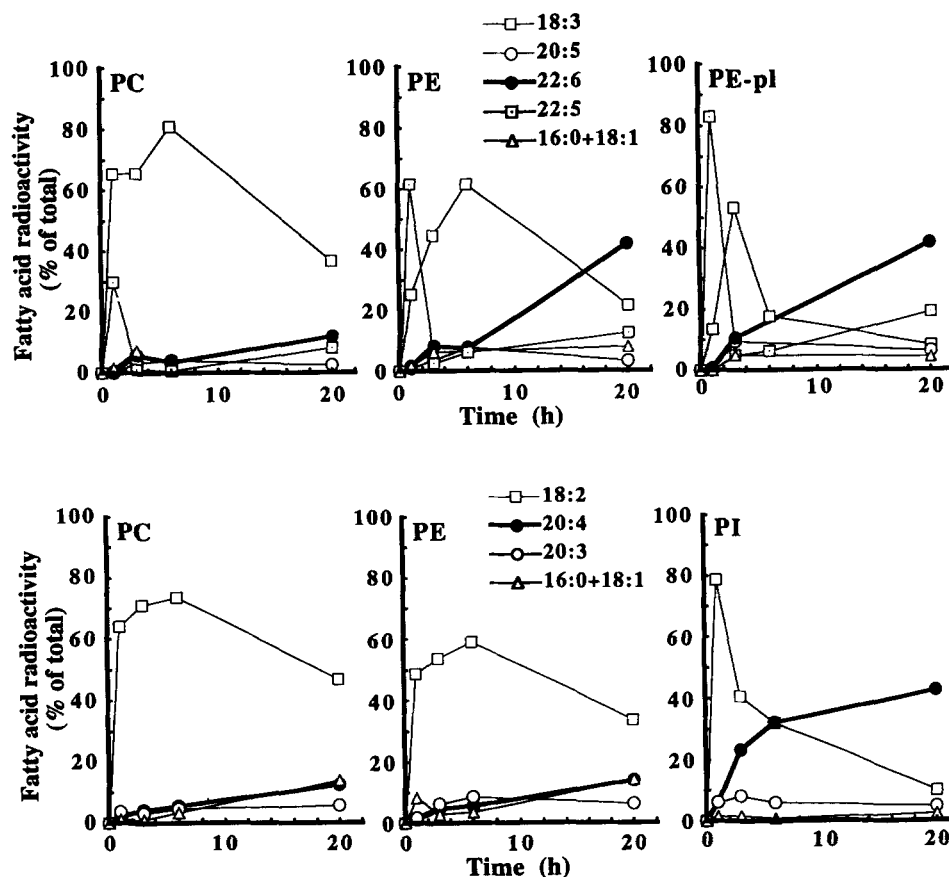


Fig. 4. Radioactivity of fatty acids in various lipid fractions after intracranial [$1\text{-}^{14}\text{C}$]linolenic acid (upper panel) and [$1\text{-}^{14}\text{C}$]linoleic acid (lower panel) injection. Phospholipid spots separated on two-dimensional TLC (see Materials and Methods) were scraped off the plate and the fatty acids were recovered after hydrolysis of the PL by KOH in ethanol. The free fatty acids were derivatized with phenacyl bromide and separated and identified by reverse phase HPLC. The eluate was collected at 0.5-min intervals and the radioactivity was counted in each fraction. The total radioactivity in each identified fatty acid peak was expressed as percent of total eluate radioactivity. Values are means of 2–3 fetuses from two pregnant rats.

peared in all the fractions examined, 2.5% in PI, 14.0% in PC, and 14.2% in PE, at 20 h after the precursor injection.

As already noticed in Fig. 1, total organ radioactivity in the liver was much lower compared to that of the brain. Nevertheless, in order to rule out the possibility that elongation–desaturation of the intracranially injected label was, in fact, accomplished in the liver, experiments were done in which comparison of the total labeled precursor and long chain PUFA in the two organs was made. The amount of total fatty acid radioactivity in the fetal liver and brain tissues, 3 and 6 h after injection, are summarized in Fig. 5. Total labeled DHA in the liver at these time points comprised 30% and 20% of the amounts in brain phospholipids, while the corresponding values for labeled AA were 2% and 3%. Evidently, liver DHA and AA radioactivity were always smaller than that of the brain, ruling out any significant contribution of the liver to brain radioactivity. These differences are even greater when values are expressed on a weight basis. Interestingly, there was a relatively high proportion of label resid-

ing in the liver as a result of LNA injection in comparison to LA injection, which may relate to a selective uptake of LNA by the liver. This finding is in accord with the work

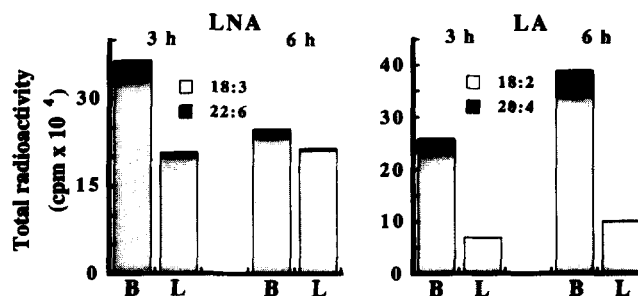


Fig. 5. Accumulation of radioactive LNA, LA, DHA, and AA, in brain (B) and liver (L) 3 and 6 h after intracranial labeled EFA injection. Liver radioactivity was examined in the total lipid extract while brain radioactivity was based on the total phospholipid fraction. Data were obtained from individual fetuses with matched brain and liver tissues. Procedure as described in legend to Fig. 4.

of Sanders and Rana (12), who showed that in the liver LNA, but not LA, was converted to long chain PUFA.

Esterification of [^3H]docosahexaenoic and [^3H]arachidonic acids

To verify the selectivity of esterification of n-3 and n-6 PUFA into designated PL and NG fractions, labeled DHA and AA were directly injected into the fetal brain. One hour after intracerebral injection, most of the FFA disappeared and the label entered the PL and NG pools (Fig. 6). Radioactivity in the NG pool (DG and TG), reached a maximal level of 42% and 33%, 5 min after DHA and AA injection, respectively. At 60 min, levels were still high in this fraction (28.8% and 20.5% for DHA and AA, respectively). A gradual, time-dependent increase in the PL fraction was noticed for both isotopes.

Examination of the individual lipid fractions revealed differential labeling with DHA as compared to AA (Fig. 7). Labeling of PC with AA, for example, was twice as high as with DHA (39.4% vs. 22.0% of total, respectively, at 60 min of incorporation); PI labeling was 5.7-fold greater (16.5% and 2.9% of total, respectively, at 60 min) (Fig. 7, panel A). In the phosphatidyl ethanolamines, PE and PE-pl, labeling with DHA predominated (Fig. 7, panel B). One hour after their intracranial administration, labeled DHA and AA constituted 18.5% and 8.2%, respectively, of total PE radioactivity and 10.7% and 3.8%, respectively, of PE-pl radioactivity. The NG fractions, DG and TG, demonstrated higher labeling with DHA than with AA (14.3% and 12.6% vs. 8.9% and 9.3%, respectively, at 1 h) with a pattern suggesting sequential labeling of DG first and TG later (Fig. 7, panel C). The PC-pl and PS fractions incorporated 2-3% of the total label at 60 min, both after DHA and AA administration (data not shown).

DISCUSSION

This report provides compelling evidence that the fetal brain is able to incorporate, elongate, desaturate, and selectively esterify radioactively labeled LA, LNA and their metabolites into plasma membrane PL.

The sources of PUFA for brain tissue PL synthesis has been a major unresolved issue in our understanding of the structural-functional relationships of these ubiquitous plasma membrane constituents. This issue is particularly important during critical periods in the developing brain, when massive accumulation of membranes takes place. A principal question has remained as to whether the brain is capable of generating long-chain PUFA from EFA precursors and to what degree it relies on the liver for PUFA (7, 30). As evident from the current studies, comparison of the long chain PUFA metabolites in brain and liver at various time points (Fig. 5), provides convincing evidence that the contribution of liver metabolism to the elongation-desaturation is indeed negligible. Although direct intracranial injection is not a physiological route for delivery of nutritional fatty acids, this approach was instrumental in order to bypass the possible contribution of the fetal liver to brain PUFA metabolism. At all time points studied, there was less radioactivity recovered in the liver than in the brain. Furthermore, up to 6 h, there was practically no labeled AA in the liver that could account for the accumulation in the brain. Traces of labeled DHA found in the liver could not account either for the relatively large quantities of labeled DHA detected in the brain.

To date, most of the studies on the contribution of the brain to elongation-desaturation of EFA were carried out in postnatal animals. Using the intraperitoneal route, Scott and Bazan (7) found that the capability of developing brain and retina tissue to form PUFA appeared very

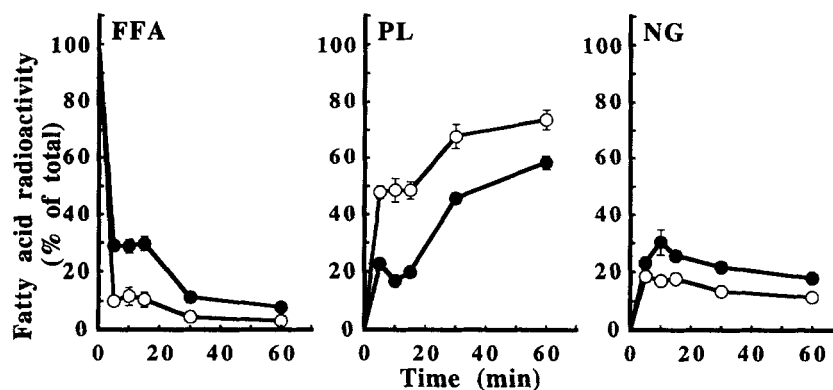


Fig. 6. Incorporation of radioactivity into the free fatty acid fraction, total phospholipid pool, and neutral lipid pool after intracranial injection of either [^3H]docosahexaenoic acid (closed circle) or [^3H]arachidonic acid (open circle). See legend to Fig. 1.

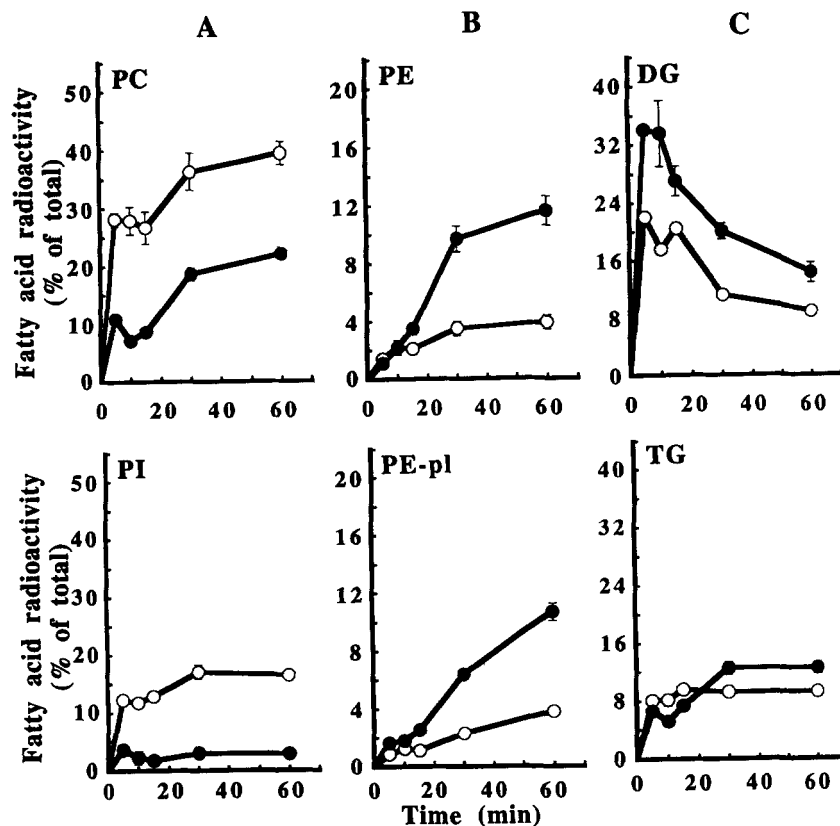


Fig. 7. Incorporation of radioactivity into individual lipid fractions after intracranial injection of either [^3H]docosahexaenoic acid (closed circle) or [^3H]arachidonic acid (open circle). See legend to Fig. 1. Panel A, PC and PI; panel B, PE and PE-pl; panel C, DG and TG.

limited. These authors concluded that PUFA is produced by elongation and desaturation of nutritional EFA by the liver and then circulated to the brain. A similar suggestion was made by Nouvelot, Delbart, and Bourre (31) while studying intermediary metabolism of dietary EFA to long-chain PUFA by the liver. On the other hand, Dhopeswarkar and Subramanian (9, 10) showed that intracranially administered radioactively labeled LNA and LA were converted into PUFA in the developing postnatal brain. Similarly, postnatal rats injected intracerebrally with LNA converted the label into PUFA (32).

Using the intracranial route of administration, Sanders and Rana (11) and Sanders and Naismith (12) were able to provide evidence for production of long chain derivatives from labeled LNA and LA precursors; little or no recycled β -oxidation products were noticed. Intraperitoneal injection, on the other hand, resulted in excess accumulation of labeled β -oxidation products in the fetal liver. Similar to their studies, we also found low amounts of labeled palmitic and stearic acids, indicating that the recycling of carbon after β -oxidation is modest. Thus, the capacity of the fetal brain to use EFA as a carbon source or synthesize PUFA independent of the liver is an important biological problem.

In a first attempt to address this issue, we found that mixed primary fetal cerebral cells in culture showed a substantial degree of elongation-desaturation capacity of both LNA and LA precursors (13, 14). Subsequent work with transformed cells of neural origin failed to provide more definitive answers as to the synthetic capacity of glia compared to neuronal cells. For example, murine neuroblastoma cells (33) did not convert LNA to DHA, while human retinoblastoma (34, 35) produced AA and DHA from LA and LNA, respectively. Similarly, a C6 glioma line did not produce DHA (36), while in a C6-astrocytoma cell line (33) AA and DHA were generated from labeled LA and LNA precursors, respectively. Primary astroblasts from newborn rat brains cultivated in culture for 21 days, converted EFA to PUFA (36). Recently, Moore et al. (17) provided evidence that postnatal cultured astrocytes had the capability to form PUFA from EFA. Using postnatal cerebral and cerebellar preparations after 12 days in culture, these authors also concluded that neurons, unlike astrocytes, were unable to convert EFA into PUFA (17). Assuming that the neuronal characteristic of these preparations has been preserved in culture, the possibility that these neurons, at discrete stages in their ontogeny, may possess the capability to produce

PUFA was not ruled out.

In this regard, the present experiments differ from those performed in the postnatal brain, mainly because the 19-day old rat fetal brain contains predominantly postmitotic neurons with a very small population of astrocytes and presumably no oligodendrocytes (37, 38). Thus, the present in vivo studies on EFA elongation-desaturation are analogous to use of neuronal-enriched in vitro preparations. Although the exact type of cells that are responsible for the elongation-desaturation is currently unknown, it would appear that the fetal brain can provide at least some of its complement of long chain PUFA for the neuronal plasma membrane build up through its own metabolism.

In the present study we have extended earlier in vivo studies (11, 12) and examined the distribution of the labeled PUFA in various esterified and nonesterified lipid fractions. First, we showed that the NG species, along with PC, turned out to be the most efficient metabolic pools for LNA, LA, DHA, and AA esterification. Second, DHA and AA, elongation-desaturation metabolites of LNA and LA, were mainly esterified, as expected, in the PE and PI species, respectively. The differential incorporation of the n-3 and n-6 fatty acids into the various lipid fractions was also clearly demonstrated by the uptake experiments (Figs. 2 and 3). In general, PC and PI incorporated more n-6 than n-3 fatty acids, while the opposite was true for PE-pl and PE. Similarly, DG and TG incorporated more n-3 than n-6 fatty acids. From the time course of incorporation, it would appear that both [1-¹⁴C]LNA and [1-¹⁴C]LA were taken up initially by the NG pool (Figs. 2 and 3). Later, as the elongation-desaturation process progressed, more label became associated with the PL fraction. As the amounts of esterified LNA and LA in the TG and DG fractions of the fetal brain are very small (unpublished data), a very high turnover of these neutral lipids is suggested. A similar observation was documented in the liver by Scott and Bazan (7) in the postnatal animal after intraperitoneal administration of labeled LNA.

In contrast to LNA and LA uptake, radioactively labeled DHA and AA were esterified into phospholipids almost without delay. The exact mechanism for this preferential labeling pattern is not clear. It could possibly relate to the selectivity of the acyltransferase and transacylase systems to esterify the 2-position of the glycerol moiety according to the number of double bonds and polar head group characteristics (18-22, 39, 40). This is well illustrated by the rate and extent of label incorporation into the individual phospholipid fractions: PC and PI were clearly more labeled with AA than with DHA (Fig. 7A), while PE and PE-pl carried more label after DHA injection (Fig. 7B). Thus, similar to the adult rodent brain (19, 40) and retina preparations in vitro (39), the fetal brain maintains the same type of selectivity in vivo.

While the liver is probably the major source for brain long-chain PUFA in the postnatal animal, the presence of an independent metabolic pathway in the brain during intrauterine life may be an additional mechanism for modeling brain PUFA profile. ■

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