Developmental changes in rat brain membrane lipids and fatty acids: the preferential prenatal accumulation of docosahexaenoic acid

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Abstract Information on the prenatal accumulation of rat brain membrane lipids is scarce. In this study we investigated in detail the fatty acid (FA) composition of the rat brain, on each day from embryonic day 12 (E12) up to birth, and on 8 time points during the first 16 days of postnatal life, and correlated the FA changes with well-described events of neurogenesis and synaptogenesis. Between E14 and E17, there was a steep increase in the concentration of all the FAs: 16:0 increased by 136%, 18:0 by 139%, 18:1 by 92%, 20:4n–6 by 98%, 22:4n–6 by 116%, 22:5n–6 by 220%, and 22:6n–3 by 98%. After this period and up to birth, the concentration of the FAs plateaued, except that of 22:6n–3, which accumulated further, reaching an additional increase of 75%. After birth, except 22:5n–6, all FAs steadily increased at various rates. Estimation of the FA/PL molar ratios showed that prenatally the ratios of all the FAs either decreased or remained constant, but that of 22:6n–3 increased more than 2-fold; postnatally the ratios remained constant, with the exception of 22:4n–6 and 22:5n–6, which decreased. In conclusion, prenatal accumulation of brain fatty acids parallels important events in neurogenesis. 22:6n–3 is exceptional inasmuch as its steep accumulation occurs just prior to synaptogenesis.—Green, P., S. Glozman, B. Kamensky, and E. Yavin. Developmental changes in rat brain membrane lipids and fatty acids: the preferential prenatal accumulation of docosahexaenoic acid. J. Lipid Res. 1999. 40: 960–966.

Supplementary key words brain development • polyunsaturated fatty acids • essential fatty acids • phospholipids

It is traditionally accepted that docosahexaenoic acid (DHA, 22:6 n–3) accumulation by the developing rat brain occurs postnatally, during the 3 weeks before weaning (1). Actually, however, accumulation of brain fatty acids (FAs) has scarcely been studied in the prenatal rat, despite the dramatic changes occurring virtually from day to day in this period in terms of neurogenesis (2–9). Thus, in the most widely cited paper on prenatal FA accumulation (10), the brain was examined at only one time point during its embryonic life.

Many studies have dealt with the effects of dietary manipulation on brain FA composition (11–13) and indeed, showed correlations between the type and amount of dietary FAs ingested and the FA composition of the brain. However, none have examined in detail the natural accumulation of prenatal FAs under normal, n–3 FA-sufficient dietary conditions. It is now well recognized that DHA is essential for normal brain development and function (14), but its exact role is presently unknown. We postulated that as DHA is important for brain development, its prenatal accumulation pattern should reflect this fact; and if it subserves important functions, most of which are unknown, correlation of its prenatal accumulation with discrete events in neurogenesis and synaptogenesis might shed light on some of its biological functions.

In a recent study we have examined the FA content of the fetal rat brain at two time points during its development in late pregnancy (15) and concluded that the accretion rate of DHA at this developmental stage is the most rapid compared to other FAs. The implications, nutritional as well as developmental of these preliminary findings, prompted us to extend the time periods of lipid analysis, both to middle pregnancy and to the first 2 weeks of postnatal life in an effort to define the ontogeny of one of the major constituents of brain, the membrane lipids. We could demonstrate that major accumulation of all the FAs occurred between embryonic days (E)14–E17, but the only FA whose steep accretion continued up to birth, was DHA.

Abbreviations: DHA, docosahexaenoic acid; FA, fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine; PUFA, polyunsaturated fatty acids.

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MATERIALS AND METHODS

Animals and surgery

Pregnant Wistar (250–300 g body weight) rats were fed ad libitum chow containing (g/100 g diet): protein (20.0); carbohydrate (50.0); mineral mix (2.5); vitamin mix (2.0); fat (5.0); moisture, ash and fiber (20.0). The FA composition of the diet is detailed in Table 1. Dams 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, the two uterine horns were exposed, and the fetal brains were immediately placed in liquid nitrogen upon removal. The time elapsed between the incision of the fetal neck in preparation for brain removal (and thus causing fetal death) and the placement of the brain into liquid nitrogen was never more than 10 sec. Examinations were performed from E12 up to birth, on each gestational day. At E12–E13 whole heads were used, each sample consisting of 8–12 embryos. From E14 onward brains were used, each sample containing brains from 3–6 fetuses at E14–E17, 2 fetuses at E18, and from E19 onward, single fetal brains were analyzed. Postnatally, brains were examined on days 1, 3, 4, 5, 6, 7, 11, and 16.

Lipid extraction and analysis

After weighing, the brains were homogenized in hexane–isopropanol 3:2 (by vol) containing 5% butylated hydroxytoluene and 5 mg/ml henicosanoic acid (21:0) at a ratio of 1:10, using a Polytron homogenizer, according to Hajra and Radin (16). The organic layer containing the lipid extract was separated from the residual tissue by low speed centrifugation.

RESULTS

The changes in the brain weight and brain total PL content from E12 through birth and up to postnatal day (P) 15, are depicted in Fig. 1. Most of the increase in brain weight during this period was postnatal, but lipid phosphorus exhibited two steep accumulation rates, the one in the last week of prenatal life and the other in the second postnatal week.

Figures 2–5 describe the changes in the concentration of the major brain FAs during this period. Clearly evident are major increases in the proportion of practically all the FAs between E14 and E17. Palmitic acid (16:0) increased during this period by 136%, stearic acid (18:0) by 139%, and oleic acid (18:1) by 92% (Fig. 2). Both arachidonic acid (20:4n–6) and DHA increased by 98% during this period (Fig. 3). Similarly, the minor polyunsaturated FAs, docosatetraenoic acid (22:4n–6) and docosapentaenoic acid (22:5n–6), increased by 116% and 221%, respectively (Fig. 4), while the dimethyl-acetals which reflect the plasmalogens also increased substantially (Fig. 5; 16:0DMA by 197%, 18:0DMA by 74%, and 18:1DMA by 123%). The only FA whose steep accretion continued up to birth was DHA, which increased by 74% between E17 and birth (Fig. 3). Of the brain PLs, PS and PE are the ones which contain the highest proportion of DHA (Fig. 3).

Table 1. The fatty acid composition (weight %) of the chow diet

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Weight %</th>
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<tbody>
<tr>
<td>16:0</td>
<td>13.03</td>
</tr>
<tr>
<td>18:0</td>
<td>4.02</td>
</tr>
<tr>
<td>18:1</td>
<td>23.66</td>
</tr>
<tr>
<td>18:2 n–6</td>
<td>51.46</td>
</tr>
<tr>
<td>18:3 n–3</td>
<td>4.98</td>
</tr>
<tr>
<td>Total</td>
<td>97.15</td>
</tr>
</tbody>
</table>

Minor fatty acids are not reported, thus totals do not add up to 100%.

![Fig. 1. Total brain weight expressed in mg (continuous line) and total membrane lipids expressed as μg/mg lipid phosphorus per gram brain (dotted line) during development. At days −9 and −8 (E12 and E13) whole heads, rather than brains, were used. Values are means ± SEM of 4–6 samples.](image-url)
In order to determine whether the steep prenatal increase in brain DHA content was due to increased proportion of DHA-enriched PLs, to enrichment of PLs with DHA or both, the fraction of each PL type during development was determined (Table 2). It can be seen that no significant change occurred in the proportion of the individual PLs. However, as can be seen from the changes in the proportion of the individual FAs esterified to the PL molecules (Fig. 7), whereas the proportion of the majority of the FAs in the PL molecule either remained constant or decreased, the PL molecules became increasingly enriched with DHA, with the most steep enrichment occurring in the last 5 days of pregnancy. Postnatally, the concentrations of all the FAs increased at various rates (Figs. 2–5), except 22:5n–6 which decreased slightly (Fig. 4). Similarly, the molar ratios of the various FAs to the PLs remained quite constant at this period, except minor changes in 22:5n–6 and 22:4n–6 (Fig. 7).

DISCUSSION

This is the first time to the best of our knowledge that a very detailed compositional analysis of the developing brain fatty acids has been performed in rats fed an adequately supplemented diet with essential FAs. While the dependence of brain FA composition on dietary essential FA manipulation, mainly n-3 FA deficiency or supplementation, has been previously amply demonstrated (11–13), description of the temporal changes in the rat brain FA composition during development, especially prenatal development, is almost non-existent. We have previously shown (15) that at these developmental stages, most of brain FAs are structural, esterified into membrane PLs. Therefore, the present study presents an analysis of membrane lipid ontogeny.

The spatio-temporal events of rat fetal brain neurogenesis and synaptogenesis are quite well characterized. Although minor differences exist, the bulk of neuronal “birth”, i.e., the period after neuron precursors have...
stopped dividing as determined by tritiated thymidine uptake, is between E14 and E17 (2, 3, 6–8, 20–28) for most brain regions. In some areas neurogenesis starts even earlier, on E11–E12 (3, 6, 29, 30), while in other regions neurogenesis is a later event. Among the latter, the visual system should be mentioned, in which neurogenesis in some parts does not start until E18 and continues up to birth (31), and the retinal ganglion cell layer, in which neurogenesis occurs between E14 and E20 (32). Synaptogenesis starts after neurons have undergone terminal mitosis, usually after E17 (33–36), although GAP-43 immunoreactivity, which reflects elongation of axons and synaptogenesis, can already be demonstrated at E13 (37) and well-defined synapses are demonstrated in the developing neocortex as early E14 (38). Events have been described which “prepare” the brain for the onset of synaptogenesis, like growth cones which are structures that herald the appearance of synapses (33). It has been shown that serotonergic growth cones display the presence of serotonin-binding protein and endogenous 5-HT already at E15 (39), suggesting that properties that characterize the presynaptic components of mature serotonergic synapses develop in growth cones before synapses are formed.

How can the ontogeny of membrane FAs as described in the present study be put in the context of the discrete developmental crossroads highlighted above? First, we demonstrate two surges in the accumulation of lipid phosphorus (Fig. 1), the first at E14–E17 coinciding with peak neurogenesis and the second starting at the second postnatal week, representing synaptogenesis. It should be mentioned that at this second time point myelinogenesis also commences, giving rise to the well-known “growth spurt” (40), but as this period has been quite extensively studied and described (10), it will not be dealt with here further. Second, the accelerated accumulation of all the FAs between E14 and E17 (Figs. 2–5) is probably accounted for by the need of the brain for structural lipids at the height of neurogenesis. The slope of the accumulation is similar for all the FAs, and ranges between 100 and 140% increase during this period. After E17 the accumulation of

![Fig. 4. Accumulation of the minor polyunsaturated fatty acids (docosatetraenoic acid; 22:4 n-6; continuous line; and docosapentaenoic acid; 22:5 n-6; dotted line) in the brain during development. At days −9 and −8 (E12 and E13) whole heads, rather than brains, were used. Values are μg FA per gram tissue, mean ± SEM of 4–6 samples.](image)

![Fig. 5. Accumulation of the main dimethylacetals (DMA) in the brain during development. At days −9 and −8 (E12 and E13) whole heads, rather than brains, were used. (16:0DMA: continuous line; 18:0DMA: dotted line; 18:1DMA: interrupted line). Values are μg FA per gram tissue, mean ± SEM of 4–6 samples.](image)
all the FAs, except DHA, plateaus up to birth, consistent with the lag in the intensity of new membrane generation with termination of most neurogenesis and just before the spurt of synaptogenesis and myelogenesis. Interestingly, the relative quantities of the discrete FAs (except DHA) throughout the last 2 weeks of pregnancy remain constant, reflecting the FA composition of neural membranes (41).

Finally, we wish to consider in some detail the marked accumulation of DHA during the last 4 days of pregnancy (Fig. 3). As expected, the PLs that contain the highest amounts of DHA are PE and PS (Fig. 6). The relative amounts of the individual PLs did not change significantly during this period (Table 2), but the mean proportion of DHA esterified into each PL molecule increased more than twice (Fig. 7). This increase is remarkable, especially as the molar proportion of the other FAs either remained constant or even decreased during this period (Fig. 7). Spatio-temporal considerations of neurogenesis place the steep accretion of DHA at the time of maximal neurogenesis in the visual cortex and the superior colliculus in the brain (31) and at the time of generation of the ganglion cell layer in the retina (32). The high content of DHA in retinal membranes is well documented (14) but almost no information exists on the regional distribution of DHA in the brain. In a very recent study of PEs in the adult rat brain (42), it was shown that differences existed between their DHA content in several brain regions, i.e., the cerebellum PE contained more DHA than the frontal cortex and the striatum. A detailed description of DHA distribution in the various brain regions during development, however, similar to the data on neurogenesis at different times and areas, is lacking. It is tempting to speculate that

TABLE 2. Relative quantities of the main brain phospholipids during development

<table>
<thead>
<tr>
<th></th>
<th>E16</th>
<th>E18</th>
<th>E20</th>
<th>P3</th>
</tr>
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<tbody>
<tr>
<td>PI</td>
<td>5.44 ± 0.73</td>
<td>5.61 ± 0.66</td>
<td>3.33 ± 0.46</td>
<td>5.40 ± 0.39</td>
</tr>
<tr>
<td>PS</td>
<td>10.53 ± 1.52</td>
<td>12.77 ± 1.35</td>
<td>8.33 ± 0.85</td>
<td>14.58 ± 2.20</td>
</tr>
<tr>
<td>PC</td>
<td>56.70 ± 5.72</td>
<td>51.10 ± 4.76</td>
<td>56.60 ± 1.39</td>
<td>51.27 ± 4.80</td>
</tr>
<tr>
<td>PE</td>
<td>27.37 ± 4.66</td>
<td>30.53 ± 3.74</td>
<td>31.80 ± 3.70</td>
<td>28.37 ± 3.60</td>
</tr>
</tbody>
</table>

Data are percent of lipid phosphorus (mean ± SEM) determined after TLC separation of the phospholipids. Abbreviations are explained in the text.
were detailed spatial compositional data known at different developmental time points regarding DHA, some of the neurological deficits resulting from nutritional inadequacies during pregnancy could be explained and probably prevented.

In the adult rat brain, synapses are particularly enriched in DHA (43). The present study suggests that the prenatal DHA accretion spurt may indeed correlate with events of synaptogenesis. Thus, the possibility exists that the last few days of gestation prepare the cells so that the DHA-enriched membranes of synapses may be generated rapidly during synaptogenesis. Indeed, endogenous DHA levels in growth cones, the precursor structures to synapses, were shown to increase before they become mature synapses in postnatal mouse brain (44). Furthermore, DHA is a potent stimulator of diacylglycerol kinase activity in rat brain membranes (45) suggesting that it may regulate through this stimulatory effect signalling events in growth-related situations in the brain such as synaptogenesis.

The nutritional implications for the need of an adequate DHA supply during pregnancy have been studied (46) and much discussed (47, 48), without the role of DHA being actually clarified. The present study puts the need for adequate prenatal supply of DHA in perspective inasmuch as its steep accumulation just prior to extensive synaptogenesis points toward a potential role in synaptogenesis, synaptic transmission, and signal transduction. The identification of important developmental periods in the ontogeny of brain membrane lipids, especially regarding the supply of essential FAs which is influenced by extrinsic factors to the brain such as nutrition and maternal-placental circulation, suggests vulnerable time points at which events that disrupt the normal ontogenetic pattern of accumulation could produce long-lasting effects on normal development.12

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REFERENCES


