

$\Delta 6$ Desaturase in Brain and Liver During Development and Aging

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$\Delta 6$ Desaturase was measured in the mouse brain and liver using linoleic acid as substrate. During pre- and postnatal development, $\Delta 6$ desaturase in brain decreased dramatically (12-fold) up to postnatal day 21 and remained nearly constant thereafter. In liver, the activity increased approximately 9-fold between day 3 before birth and day 7 after birth. Then it decreased slightly up to weaning and was approximately constant up to 4 mo. From then on, $\Delta 6$ desaturase decreased with age (40% between 4 and 17 mo).

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Brain is one of the tissues containing high amounts of lipids which, in turn, play a role in modulating the structure, fluidity and function of brain membranes (1-3). Brain lipids contain polyunsaturated fatty acids derived from dietary essential linoleic and α -linolenic acids. More than one-third of the brain fatty acids are polyunsaturated with a prevalence of acids containing very long chains (mainly arachidonic acid, 20:4 n-6, and cervonic acid, 22:6 n-3). In fact, brain cells and organelles contain only trace amounts of linoleic acid and α -linolenic acid (4-6). Thus, either these fatty acids are rapidly and completely transformed into the longer chain fatty acids after crossing the blood brain barrier, or the fatty acids essential for the brain are in fact the very long chain fatty acids which are either synthesized in the liver or are provided with the diet. *In vitro* studies, using dissociated fetal brain cells in culture, have shown that differentiation, multiplication of the cells, and release of neuromediators are effective only if the cells are grown in the presence of 20:4 n-6 and 22:6 n-3 (7,8). Essential fatty acid deficiency is known to have dramatic effects on various organs (9-11).

During development, it is essential to provide brain cells with polyunsaturated fatty acids. Otherwise, irreparable damage can occur. Polyunsaturated fatty acid requirements during development are particularly critical because the turnover of brain membranes is quite slow—more than one year for myelin fatty acids (12) and because renewal of neurons and oligodendrocytes is minimal. It has been hypothesized that one aspect of aging could be the reduced activity of $\Delta 6$ desaturase which would impede membrane renewal (13).

Studies on desaturase, though numerous in liver but rare in brain, have generally been limited to one tissue at only one or two time points. Unfortunately, only few studies have been undertaken to follow $\Delta 6$ desaturase activity during development, and nothing is known in regard to $\Delta 6$ desaturase activity in brain

during aging. In a study that focussed on early development (up to 30 days after birth in the rat), Strouve-Vallet and Pascaud (14) found that $\Delta 6$ desaturase activity in rat liver microsomes remained constant, but dramatically decreased in brain microsomes. Cook (15) obtained similar results during early development in rat brain homogenate, but reported markedly increased activity in liver from day 4 up to day 32 after birth. Purvis *et al.* (16) found that desaturase activities in brain microsomes of newborn pigs at 60 days gestation and at 5 wk post-partum were nearly identical. In contrast, $\Delta 6$ desaturase activity was approximately 3-fold higher in the liver. Sanders and Rana (17) found that the activity in 21-day fetuses was approximately 3-times higher in brain than in liver. In adult and fetal human liver, $\Delta 6$ desaturase is the rate limiting step of arachidonic synthesis (18). Interestingly, $\Delta 6$ desaturase has been reported to peak 3 days after surgery in regenerating rat liver (19).

As polyunsaturated fatty acids appear especially important to maintain functional structures in brain (20-23), this work was undertaken to determine $\Delta 6$ desaturase activity in both brain and liver during development and aging. The mouse was chosen as model because it is known that desaturating activity in mice is lower than that in rats (24,25) and thus is closer to that in humans, taking into account the ratio of very-long-chain polyunsaturated fatty acids to their precursors in the blood and liver.

MATERIALS AND METHODS

Mice were bred in our laboratory (C3H-SWV strain; similar results were obtained with Swiss strain) and fed standard chow (Iffa-Credo, l'Arbresle, France). As young animals were used prior to weaning, all animals had free access to chow. Animals were killed by decapitation. Livers and brains were immediately excised, rinsed with ice-cold physiological saline, blotted, and homogenized in a Potter apparatus.

Incubations were performed essentially as described by Cook (15) and as modified by Blond *et al.* (26), Blond and Lemarchal (27), and Narce and Poisson (28). Briefly, tissues were homogenized in 0.25 M saccharose, 0.05 M phosphate, and 2 mM glutathione, pH 7.4 (2 g fresh weight tissue/5 mL buffer). Homogenates were centrifuged for 15 min at 12000 $\times g$ in a Sorvall centrifuge, supernatant was carefully taken up, and in case of liver homogenates diluted two-fold with buffer. Protein was measured according to Lowry *et al.* (29). Incubation media (2 mL) contained variable amounts of protein, Na_2HPO_4 (50 mM), ATP (7.5 mM), MgCl_2 (3.8 mM), NADPH (0.2 mM), NADH (0.5 mM), CoA (0.2 mM), and [^{14}C]linoleic acid (100 nmol, 2 μCi , 20 μL). After thirty minutes, incubations were stopped by addition of 0.5 N KOH in ethanol. After adding unlabelled fatty acids (commercial grade) as carriers (30 μg each of 18:2 n-6, 18:3 n-6, 20:3 n-6, 20:4 n-6, 20:5 n-3, and 22:6 n-3),

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Abbreviations: ATP, adenosine 5'-triphosphate; b.p., boiling point; CoA, coenzyme A; KOH, potassium hydroxide; NADH, reduced NAD (nicotinamide adenine dinucleotide); NADPH, reduced NADP (NAD phosphate).

lipids were saponified at 90° for 30 min. After acidification (0.4 mL 10 N HCl), fatty acids were extracted 2-times with 5 mL of hexane and were methylated with 14% boron trifluoride in methanol for one hour. Methyl esters were purified by thin-layer chromatography using petroleum hydrocarbon (b.p. 40°–60°)/diethyl ether (80:20, v/v) as developing solvent. Radioactive methyl esters were visualized by autoradiography, the fractions were extracted 2-times with 3 mL of petroleum hydrocarbon and 2-times with diethyl ether, the solvent was evaporated in a stream of nitrogen, and the lipids were further resolved according to their degree of unsaturation by argentation thin-layer chromatography. Silver nitrate impregnated silica gel plates (30% AgNO₃) were prepared by immersing silica gel plates into a 10% AgNO₃ solution in acetonitrile for 15 min and drying for 15 min at 100°. After development with petroleum hydrocarbon (b.p. 40°–60°)/diethyl ether (50:50, v/v), fractions were visualized by autoradiography and by staining with 0.1% dichlorofluorescein, then were scraped off, and counted in a scintillation counter.

RESULTS AND DISCUSSION

With brain homogenate, $\Delta 6$ desaturase activity was linear up to 4 mg protein per mL incubation medium at all ages. With liver homogenate, it was linear up to 2 mg protein/mL up to 3 days after birth. Thereafter $\Delta 6$ desaturase activity was linear up to 1 mg/mL. Figure 1 shows $\Delta 6$ desaturase activity during development and aging in brain and in liver. In liver, the activity increased approximately 9-fold from day 18 at fetal age up to day 7 after birth. It decreased by 44% up to weaning and was constant then up to 4 mo. Interestingly, $\Delta 6$ desaturase activity decreased by 40% during aging. In brain, the activity dramatically decreased

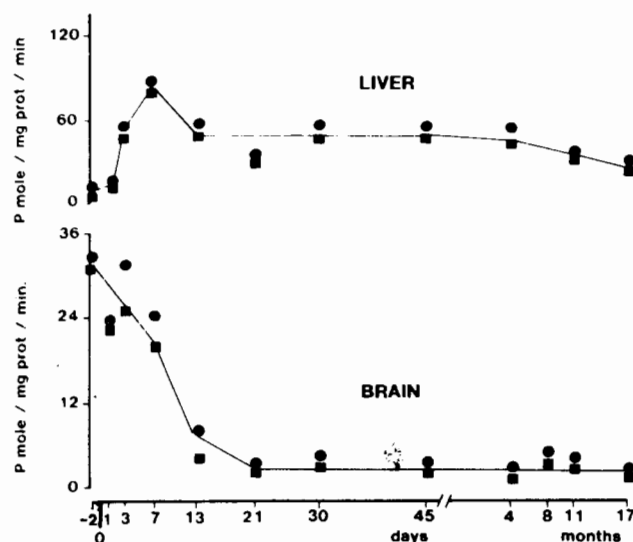


FIG. 1. $\Delta 6$ Desaturase during development and aging in mouse brain and liver homogenates. ●, total synthesis of polyunsaturated fatty acids from linoleic acid (mainly γ -linolenic acid, with small amounts of arachidonic acid and trace amounts of adrenic acid); ■, γ -linolenic acid synthesized from linoleic acid.

during early development: between day 18 of gestational age and weaning it was reduced about 14-fold, but remained nearly stable during adulthood and aging. These results are consistent with those of Purvis *et al.* (16) which showed that preterm desaturase activities in both pig liver and pig brain were lower than at term. They also are consistent with the data of Sanders and Rana, (17) that showed that activity in 21-day fetal rat brain is 3.4-fold higher than in liver, and those of Strouve-Vallet and Pascaud (14) for the 4-to-30 day postnatal period in both liver and brain, although the latter did not observe the day-7 peak in liver or any activity in brain after 21 days. In agreement with Cook (15) we found a decrease in brain activity during early development, but in disagreement with him we did not find any increased activity in the liver after the 4th day. Our results are also in agreement with those of Ravel *et al.* (30) who found that the elongation rate of linoleic acid in fetal rat liver was lower than in maternal liver. However, these authors found that there was no significant difference between liver $\Delta 6$ activity in the fetus and in the pregnant rat.

$\Delta 6$ Activity in brain is very high during early development up to 7 days after birth. This corresponds to the period of neuronal and glial multiplication, the latter event being at a peak at 3–5 days after birth in the mouse. Early brain development requires large quantities of polyunsaturated fatty acids for membrane synthesis. Interestingly, $\Delta 6$ activity does not peak during myelination, although myelin contains large amounts of polyunsaturated fatty acids. The same pattern was found for $\Delta 9$ desaturase activity (31). Thus, polyunsaturated fatty acids required for myelination are either accumulated in the oligodendrocytes before myelination or possibly are supplied through the blood stream. This is in contrast to the synthesis of saturated and monounsaturated long-chain and very-long-chain fatty acids which peaks during myelination and is impaired in neurological dysmyelinating mutants (32). Interestingly, chain lengthening of eicosapentaenoic acid (EPA) is less affected in these mutants than elongation of erucic and arachidic acids (33).

The question remains whether the residual $\Delta 6$ desaturase activity after day 21 is sufficient for synaptogenesis and, later on, to support the turnover of brain membranes. If it is not, the very-long-chain fatty acids would have to be synthesized by the liver. As liver synthesis decreases during aging this source may be insufficient.

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