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Delta-6 desaturation of alpha-linolenic acid in brain and liver during development and aging in the mouse

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During pre- and postnatal brain development, delta-6 desaturase decreased dramatically (approx. 4-fold) up to weaning, slowly (2-fold) up to 3 months, and remained nearly constant and extremely low thereafter. In contrast, in liver, the activity increased approximately 7-fold between day 3 before birth and day 11 after birth, then decreased slightly up to weaning, and was constant up to 9 months. Subsequently, the activity decreased during aging (4-fold between 9 and 26 months). During aging, the reduced activity in liver and the very low activity in brain raise the question of the origin of polyunsaturated fatty acids participating in the physiological turnover of brain membranes.

To understand some of the physiological alterations during brain development and aging, it is very important to determine the origin of membrane polyunsaturated fatty acids. They are practically all structural and are not related to energy metabolism. They participate directly in the functioning of nervous membranes, as well as in all other organs. Brain lipids contain a very high amount of polyunsaturated fatty acids, derived from the essential fatty acids, linoleic and alpha-linolenic acid. Dietary polyunsaturated fatty acids influence directly the fatty acid composition of the membranes [20], and they are particularly important for cerebral development [15].

Concerning specifically the (n-3) fatty acids, it has been previously shown that a deficiency in alpha-linolenic acid in the diet alters dramatically the fatty acid composition of various organs, including the brain [6, 7, 16, 30, 33]. Moreover, the speed of recuperation from these abnormalities is extremely slow for brain cells [8], brain organelles [34] and brain microvessels [21]. A diet deficient in alpha-linolenic acid alters brain membrane enzymatic activities [1, 7, 18], reduces the amplitude of electrophysiological parameters such as the electroretinogram [7, 25], alters the resistance to poisons of the nervous system [7] and reduces the performance of learning tasks [7, 24, 33].

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In fact, brain cells and organelles contain only trace amounts of alpha-linolenic acid, but are extremely rich in longer and more unsaturated chains such as docosahexaenoic acid (cervonic acid, 22:6 n-3). It is not clear if the precursor is rapidly and completely transformed into longer chains after crossing the blood–brain barrier, or if the fatty acids essential for the brain are the very long chain fatty acids which are either synthesized in the liver or are possibly provided by the diet. Moreover, in dissociated brain cells in culture [5], it has been shown that differentiation, multiplication of the cells and release of neuromediators only occur in the presence of 22:6 n-3 (added to 20:4 n-6).

Moreover, in humans it has been shown that alpha-linolenic deficiency alters nervous function [2, 19] and a dominant disease of modern society is n-3 fatty acid deficiency [28]. Loss of delta-6 desaturase has been speculated as being a key factor in aging [22].

As desaturation of linoleic acid has been found to be altered during development and aging [4, 9], it is extremely important to investigate (under the same conditions) the delta-6 desaturation of alpha-linolenic acid, the first step in the synthesis of 22:6 n-3, since it is not clear whether linoleic and alpha-linolenic acid are desaturated by the same enzymes.

Mice were bred in our laboratory (Swiss strain) and fed standard chow (Iffa-Credo, l'Arbresle, France). Animals were killed by decapitation. Brains and liver were immediately excised, rinsed with ice-cold physiological saline, blotted and homogenized with a Potter apparatus.

Incubations were performed as previously published [9], except that alpha-linolenic acid was the substrate. 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 12,000 × *g*, and supernatants were carefully taken up. Incubation media (2 ml) contained variable amounts of protein, and (in mM) Na_2HPO_4 50, ATP 7.5, $MgCl_2$ 3.8, NADPH 0.2, NADH 0.5, CoA 0.2, and [1-^{14}C]alpha-linolenic acid (100 nmol, 2 μCi, 20 μl). After 30 min, incubations were stopped by addition of 2.0 N KOH in ethanol. Unlabelled fatty acids (commercial grade) were added as carriers and lipids were saponified. After acidification, fatty acids were extracted twice 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°C)/diethyl ether (80:20, v/v) as developing solvent. Radioactive methyl esters were visualized by autoradiography, the fractions were extracted twice with 3 ml of hexane and twice with diethyl ether, and the lipids were further resolved according to their degree of unsaturation by argentation thin-layer chromatography [9] (30% $AgNO_3$). After development with petroleum hydrocarbon (b.p. 40–60°C)/diethyl ether (50:50, v/v), radioactivity in fatty acids was determined using automatic scanning (Berthold).

Linearity with protein concentration and length of incubation were checked. Statistical analysis was performed with ANOVA.

For this study the mouse was chosen instead of the rat, because body and organ weight gain in the mouse is nearly nil during adulthood and aging, in contrast to the rat. Moreover, it is generally accepted that desaturation in the mouse is less active than in the rat, and consequently closer to the situation in humans. This has been deduced after examining the ratio between long polyunsaturated chains and primers [23], but not by measuring the enzymatic activities.

Nearly all previously published desaturation results have presented data on desaturation of linoleic acid [3, 10, 12–14, 17, 26, 27, 31]. In contrast, desaturation of alpha-linolenic acid has rarely been measured in brain and/or liver and only in the rat [14, 29, 32].

In the present study we found that delta-desaturase activity in brain homogenate was linear up to 4 mg protein per ml incubation medium at all ages. With liver homogenate, it was linear up to 2.5 mg protein/ml up to 5 days after birth; thereafter it was linear up to 1.5 mg/ml.

Fig. 1 shows that liver activity increased approximately 7-fold from day 18 of fetal age up to day 11 after birth. It decreased by 50% up to weaning and was constant then up to 9 months. Very interestingly, delta-6 desaturase decreased 4-fold during aging. Schematically, this pattern is similar to the one we have previously found for desaturation of linoleic acid [9], but delta-6 desaturation is 2-fold more active with alpha-linolenic acid (this study) than with linoleic acid [9]. Desaturase alteration during aging is much more marked with alpha-linolenic acid (this study, 4-fold) than with linoleic acid (ref. 21; 40%). Interestingly, desaturase in rat liver microsomes decreases up to 9 months [32]. The different time pattern raises the question of whether linoleic and alpha-linolenic acid are desaturated by the same delta-6 desaturating enzyme.

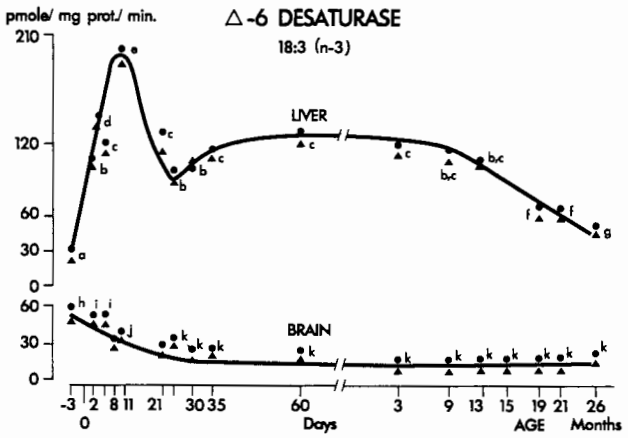

Fig. 1. Delta-6 desaturation of alpha-linolenic acid during development and aging in mouse brain and liver homogenates. ●, total synthesis of polyunsaturated fatty acids from alpha-linolenic acid (mainly stearidonic acid 18:4 n-3; with small amounts of eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3)); ▲, stearidonic acid synthesized from alpha-linolenic acid. Each point is the mean value from at least 4 experiments. Each experiment used at least 5 animals. Points not bearing the same superscript are significantly different at $P \leq 0.05$.

Early brain development requires large quantities of polyunsaturated fatty acids for membrane synthesis. Interestingly, the delta-6 desaturase activity decreased dramatically during pre- and postnatal development (4-fold) up to weaning; the highest activity was found during the perinatal period, which corresponds to neuronal and glial multiplication. Curiously, the activity did not peak during myelination, although myelin contains large amounts of polyunsaturated fatty acids; the same pattern has been found for desaturation of linoleic acid [9] and oleic acid through delta-9 desaturation [10].

As shown in Fig. 1, delta-6 desaturase activity decreased 2-fold between weaning and 9 months to a low and nearly stable level thereafter. This raises the question of whether this residual activity is sufficient to provide the polyunsaturated fatty acids necessary for the physiological turnover of all brain membranes. If not, these fatty acids must be provided by the liver, but in this

organ, desaturating activity diminishes during aging. Thus, during aging, provision of stearidonic acid in the diet could be of interest, to provide the substrate for chain lengthening so as to by-pass the affected desaturase.

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