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ALTERATIONS IN EIGHTEEN-CARBON SATURATED, MONOUNSATURATED AND POLYUNSATURATED FATTY ACID PEROXISOMAL OXIDATION IN MOUSE BRAIN DURING DEVELOPMENT AND AGING.

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SUMMARY

Peroxisomal oxidation was measured in mouse brain homogenate by adding cyanide to the test tube (which inhibits mitochondrial oxidation). Eighteen-carbon fatty acids (saturated, monounsaturated and polyunsaturated) were oxidized by brain peroxisomes. At nearly all ages, oxidation of oleic acid was higher (about 2 fold) than oxidation of other eighteen-carbon fatty acids. In contrast to other fatty acids, stearic acid oxidation decreased regularly up to weaning (6 fold) and was stable thereafter. Oleic acid oxidation increased up to weaning, decreased during development up to day 70 and remained subsequently nearly stable. Linoleic acid and alpha-linolenic acid oxidation increased up to weaning, decreased up to day 105 and was nearly stable thereafter. Alpha-linolenic acid oxidation about was two fold lower than linoleic acid oxidation. Interestingly, peroxisomal oxidation for all fatty acids examined declined during aging, between day 365 and day 450.

INTRODUCTION.

Polyunsaturated fatty acids derived from the dietary precursors linoleic and alpha-linolenic acids control the structure and function of membranes, including those in the brain. Brain contains minute amounts of linoleic and alpha-linolenic acid; consequently renewal of membrane fatty acids could be under the control of the level of these fatty acids. Thus it is important to determine whether this level can be affected by oxidation, particularly in peroxisomes. Moreover, a number of peroxisomal diseases in humans affect the fatty acid profile in the brain (1), and fatty acid analysis is the basis for diagnosis of peroxisomal disorders with neurological involvement (2). Only very limited information is available on eighteen-carbon atom

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beta-oxidation, and no studies have been performed during development and aging. However, it has been reported that stearic and lignoceric acids are oxidized in brain peroxisomes (3) and inhibition in vivo of peroxisomal oxidation induces alterations in rat brain development and changes in the brain fatty acid profile (4, 5). Moreover, peroxisome proliferator-activated receptors are localized in the brain (6).

It is well known that saturated and unsaturated fatty acids are chain-shortened by beta-oxidation in mitochondria and peroxisomes, particularly in the liver. Mitochondria oxidize the major portion of the long chain fatty acids (C16 - C18) which are an important source of metabolic fuel. Peroxisomal oxidation, which in animal cells is not complete since there is only a limited number of cycles for beta-oxidation, is responsible for chain-shortening of very long chain fatty acids including very long chain polyunsaturated fatty acids (7), as well as other molecules (8). Thus liver peroxisomal beta-oxidation provides acetyl-coA for the anabolic functions of the cells; peroxisomal beta-oxidation enzymes are unique proteins with molecular properties that are quite different from those of their mitochondrial counterparts and they are encoded by different nuclear genes (9).
Stearic acid beta-oxidation in liver peroxisomes is six-fold higher than oxidation in mitochondria (3). Gamma-linolenic acid is highly oxidized in liver, in contrast to arachidonic acid; oleic acid and docosahexaenoic acid are oxidized (10) in rat liver. Peroxisomal oxidation rates decrease markedly with substrates longer than eighteen carbon atoms both in homogenates (11) and in isolated liver peroxisomes (10, 12-14)

We have recently investigated liver peroxisomal beta-oxidation activity on stearate, oleate, linoleate and alpha-linolenate as a funtion of age in two mouse strains (15). Each fatty acid showed a similar beta-oxidation activity pattern with age characterized by a rapid increase (200%) from day 2 to 20 followed by a dramatic weaning-related decrease (70%) from day 20 to 22. There was a further increase (260%) from day 22 to 75, then a plateau up to day 300, and finally an age-related decrease (70%) from day 300 to 540. Oleic, linoleic and alpha-linolenic acids were respectively 5-, 7.5- and 9-fold more degraded than stearic acid. Interestingly, a longer-lived strain exhibited higher activity for peroxisomal beta-oxidation.

Comparison with various liver peroxisomal-related enzymes (16) suggested that aging decreases the peroxisomal polyunsaturated fatty acid oxidizing system via a specific decrease in acyl-CoA-oxidase activity, and that clofibrate treatment triggers peroxisomal proliferation. Thus, the aging-related decrease in peroxisomal activities might be due to an alteration in peroxisome synthesis.

These experiments were designed to determine the level of peroxisomal oxidation of the various 18-carbon fatty acids in the brain and whether the level is altered during development and aging. The results are very important: if alterations are present, it suggest that fatty acids necessary for membrane synthesis and renewal are altered, and that the supply of precursors for eicosenoids might be compromised.

MATERIAL AND METHODS.

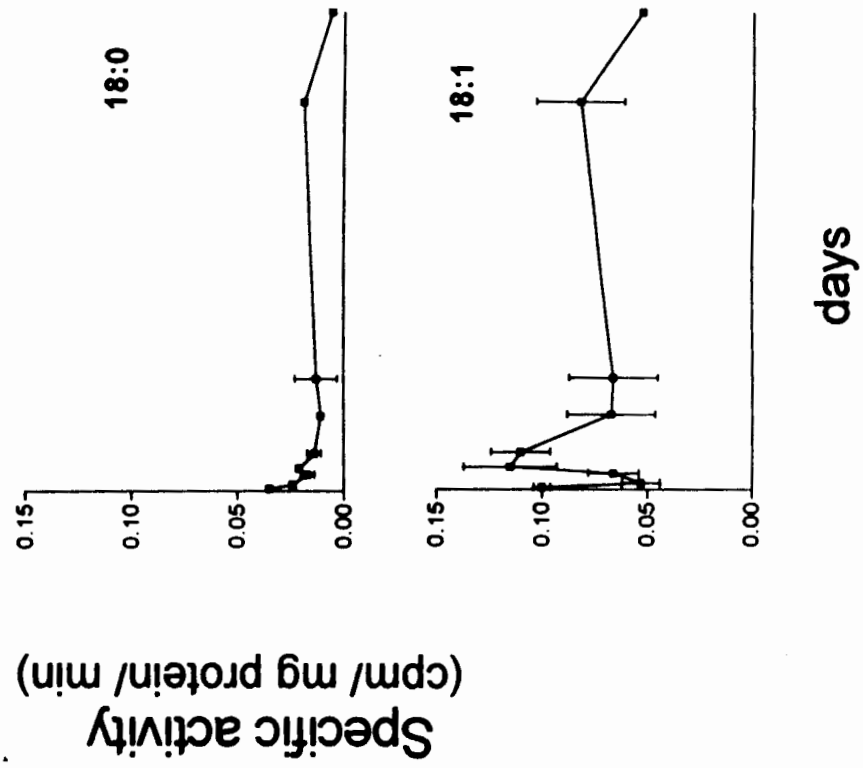
Preparation of brain homogenates and incubations with labeled fatty acids were performed as previously described for the liver (15, 16).
Preparation of fatty acid solutions: [1-^{14}C]-fatty acids (NEN, France) were solubilized according to Singh and Kishimoto (17). [1-^{14}C]-fatty acids (specific activity: about 52 mCi.mmol^{-1}) were prepared as 100 µM stock solutions. [1-^{14}C]-stearic acid, [1-^{14}C]-oleic acid, [1-^{14}C]-linolenic acid and [1-^{14}C]-alpha-linolenic acid were dried under N_2, and solubilized in a solution of 100 mM Tris-HCl (pH 8.5), 10 mg/ml alpha-cyclodextrin solution. Solubilization was complete after 30 min incubation in a sonicated water bath at room temperature.

Animals: Animals were female CSWV or OF1 mice. They were bred in our laboratory and fed a standard AO3 diet (UAR, France). The light/dark cycle was 12h/12h and room temperature 24°C.

Brain homogenate preparation: Three homogenates were prepared for each age except for day 20, 22, 300, 365, 450 and 500 when five homogenates were used. Animals were killed by cervical dislocation and weighed. The brain was rapidly removed, weighed and homogenized in 0.25 M sucrose, 2 mM MOPS-NaOH (pH 7.4) buffer (1:5 w/v). Automatic homogenization was carried out for 30 sec at 300 rpm with a Thomas teflon B pestle. The homogenate was centrifuged for 5 min at 4°C at 3000*g*. The upper phase, preserved in ice, was immediately used for incubations.

Fatty acid oxidation assays: Assays were carried out in duplicate essentially as described by Wanders et al. (18, 19) with inhibition of mitochondrial beta-oxidation by cyanide. Briefly, incubations (10 min.) were performed after addition of mouse brain homogenate to 10 µM [1-^{14}C]-fatty acid, 10 mM ATP, 1 mM NAD, 5 mM $MgCl_2$, 200 µM CoA, 5 mM KCN, 50 mM MOPS pH 7.4, 300 mM sucrose. Lipids were removed by methanol/chloroform/heptan (1.41:1.25:1.00, by vol) extraction. Remaining [1-^{14}C]-acyl esters in 0.1 M sodium acetate were hydrolyzed with NaOH at 50°C for 45 min and subsequently removed by identical methanol/chloroform/heptan extraction after acidification with H_2SO_4. Radioactivity was determined in 500 µl of the remaining aqueous phase by scintillation counting.

Protein assays were performed in triplicate according to Lowry with bovine serum albumin as standard.
Statistical significance of means was calculated by analysis of variance (ANOVA) using Student-Newman-Keuls test for multiple comparisons and with Student's t test for comparison of two groups. The Mann-Whitney test was also used when the


18:0
18:1
0.15
0.10
0.05
0.00
Specific activity
(cpm/ mg protein/ min)
days

Peroxisomal oxidation of 18-carbon fatty acids in the brain during development and aging.
Deviations were much higher with oleic acid than with other fatty acids.
Stearic acid: 18:0; oleic acid: 18:1; linoleic acid: 18:2; alpha-linolenic acid: 18:3.

conditions of parametrics tests were not met. Difference was considered significant at $p < 0.05$. SigmaStats programe package (Jandel Sceintific Ekrath Germany) was used to perform the statistical analysis.

RESULTS AND DISCUSSION

In intact mitochondia, fatty acid oxidation is tightly coupled to the respiratory chain, so that beta-oxidation stops when respiration is inhibited, for example by cyanide.
The physiological function of peroxisomal peroxidation is still controversial, and not well defined. It is however clear that this process plays a role in chain shortening of long and very long chain fatty acids. In the liver the process is more active following induction by a high fat diet (14), and impaired fatty acid oxidation is the cause of accumulation of very long chain fatty acids in human peroxisomal diseases such as adrenoleukodistrophy (1, 20).

Interestingly, peroxisomal beta-oxidation of lignoceric acid (a 24-atom saturated fatty acid) in brain (21) increases dramatically during development up to weaning, and slightly decreases shortly after (22), in contrast to oxidation of eighteen-carbon fatty acids in this study.
It is not clear whether the balance between mitochondrial and peroxisomal oxidation (8) controls the level of precursors in brain; however, besides peroxisomal oxidation, mitochondrial oxidation is potent in liver (23).

Compared to our previous results (15, 16), oxidation for all fatty acids was much higher in the liver than in the brain. In contrast to the brain in the liver, at all ages, linoleic and alpha-linolenic oxidation was 2- and 4-fold more active than oxidation of oleic and stearic acid, respectively. Activities increased up to day 6 and decreased slightly thereafter. Peroxisomal oxidation of 18:0 and 18:1 was of the same order of magnitude in brain and liver, thus ensuring the physiological turn-over of membrane fatty acids (largely present in myelin, for example). In contrast, 18:2 and 18:3 peroxisomal oxidation was lower in brain than in liver, probably due to the very minute amount of these two fatty acids in the nervous tissues. It is difficult to normalise for peroxisome numbers between different ages. Protein content normalisation allows poorly for this. However, we have previously shown that a peroxisomal enzyme, catalase, present decreasing activity between 7 and 60 days (25) and increasing activity between 2 and 12 months and is stable afterward (25).
These results show that peroxisomal oxidation for each eighteen-carbon fatty acid is different during development and aging. Further experiments must now be performed

with purified perxisomes, but the technique used for isolating peroxisomes from brain is much more complex than for liver (22, 26) and does not provide ultra-pure peroxisomes. As contaminants have a high rate of fatty acid metabolism, measurement of fatty acid metabolism in peroxisomes requires ultra-pure organelles. In conclusion, these results clearly demonstrate that eighteen-carbon-atom fatty acids are oxidized by brain peroxisomes, thus possibly controlling very long chain metabolism and the maintenance of membrane fatty acid composition.

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