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STEARYL-ALCOHOL BIOSYNTHESIS FROM STEARYL-CoA IN MOUSE BRAIN MICROSOMES IN NORMAL AND DYSMYELINATING MUTANTS (QUAKING AND JIMPY)

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SUMMARY

In mouse brain microsomes, stearyl-CoA is reduced into stearyl-alcohol in the presence of NADPH (NADH is hardly active). Using both NADPH and NADH does not increase the synthesis. This synthesis is reduced by 60% when using stearic acid + CoA + ATP + Mg^{2+} instead of stearyl-CoA, thus showing that stearic acid must be activated before reduction, stearyl-CoA being the substrate. Stearyl-alcohol synthesis increases during development and parallels the plasmalogens deposition; this biosynthesis is 51% normal in the Quaking mouse brains (but at least 18% of this synthesis is used for plasmalogens found outside myelin).

Several studies in vivo [2,13,14] and in vitro[10] have shown that fatty alcohols are the precursors of ether-linked long chains in glycerolipids in various biological materials (including brain). Moreover, free fatty alcohols have been detected in very low concentration in mammalian brain [15], but the biosynthesis of fatty alcohols has not been elucidated in nervous tissue. As fatty alcohols showed a high specific radioactivity after intracerebral injection of radioactive palmitic acid [13], the formation of the long chain fatty alcohol by reduction of the corresponding fatty acyl-CoA is a likely pathway, as brain has various enzymes for the synthesis of fatty acids [3,5,12] and as this organ is able to synthesize fatty alcohols from fatty acids in the presence of CoA: thus acyl-CoA was postulated to be the primer for alcohol synthesis in rat brain [11].

Thus the purpose of this work is to confirm that mouse brain microsomes are able to synthesize stearyl-alcohol from stearyl-CoA. Preliminary results have shown that besides fatty acids biosynthesis from acyl-CoA and malonyl-CoA, some hydroxylated compounds are formed, identified as fatty alcohols (unpublished data). As fatty alcohols are involved in the synthesis of plasmalogens, this mechanism may be an important event during brain development,

for these phospholipids are concentrated in myelin membrane [16]. Therefore, studies were also undertaken in the Quaking and Jimpy dysmyelinating mutant mice: in these animals no data have been presented on fatty alcohol biosynthesis.

C57Bl/6J mice and Jimpy and Quaking mutant mice are raised in our laboratory. Microsomal preparations from 18-day-old mice have been previously described [3]. Briefly, tissue is washed and homogenized in cold 0.32 M sucrose, potassium phosphate 0.1 M, pH 7, NaCl 0.9%. It is first centrifuged at 17,500 × *g* and the pellet containing cellular debris, mitochondria, synaptosomes and myelin is discarded. The supernatant is then spun at 100,000 × *g*. The purity is checked by electron microscopy and marker enzymes [4]. Routinely the incubation mixture contains 50 μM [1-^{14}C] stearyl-CoA (2 μCi/μmole), 500 μM NADPH and 1 mg protein. The final volume is made up to 1 ml with phosphate buffer. After 30 min at 37°C, the reaction is stopped with 5 ml of chloroform-methanol 2:1 (v/v) containing a mixture of standards of stearic acid, stearyl-alcohol and stearyl aldehyde (these standards were purchased from Supelco). After vigorously stirring for 1 min, the upper phase is studied by thin-layer chromatography (with Merck 60 F 254 plates) with chloroform-acetic acid 90:10 (v/v). This technique separates alcohol (R_f = 0.76), acid (R_f = 0.85) and aldehyde (R_f = 0.93) from other lipids. Each compound is extracted with 2 × 5 ml chloroform-methanol 2:1 and rechromatographed by thin-layer chromatography with a solvent system made of petroleum ether-ether-acetic acid 70:30:2 (v/v/v). Thus, pure alcohol, acid and aldehyde are isolated (R_fs being, respectively, 0.25, 0.42 and 0.58) and their radioactivity is measured by counting in Cab-O-sil mixture.

For plasmalogen measurements, a lipid extract was performed as previously described [1] and plasmalogens were determined by iodine assay [9].

The acyl-CoAs were synthesized from fatty acids and CoA [8]. Seventy-five μmole of stearic acid dissolved in 6 ml dichloremethane were incubated for 5 min, at 39°C in the presence of collidine (10.8 μl), then 10.8 μl ethyl-chloroformiate were added and the mixture was left at room temperature for 10 min. Dichloromethane was then evaporated to dryness and the residue was dissolved in freshly distilled tetrahydrofurane (T.H.F.) (1.5 ml). The non-soluble collidinium chloride was spun down by a brief centrifugation at 6,000 rpm. The supernatant was collected and the pellet was washed with T.H.F. (1.5 ml). The yield of the reaction was 83% when measured by hydroxamate method.

Ninety μmole of CoA were dissolved in 900 μl distilled water, 150 μl tris · HCl buffer 1 M, pH 8.5, and 150 μl pyridine. The anhydride was then added to this mixture (gently and under N_2). After vigorously stirring, the mixture was buffered at pH 7 by 1 N HCl and was extracted three times with 10 ml ether. The stearyl-CoA was then precipitated by 10% perchloric acid added dropwise and the mixture was re-extracted with ether (acyl-CoA forms a ring at the interface). The remaining ether was evaporated by N_2; the test tube was centrifuged for 15 min at 17, 500 × *g*. The pellet was dissolved in water and the pH was adjusted to 5.5 with 1N NaOH. The solution was then extracted with 5 volumes of chloroform-methanol 2:1 at 4°C for 1 h; the upper phase contained pure

stearyl-CoA. The yield of synthesis was 30%, starting from stearic acid.

The purity was checked by thin-layer chromatography on silica gel plates visualized under UV (350 nm); the eluting solvent was butanol-water-acetic acid 50:30:20 (v/v/v). The R_f values for stearic acid, stearyl-CoA and CoA are, respectively, 0.70, 0.55 and 0.15.

We used 130 normal, 35 Quaking and 45 Jimpy mice.

RESULTS AND DISCUSSION

NADPH is absolutely necessary for reduction: the fatty alcohol synthesis is regularly increasing up to 1 mM NADPH. Without NADPH some synthesis occurs (8% of the activity at 500 μM NADPH), presumably because there is still sufficient endogenously reduced pyridine nucleotide. No activity is detectable with boiled enzyme. NADH cannot replace NADPH: thus NADPH alone will be used, since adding NADH in the presence of NADPH does not significantly change the synthesis. When using stearic acid, ATP, CoA and Mg^{2+} instead of stearyl-CoA, the yield of fatty alcohol synthesis is reduced by 60% (as fatty acid must be activated and then reduced this two steps reaction probably reduces the yield). In the absence of ATP the yield of fatty alcohol synthesis is nil. The rate of formation of stearyl-alcohol is found to be nearly linear up to 1 mg protein; under the conditions described, it is also linear up to 30 min of

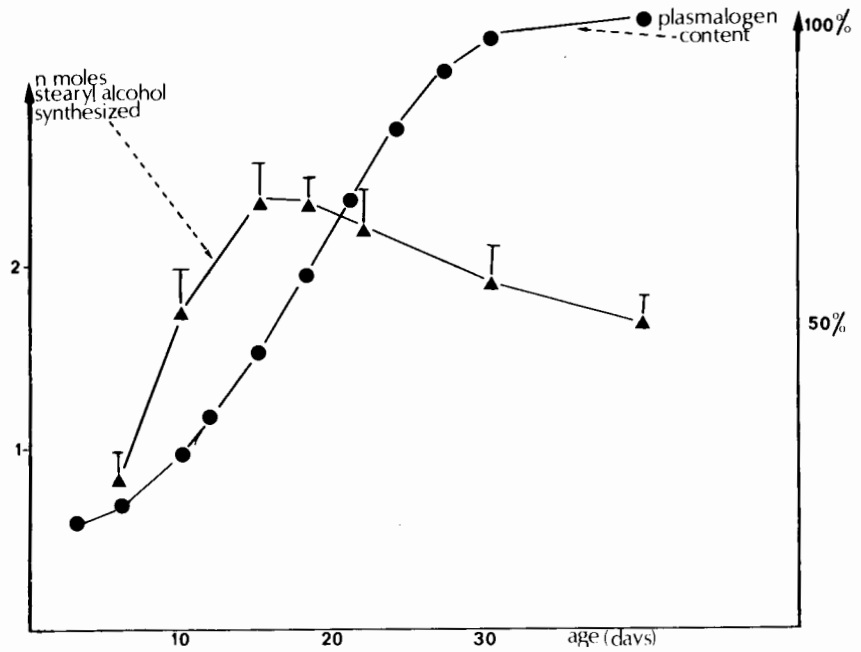

Fig. 1. Stearyl-alcohol biosynthesis and plasmalogen content during brain development. •, plasmalogen content; ▲, Stearyl-alcohol biosynthesis.

incubation. A minute amount of fatty aldehyde is detected. Thus, there is no conclusive evidence for the formation of fatty aldehyde as an intermediate as has been proposed [11]. From these studies the conversion of stearic acid to stearyl-alcohol in mouse brain may be envisaged as:

$$\text{stearic acid} \xrightarrow[\text{ATP Mg}]{\text{CoA}} \text{stearyl-CoA} \longrightarrow \text{stearyl-alcohol}$$

This confirm the results obtained in rat brain where palmitic acid is transformed into hexadecanol in the presence of CoA and ATP [11].

This pathway of reduction of activated fatty acid is probably universal since several studies indicate that fatty acids are good precursors of ether-linked long chain moieties in glycerolipids in various biological systems. However, this pathway does not exclude that fatty alcohols could be formed by oxidation of hydrocarbons, as these compounds have been recently detected in brain [6].

In Fig. 1 is shown the plasmalogen content in the normal brain as a function

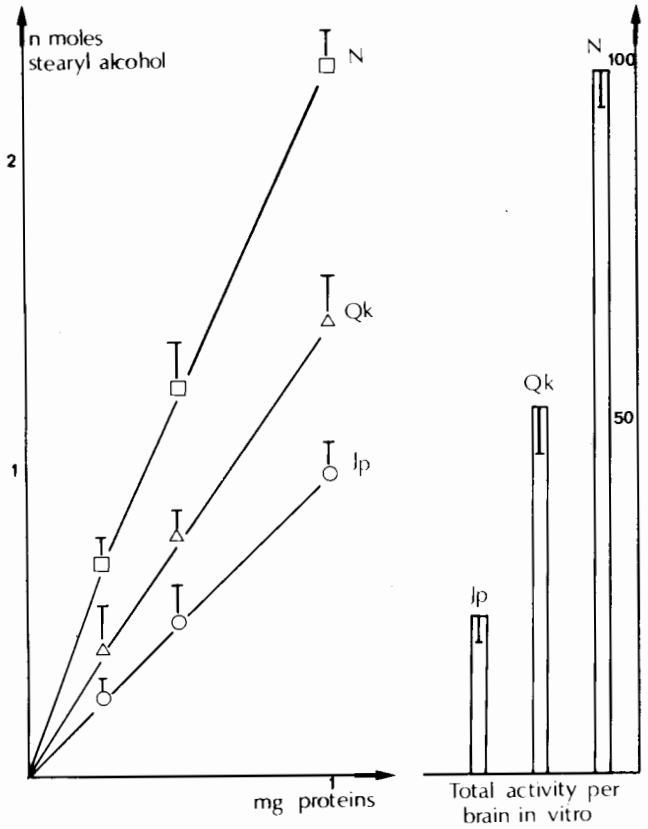

Fig. 2. Stearyl-alcohol biosynthesis in normal and dysmyelinating mutants (Quaking and Jimpy). N, normal; Qk, Quaking; Jp, Jimpy.

of age. The highest speed of deposition is around 15 days of age (between 12 and 20 days); very young animals (3-day-old) contain 18% of the adult level. In the adult the values are 14.9 ± 0.9 μmole/g brain, or 6.1 ± 0.6 μmole/brain. In Quaking these values are, respectively, 9.1 ± 0.8 and 3.6 ± 0.5. This curve is similar to the one obtained in the rat [16]. Stearyl-alcohol biosynthesis is maximal at 15 days and slowly decreases thereafter. This unexpected light decrease is probably due to the occurrence of plasmalogens beside myelin and to a greater turn-over of plasmalogens than other myelin lipids.

In Fig. 2 is shown stearyl-alcohol biosynthesis in 18-day-old normal and dysmyelinating mutants. The synthesis is 51% normal in Quaking and 23% normal in Jimpy. In Jimpy (in which there is no myelin) this synthesis reflects the plasmalogen deposition outside myelin, which can be estimated to be at least 18% in the normal animal (as 6-day-old mice contain 18% of the plasmalogens content of the adult animals, although at this stage brain is devoided of myelin. In this respect the synthesis in the Quaking is reduced by 49%; but 18% at least being used outside myelin, the decrease in stearyl-alcohol biosynthesis for myelin elaboration can be estimated to be about 70%.

When considering the phosphotransferase, the rate of synthesis of ether lipids is less with mutant brain microsomes than with microsomes from littermate controls [7]: according to our results it appears that the deficiency of plasmalogens in the mutants is also due to another enzymatic defect in the metabolism of plasmalogens at the level of fatty alcohols.

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REFERENCES

1 Baumann, N.A., Jacque, C., Pollet, S. and Harpin, M.L., Fatty acid and lipid composition of the brain of a myelin deficient mutant, the Quaking mouse, Europ. J. Biochem., 4 (1968) 340—344.
2 Bell, O.E., Blank, M.L. and Snyder, F., The incorporation of ^{18}O and ^{14}C from long chain alcohols into the alkyl and alk-1-enyl ethers of phospholipids of developing brain, Biochim. biophys. Acta (Amst.), 231 (1970) 577—593.
3 Bourre, J.M., Pollet, S.A., Dubois, G. et Baumann, N.A., Biosynthèse des acides gras à très longues chaînes dans les microsomes de cerveau de souris, C.R. Acad. Sci. (Paris), 271 (1970) 1221—1223.
4 Bourre, J.M., Pollet, S.A., Daudu, O.L. and Baumann, N.A., Evolution in mouse brain microsomes of lipids and their constituents during myelination, Brain Res., 51 (1973) 225—239.
5 Bourre, J.M., Daudu, O.L. and Baumann, N.A., Lignoceric acid in the developing brain. Activities of mitochondrial acetyl-CoA dependent synthesis and microsomal malonyl chain elongating system in relation to myelination, Europ. J. Biochem., 72 (1977) 41—47.

6 Bourre, J.M., Cassagne, C., Larrouquere-Regnier, S. and Darriet, D., Occurrence of alkane in brain myelin. Comparison between normal and Quaking mouse, J. Neurochem., 29 (1977) 645—648.
7 Dorman, R.V., Freysz, L. and Morrocks, L.A., Synthesis of ethanolamine phosphoglycerides by microsomes from the brains of Jimpy and Quaking mice, J. Neurochem., 29 (1977) 231—233.
8 Goldman, P. and Vagelos, R., Chemical synthesis of acyl CoA esters, J. biol. Chem., 236 (1961) 2620—2629.
9 Gottfried, E.L. and Rapport, M.M., The biochemistry of plasmalogens, J. biol. chem., 237 (1962) 329—333.
10 Hajra, A.K. Biosynthesis of alkyl-ether containing lipid from dihydroxyacetone phosphate, Biochem. Biophys. Res. Commun., 164 (1969) 486—492.
11 Natrajan, Y. and Sastry, P.S., Conversion of (1-^{14}C)palmitic acid to (1-^{14}C)hexadecanol by developing rat brain cell free preparation, J. Neurochem., 26 (1976) 107—113.
12 Paturneau-Jouas, M., Baumann, N. and Bourre, J.M., Elongation of palmityl-CoA in mouse brain mitochondria. Comparison with stearyl-CoA, Biochem. Biophys. Res. Commun., 4 (1976) 1326—1334.
13 Schmid, H.H. and Takahashi, T., Reductive and oxidative biosynthesis of plasmalogens in myelinating brain J. biol. Chem., 11 (1970) 412—419.
14 Stoffel, W. and Lekim, D., On the stereospecificity of the biochemical dehydrogenation of the 1-O-alkyl glyceryl to the 1-O-alk-1′-enyl glyceryl ether bond, Hoppe-Seyler's Z. Physiol. Chem., 352 (1971) 501—511.
15 Takahashi, T. and Schmid, T., Long chain alcohol in mammalian tissue, Chem. Phys. Lipids, 4 (1970) 243—246.
16 Wells, M.A. and Dittmer, J.C., A comprehensive study of the postnatal changes in the concentration of the lipids of developing rat brain, biochemistry, 6 (1967) 3169—3175.