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THE ACTIVITY OF PARTIAL REACTIONS IN THE CHAIN ELONGATION OF PALMITOYL-CoA AND STEAROYL-CoA BY MOUSE BRAIN MICROSOMES

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Abstract—Partial reactions in the overall chain elongation of palmitoyl-CoA and stearoyl-CoA by mouse brain microsomes have been analyzed. The rate of the initial condensation reaction between palmitoyl-CoA and malonyl-CoA was more than 5 times greater than the rate obtained with stearoyl-CoA, and in both cases good agreement between condensation and overall chain elongation rates was observed.

By contrast, both β-hydroxyoctadecanoyl-CoA and β-hydroxyeicosanoyl-CoA were quite rapidly dehydrated by brain microsomes at similar rates. Similar results were obtained with 2-*trans*-octadecenoyl-CoA and 2-*trans*-eicosenoyl-CoA in which both substrates were rapidly reduced at nearly the same rate in the presence of NADPH. In all cases, intermediate reactions subsequent to condensation were much more rapid than overall chain elongation. These results suggest that the mechanism of malonyl-CoA-dependent fatty acid chain elongation in brain microsomes is similar to that observed in other tissues, and are consistent with an overall regulation of chain elongation mediated primarily by the initial condensation reaction.

THE MALONYL-CoA-dependent fatty acid chain elongation system in microsomes represents a potentially important means of modifying fatty acids obtained from *de novo* synthetic activity or the diet to meet the requirements of a particular tissue. Although early studies were primarily concerned with the hepatic system (ABRAHAM *et al.*, 1961; STOFFEL & ACH, 1964; NUGTEREN, 1965), fatty acid elongation in brain has proven to be of considerable interest due in part to the prominence of very long chain saturated and mono-unsaturated fatty acids in the sphingolipids of myelin (O'BRIEN, 1965) and to the availability of mutant mice defective in both myelination (SIDMAN *et al.*, 1964) and fatty acid biosynthetic activity (BOURRE *et al.*, 1973*a*; 1977).

In 1965, Nugteren established that β-keto, β-hydroxy and *trans*-α,β-unsaturated intermediates were formed during fatty acid chain elongation in rat liver microsomes. A recent investigation of partial reactions in the hepatic fatty acid chain elongation sequence employing representative saturated and polyunsaturated substrates has demonstrated that in each case the rates of the initial condensation reaction between fatty acyl-CoA and malonyl-CoA show good agreement with those of overall chain elongation, while subsequent partial reactions are much more rapid than chain elongation (BERNERT & SPRECHER, 1977). The object of this study was to establish whether the same malonyl-CoA-dependent chain elongation system is operative in mouse brain microsomes, and whether the considerably slower rate of stearoyl-CoA elongation relative to that of palmitoyl-CoA in this tissue may be attributed to a difference in condensation activity. Evidence for the existence of separate systems acting on these two substrates has been provided (BOURRE *et al.*, 1970; 1973*a*; GOLDBERG *et al.*, 1973).

MATERIALS AND METHODS

Materials. Malonyl-CoA, NADPH, palmitoyl-CoA and stearoyl-CoA were obtained from Pabst-Biochemical, while bovine serum albumin containing less than 0.005% free fatty acids was purchased from the Sigma Chemical Co., St. Louis, MO (U.S.A.). [1-^{14}C]Palmitoyl-CoA and [1-^{14}C]stearoyl-CoA were products of N.E.N. Chemicals (Dreieichenhain, West Germany) and were diluted to a specific activity of 2 Ci/mol with the unlabeled analogue. [3-^{14}C]DL-β-hydroxy-18:0-CoA (0.49 Ci/mol), [3-^{14}C]DL-β-hydroxy-20:0-CoA (0.15 Ci/mol), [3-^{14}C]2-*trans*-18:1-CoA (0.16 Ci/mol) and [3-^{14}C]2-*trans*-20:1-CoA (0.38 Ci/mol) were chemically synthesized using the procedures previously described (BERNERT & SPRECHER, 1977).

Microsome preparation. Normal 18-day-old C57-black mice of either sex were used. The pooled brains were washed and homogenized in cold 0.32 M-sucrose, 0.1 M-phosphate buffer, pH 7. Microsomes were then prepared as previously described by BOURRE *et al.* (1973*b*).

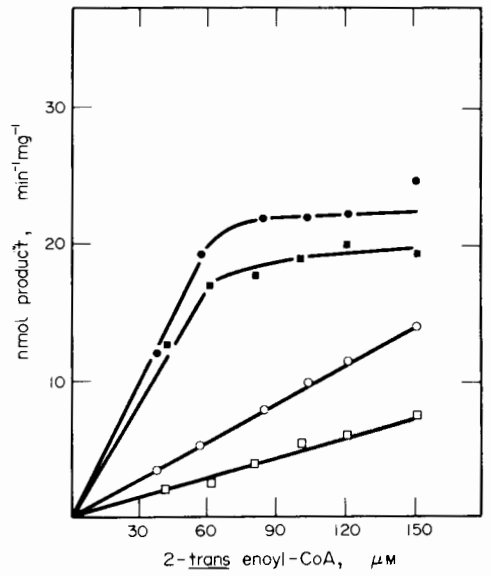

FIG. 4. Kinetics of the enoyl-CoA reductase reaction indicating the simultaneous formation of stearic acid (●) and β-hydroxy 18:0 (○) when [3-^{14}C]2-*trans* 18:1-CoA was the substrate, and of arachidic acid (■) and β-hydroxy 20:0 (□) when [3-^{14}C]2-*trans* 20:1-CoA was employed as substrate. Conditions of assay were as described in Materials and Methods.

tial reactions investigated in this study confirmed that the slowest by far was that of condensation (Table 1). Such an observation is consistent with an overall regulation of the chain elongation process mediated by this first committed step. Further support for this hypothesis is provided by a comparison of the partial reaction rates obtained in this study with overall chain elongation rates calculated from the data of BOURRE *et al.* (1975*b*) (Table 1), which were obtained under very similar reaction conditions. While a close relationship between chain elongation and condensation activities could be discerned, both β-hydroxy acyl-CoA dehydrase and 2-*trans* enoyl-CoA reductase reactions occurred at rates at least 10–20 times greater than chain elongation. Both the dehydrase and enoyl reductase reactions displayed some selectivity in that the 20 carbon substrates were converted somewhat more slowly than were the 18 carbon analogs. However, in neither case were the differences as marked as they were in the condensation reaction.

DISCUSSION

In vivo studies have established that the essential fatty acid pattern in brain lipids may be altered by dietary manipulation (HOLMAN & MOHRHAUER, 1963; SUN, 1972); and it has been shown that non-essential fatty acids also may be taken up intact by the brain when either fed or injected (DHOPESHWARKAR & MEAD, 1969; GOZLAN-DEVILLIERRE *et al.*, 1976). Nevertheless, endogenous synthesis is probably of considerable importance in this tissue, especially in the production of the very long chain and α-hydroxylated acids characteristic of myelin. It is well-established that brain microsomes are capable of elongating a variety of long chain fatty acids by a malonyl-CoA-dependent process (AEBERHARD & MENKES, 1968; BOURRE *et al.*, 1970, 1973*a*, 1975*a*, 1976*a*, 1977; GOLDBERG *et al.*, 1973; BROPHY & VANCE, 1975). However, whether the mechanism of this reaction is similar to that occurring in liver which involves the production of at least three distinct intermediates in the overall process (NUGTEREN, 1965) has not previously been determined. The results of this study indicate that chain elongation intermediates may be recovered as partial reaction products, and are active as substrates when in their CoA thioester form, as is the case in the hepatic system.

We have previously reported that the inclusion of albumin in the incubation is essential in assays of both condensation and enoyl-CoA reductase reactions with rat liver microsomes, while neither the hydroxy acyl-CoA dehydrase nor overall chain elongation rates are greatly affected by its presence (BERNERT & SPRECHER, 1977). The albumin probably is involved in product protection during condensation assays, while the effect on enoyl-CoA reductase reactions is more complex (BERNERT & SPRECHER, in press). Although the effect of albumin on partial reactions in the brain chain elongation system has not been investigated, it was routinely included at a constant molar ratio of 2 relative to substrate concentration

TABLE 1. COMPARISON OF REACTION RATES IN THE CHAIN ELONGATION OF PALMITIC AND STEARIC ACID

Initial substrate	Condensation*	β-Hydroxyacyl-CoA dehydrase†	Enoyl-CoA reductase	Overall chain elongation‡
16:0	0.57 ± 0.01 2 (48)	9.8 ± 0.8 2 (45)	22.0 1 (30)	0.60 ± 0.08
18:0	0.09 ± 0.01 2 (48)	6.1 ± 0.2 2 (45)	19.5 1 (30)	0.04 ± 0.01

Results are expressed as nmols of product formed min^{-1} (mg protein)$^{-1}$ ± S.E.M. The number of microsomal preparations for each value is indicated immediately below the rate, with the total number of pooled brains given in parentheses.

* Determined at a substrate concentration of 50 μM.

† Determined at a substrate concentration of 100 μM.

‡ Calculated from the data of BOURRE *et al.*, 1975*b*.

in all assays. Since the total albumin concentration in the incubation was thus variable when *v/s* curves were analyzed, the gradual rise in rate observed in the condensation reaction with palmitoyl-CoA at substrate concentrations above 35–40 μM (Fig. 1) may have reflected a less than optimum acyl-CoA:albumin molar ratio for this reaction. It might be noted that an essentially normal *v/s* curve was obtained for this reaction when malonyl-CoA was varied at a constant palmitoyl-CoA (and albumin) concentration (Fig. 2). The inclusion of albumin in all reaction assays also reduces the possibility that substrate deacylation influenced reaction rates since BROPHY & VANCE (1976) noted that the addition of albumin to their incubations essentially eliminated acyl-CoA hydrolase activity.

There is good evidence that two different chain elongation systems are involved in the elongation of palmitoyl-CoA and stearoyl-CoA by mouse brain microsomes (BOURRE *et al.*, 1970; POLLET *et al.*, 1973; GOLDBERG *et al.*, 1973). The results of this study suggest that this dichotomy may involve the condensation reaction, in which one or more separate enzymes acting on these two substrates might serve to regulate their respective chain elongation activities. It would be interesting to determine whether a variation in condensation activity towards palmitoyl-CoA and stearoyl-CoA during early myelogenesis could account for the selective increase in overall chain elongation activity observed with these two substrates during this period (BOURRE *et al.*, 1976*b*).

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