Carnitine acyltransferase activities in rat brain mitochondria

Bimodal distribution, kinetic constants, regulation by malonyl-CoA and developmental pattern

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1. Carnitine palmitoyltransferase and carnitine octanoyltransferase activities in brain mitochondrial fractions were approx. 3–4-fold lower than activities in liver. 2. Estimated K_m values of CPT₁ and CPT₂ (the overt and latent forms respectively of carnitine palmitoyltransferase) for L-carnitine were 80 μ M and 326 μ M, respectively, and $K_{0.5}$ values for palmitoyl-CoA were 18.5 μ M and 12 μ M respectively. 3. CPT₁ activity was strongly inhibited by malonyl-CoA, with I_{50} values (concn. giving 50% of maximum inhibition) of approx. 1.5 μ M. In the absence of other ligands, [2-¹⁴C]malonyl-CoA bound to intact brain mitochondria in a manner consistent with the presence of two independent classes of binding sites. Estimated values for $K_{D(1)}$, $K_{D(2)}$, N_1 and N_2 were 18 nM, 27 μ M, 1.3 pmol/mg of protein and 168 pmol/mg of protein respectively. 4. Neither CPT₁ activity, nor its sensitivity towards malonyl-CoA, was affected by 72 h starvation. 5. Rates of oxidation of palmitoyl-CoA (in the presence of L-carnitine) or of palmitoylcarnitine by non-synaptic mitochondria were extremely low, indicating that neither CPT₁ nor CPT₂ was likely to be rate-limiting for β -oxidation in brain. 6. CPT₁ activity relative to mitochondrial protein increased slightly from birth to weaning (20 days) and thereafter decreased by approx. 50%.

Although the adult brain has a marked preference for glucose as its major respiratory substrate (McIlwain & Bachelard, 1971), the developing brain utilizes both glucose and ketone bodies (Page *et al.*, 1971; Cremer & Heath, 1974; Land *et al.*, 1977; Booth *et al.*, 1980). However, there are reports from experiments *in vivo* and *in vitro* (Vignais *et al.*, 1958; Allweis *et al.*, 1966; Little *et al.*, 1969; Roux *et al.*, 1970; Spitzer & Wolf, 1971) that brain can oxidize long-chain fatty acids, albeit at a rate considerably lower than that of liver. Furthermore, maximal rates of ¹⁴CO₂ release from

[¹⁴C]palmitate were only obtained in the presence of L-carnitine, CoA and malate (Beattie & Basford, 1965), suggesting that CPT activity was involved in the translocation of acyl groups into mitochondria before β -oxidation. Such transport would also be required for mitochondrial elongation of acyl-CoA, a pathway which is known to occur in brain (Cook & Spence, 1974; Bourre *et al.*, 1977). Previous measurements of CPT activity in human (Norum, 1966) and rat (Abdel-Latif *et al.*, 1973; Bresolin *et al.*, 1982) brain preparations were made by assays that either do not give quantitative estimates of enzyme activity or do not give information on the bimodal distribution of CPT across the mitochondrial membrane. [These assays can be criticized because: of the use of long incubation times; of the use of excessively high concentrations of acyl-CoA or acylcarnitine, which in the absence of albumin have detergent effects on mitochondrial membranes and alter the kinetics of CPT (see Bremer & Norum, 1967); the isotope-exchange assay probably measures total CPT activity.] The latter point is important in

Abbreviations used: CPT₁, CPT₂, COT₁ and COT₂, the overt and latent forms, respectively, of carnitine palmitoyltransferase (EC 2.3.1.21) and carnitine octanoyltransferase; I_{max} , maximal percentage inhibition of CPT₁, or COT₁, by malonyl-CoA; I_{50} , the concentration of malonyl-CoA giving 50% of I_{max} ; $K_{D(1)}$, $K_{D(2)}$, N_1 and N_2 , the dissociation constants and maximal binding capacities of the high-affinity (1) and low-affinity (2) malonyl-CoA-binding sites; $K_{0.5}$, the concentration of acyl-CoA required for 50% of the measured V_{max} .

tissues such as white adipose tissue, where overt CPT₁ activity is very low in relation to total (overt plus latent) CPT activity (Saggerson & Carpenter, 1981a). In addition, the brain contains different populations of mitochondria, which show different enzyme complements (see Leong *et al.*, 1984). Hence CPT activity was determined in both synaptic and non-synaptic mitochondria from forebrain. Some basic characteristics of CPT₁ and CPT₂ in non-synaptic mitochondria were determined, and we report that, as in all other tissues studied (McGarry *et al.*, 1978; Saggerson & Carpenter, 1981a), CPT₁ activity in brain is strongly inhibited by malonyl-CoA. In view of the changing substrate requirements in the neonatal brain, the developmental profile of CPT in non-synaptic mitochondria is also reported.

Materials and methods

Chemicals

The sources of chemicals have been described previously (Booth & Clark, 1978; Bird & Saggerson, 1984).

Animals

Wistar-strain rats were bred and maintained in the animal colony at St. Bartholomew's Hospital Medical College, London. Birth dates were noted after daily inspection. Before 21 days of age both male and female animals were used, but after that age only males were used. At 21 days of age rats were weaned on to Rat and Mouse Breeding Diet No. 3 (Stepfield, Witham, Essex, U.K.), with water freely available. Starved animals were killed between 09:00 and 10:00h. Adult animals were 50–60 days of age.

Preparation of mitochondria

The subcellular fractionation was carried out on the forebrains of 4–20 animals, depending on age. The preparation of the two mitochondrial fractions, non-synaptic (free) and synaptic, was essentially that of Lai *et al.* (1977), with modification by Booth & Clark (1978), except that for preparations of mitochondria from young animals the crude mitochondrial pellet was taken up in a final concentration of 11% Ficoll, rather than 10% used for an adult preparation. Mitochondria were resuspended in 320mM-sucrose/1mM-EDTA/10mM-Tris/HCl (pH 7.4) to a final concentration of about 10mg of protein/ml. When malonyl-CoA-binding assays were performed, 1mM-dithiothreitol was also present.

Rates of palmitoyl-group oxidation

Rates of oxygen uptake by purified non-synaptic mitochondria were determined with a Clark-type

oxygen electrode as described previously (Clark & Nicklas, 1970), except that the mitochondrial pellets were washed with isolation medium containing 0.5mg of bovine serum albumin/ml and the respiration medium contained 75mM-mannitol, 25mM-sucrose, 5mM-phosphate/Tris, 20mM-Tris/HCl (pH 7.4), 100mM-KCl, 0.5mM-EDTA, 1.3mg of bovine serum albumin/ml, 1μM-carbonyl cyanide chlorophenylhydrazone, 2.5mM-malate, 10μM-fluorocitrate, 2.5mM-malate and 0.75mM-arsenite. The final volume was 0.5ml, containing approx. 2mg of mitochondrial protein. This system was similar to that used previously by Harper & Saggerson (1975) to measure purely palmitoyl-group oxidation, without contribution from the tricarboxylic acid cycle, in liver or adipocyte mitochondria.

Enzyme assays

CPT and COT assays were performed in a final volume of 1.0ml at 25°C in an incubation medium containing 60mM-KCl, 150mM-sucrose, 25mM-Tris/HCl (pH 7.4), 1mM-EDTA, 1mM-dithiothreitol, fatty acid-poor albumin (1.3mg/ml) and approx. 0.2mg of mitochondrial protein plus malonyl-CoA and either palmitoyl-CoA or octanoyl-CoA at the concentrations indicated in the legends to the Table and Figures. After a 2min preincubation period at 25°C, reactions were started by the addition of L-carnitine (0.4μmol, except in experiments where L-carnitine concentration was varied), plus either 1μCi of DL-[Me-³H]carnitine or 0.5μCi of L-[Me-³H]carnitine, and were continued for 4min. Acyl[³H]carnitine was extracted into butan-1-ol and determined by liquid-scintillation counting (Saggerson *et al.*, 1982).

With intact mitochondria this assay essentially estimates CPT₁ activity. Determination of CPT₂ activity requires breakage of the mitochondrial membrane. Several methods were tested (e.g. omitting albumin from the assay, including Triton X-100 and sonication of mitochondria). Sonication (2min on ice at maximum power, 8μm peak-to-peak, with an M.S.E. ultrasonicator) was found to yield the maximum total CPT activity. CPT₂ activity was then calculated by subtracting CPT₁ activity from total activity. Acyl-CoA hydrolase activities were estimated in the same incubation medium, except that dithiothreitol was omitted, and 0.1mM-5,5'-dithiobis-(2-nitrobenzoic acid) was included. Hydrolase activity was calculated from the rate of change in *A*₄₁₂ measured in a Pye-Unicam SP8100 spectrophotometer. With 40μM-palmitoyl-CoA as substrate, the rates of deacylation were approx. 1.5 and 15nmol/min per mg of mitochondrial protein in whole and sonicated mitochondria respectively. Similar rates were

obtained with 40 μM -octanoyl-CoA, and with intact mitochondria there was no detectable rate of malonyl-CoA (20 μM) deacylation.

Quantitative measurements of [2- ^{14}C]malonyl-CoA binding to intact mitochondria were made as described by Bird & Saggerson (1984). The assays were carried out in 1.5 ml plastic tubes in the same incubation medium as used for the CPT assays, except that the concentrations of KCl, sucrose and mitochondrial protein were 40 μM , 185 μM and approx. 1.5 mg/ml respectively. Long-chain acyl-CoA derivatives were omitted from the assays, and [^{14}C]malonyl-CoA concentration was varied over the range 0.01–30 μM . After a 20 min incubation at 0–4°C, the tubes were rapidly centrifuged for 2 min in an Eppendorf 5412 centrifuge, and the supernatants were discarded. The mitochondrial pellets were solubilized in Triton X-100 (10%, v/v) and then transferred to vials for liquid-scintillation counting. The amount of specifically bound malonyl-CoA was calculated, after correction for the non-specific entrapment of [^{14}C]malonyl-CoA, and the data were analysed by computer by a mathematical model describing two independent classes of binding sites (Bird & Saggerson, 1984). The analysis was commenced with the 0.01 μM point.

Protein concentration was determined (Lowry *et al.*, 1951) with bovine serum albumin as standard.

Results

CPT and COT activities in synaptic and non-synaptic mitochondria

Both CPT and COT activities were readily demonstrable in the non-synaptic mitochondrial fraction isolated from young-adult rat brains (Table 1). CPT₁ activity was 3–4-fold lower than that reported for the liver and kidney-cortex enzymes, but was greater than that found in white

adipocytes and mammary-gland mitochondria (Saggerson & Carpenter, 1981a). In the last two tissues, however, a large proportion (approx. 95%) of total CPT activity was latent (CPT₂). This type of distribution was not apparent in brain mitochondria, where CPT₂ activity with 40 μM -palmitoyl-CoA was approximately double that of the overt CPT₁ activity. In liver, the two activities are approximately equal (Saggerson & Carpenter, 1981a). Comparison of relative activities at 100 μM -palmitoyl-CoA is not appropriate, owing to substrate inhibition of CPT₂ activity (see the following section).

Overt (CPT₁) activity in synaptic mitochondria was 52–70% greater than that in non-synaptic mitochondria. The latent (CPT₂) activity was slightly higher (22%) in synaptic as compared with non-synaptic mitochondria, but only when measured with 40 μM substrate. At 100 μM substrate concentration, the CPT₂ activity was decreased to 60% of the non-synaptic-mitochondrial activity. This may indicate a difference in the characteristic inhibition of CPT₂ in the two populations of mitochondria. The differences noted between the two populations of mitochondria may indicate a difference in the importance of CPT to metabolism at these sites in the brain. In contrast with liver (Saggerson *et al.*, 1982), starvation (up to 72 h) failed to cause any change in either overt or latent CPT activity in non-synaptic brain mitochondria (results not shown). COT₂ activity, in contrast with CPT₂ activity, was apparently not susceptible to substrate inhibition (Table 1), and the observation that activity with 100 μM -octanoyl-CoA was approximately double that with 40 μM substrate suggests that COT₂ has a relatively low affinity for its acyl-CoA substrate compared with COT₁. We are unaware of any previous determinations of COT activity in mammalian brain, and the nature and function of carnitine octanoyltransferases remain unclear (see Bremer, 1983).

Table 1. *Overt and latent activities of carnitine palmitoyltransferase and carnitine octanoyltransferase in rat brain mitochondria*

Overt and latent carnitine acyltransferase activities were determined with the indicated concentrations of palmitoyl-CoA (CPT) or octanoyl-CoA (COT). For the non-synaptic mitochondrial fraction, values are means $\pm$ S.E.M. for five separate experiments. For the synaptic mitochondria, values are means of two separate experiments $\pm$ their range.

Enzyme	Enzyme activity (nmol/min per mg of mitochondrial protein)					
	Non-synaptic mitochondria				Synaptic mitochondria	
	[Palmitoyl-CoA]		[Octanoyl-CoA]		[Palmitoyl-CoA]	
	40 μM	100 μM	40 μM	100 μM	40 μM	100 μM
Overt activity	0.89 $\pm$ 0.08	1.05 $\pm$ 0.11	0.71 $\pm$ 0.02	0.79 $\pm$ 0.02	1.35 $\pm$ 0.18	1.79 $\pm$ 0.15
Latent activity	1.94 $\pm$ 0.15	1.00 $\pm$ 0.12	0.75 $\pm$ 0.06	1.60 $\pm$ 0.16	2.36 $\pm$ 0.70	0.59 $\pm$ 0.26

Kinetic characteristics of CPT in non-synaptic mitochondria

The kinetics of brain CPT₁ and CPT₂ with respect to palmitoyl-CoA concentration (Fig. 1a) were similar to those observed previously for the liver enzyme (Saggerson & Carpenter, 1983) in that CPT₁ activity was maximal with 80–100 μ M-palmitoyl-CoA with no apparent substrate inhibition, whereas CPT₂ activity was markedly inhibited at acyl-CoA concentrations greater than 40 μ M. Lineweaver–Burk plots for both activities were non-linear (reflecting in part the fact that the free palmitoyl-CoA concentration in the assay was not known), and $K_{0.5}$ values (using maximal assayable activity as $V_{\max}$) were of the same order as estimates made in similar assay conditions for the liver enzymes, i.e. 20–26 μ M (Saggerson *et al.*, 1982; Saggerson & Carpenter, 1983). The estimated K_m value of CPT₁ for L-carnitine (80 μ M; Fig. 1b) was also similar to values found for the hepatic enzyme (E. D. Saggerson & C. A. Carpenter, unpublished work), and lies within the range of carnitine concentrations reported in rat brain (Bohmer & Molstad, 1980; Bresolin *et al.*, 1982). The K_m value of CPT₂ for L-carnitine was considerably greater (326 μ M), although accurate determination of the latter value is difficult because of uncertainties about the effects of sonication on the kinetics of CPT.

Like CPT₁ from all other tissues studied, the brain enzyme was sensitive to inhibition by

malonyl-CoA (Fig. 2). I_{50} values were intermediate between those in tissues where CPT₁ is highly sensitive to malonyl-CoA (e.g. heart, skeletal muscle) and those where the enzyme is moderately sensitive to inhibition (e.g. liver, kidney cortex) (Saggerson & Carpenter, 1981a). The concentration of malonyl-CoA in brain has not been determined, but one may predict its presence, since lipogenesis is active in brain (Dhopeswarkar *et al.*, 1969) and malonyl-CoA is required for the microsomal elongation of fatty acids (Cook & Spence, 1974). As with the liver enzyme (Saggerson & Carpenter, 1981a), COT₁ activity was even more strongly inhibited by malonyl-CoA than was CPT₁ (Fig. 2). It was noted that CPT from animals of 1–20 days of age was still malonyl-CoA-sensitive, with I_{50} values in the range 2.5–4.0 μ M. In addition, the inhibition by malonyl-CoA of CPT₁ in adult brain non-synaptic mitochondria was not changed by starvation.

Malonyl-CoA binding to mitochondria

Scatchard plots of the specific binding of [¹⁴C]malonyl-CoA to intact mitochondria were non-linear (Fig. 3), but could be resolved satisfactorily by a model describing two independent classes of binding sites. In the absence of other ligands, the tightness of binding of malonyl-CoA at the high-affinity sites ($K_{D(1)} = 18$ nM) was again intermediate between that previously determined for heart ($K_{D(1)} = 11$ nM) and liver ($K_{D(1)} = 40$ –100 nM) high-

Fig. 1. Concentration-dependence of (a) palmitoyl-CoA and (b) L-carnitine for carnitine palmitoyltransferase activity. The free-mitochondrial fraction was isolated from four to six 50-day-old rat brains. Values are the means $\pm$ their range of two separate experiments. (a) L-Carnitine concentration was fixed at 400 μ M. $K_{0.5}$ values for palmitoyl-CoA were 18.5 ± 0.5 μ M and 11.8 ± 0.2 μ M for CPT₁ and CPT₂ respectively. ●, CPT₁; ○, CPT₂. (b) Palmitoyl-CoA concentration was fixed at 40 μ M. Lineweaver–Burk plots were linear, and estimated K_m values for L-carnitine were 80 ± 6 μ M and 326 ± 68 μ M for CPT₁ and CPT₂ respectively.

Fig. 2. Inhibition of carnitine palmitoyltransferase and carnitine octanoyltransferase by malonyl-CoA

The non-synaptic mitochondrial fraction was isolated from four to six 50-day-old rat brains. Values are means $\pm$ S.E.M. for four separate experiments. L-Carnitine concentration was 400 μ M, and palmitoyl-CoA and octanoyl-CoA concentrations were 40 μ M. For CPT₁ (●), plots of $(\% \text{ inhibition})^{-1}$ versus [malonyl-CoA] were linear, and I_{max} and I_{50} values were $86 \pm 5\%$ and $1.6 \pm 0.4 \mu\text{M}$ respectively. For COT₁ (○), such plots were non-linear, and estimates of I_{max} and I_{50} were 74% and $<0.25 \mu\text{M}$ respectively.

Fig. 3. Scatchard plot of the binding of $[2\text{-}^{14}\text{C}]\text{malonyl-CoA}$ to non-synaptic mitochondria

The mitochondrial fraction was isolated from four to six 50-day-old rat brains. Specific $[^{14}\text{C}]\text{malonyl-CoA}$ binding was determined after a 20 min incubation. Values are the means of three separate experiments. Computer analysis of individual Scatchard plots by using a two-site model for binding gave the following values ($\pm$ S.E.M.): $K_{D(1)} = 18 \pm 1 \text{ nM}$; $K_{D(2)} = 27 \pm 9 \mu\text{M}$; $N_1 = 1.32 \pm 0.16 \text{ pmol/mg of protein}$; $N_2 = 168 \pm 35 \text{ pmol/mg of protein}$ (the latter value is mean $\pm$ range for two determinations).

Fig. 4. Relationship between occupancy of high-affinity $[^{14}\text{C}]\text{malonyl-CoA}$ -binding sites on brain mitochondria and inhibition of CPT₁ by malonyl-CoA

The non-synaptic mitochondrial fraction was isolated from eight 50-day-old rat brains. Specific $[^{14}\text{C}]\text{malonyl-CoA}$ binding was determined after a 20 min incubation in the presence of 20 μM -palmitoyl-CoA. Scatchard analysis of the data showed $K_{D(1)} = 0.46 \mu\text{M}$ and $N_1 = 6.5 \text{ pmol/mg of protein}$. The latter value represented 100% occupancy of high-affinity binding sites. CPT₁ activity was measured with 20 μM -palmitoyl-CoA and 400 μM -L-carnitine. The I_{50} for inhibition by malonyl-CoA was 0.45 μM . ●, Percentage inhibition of CPT₁; ○, percentage occupancy of high-affinity malonyl-CoA sites $[(100/N_1) \times \text{amount bound}]$.

affinity binding sites (Bird & Saggerson, 1984). However, in the absence of other ligands maximal binding capacity at these sites in brain was only about one-fifth of that of the liver and heart.

To correlate malonyl-CoA binding with inhibition of CPT₁ activity, we determined both enzyme activity and specific $[^{14}\text{C}]\text{malonyl-CoA}$ binding in the presence of 20 μM -palmitoyl-CoA; the latter concentration was chosen, since under these conditions binding data are still readily amenable to graphical Scatchard analysis (Bird & Saggerson, 1984). Palmitoyl-CoA increased both the $K_{D(1)}$ and N_1 values (cf. Figs 3 and 4), such that N_1 was now comparable with the value found in heart mitochondria under the same conditions (Bird & Saggerson, 1984). Fig. 4 shows that there was a good correlation between occupancy of high-affinity malonyl-CoA-binding sites and inhibition of CPT₁ activity, and we conclude that, as in heart mitochondria (Bird & Saggerson, 1984), the high-affinity binding sites are functional in mediating

the inhibitory effects of malonyl-CoA. The nature and importance of the low-affinity sites are unknown.

Rates of palmitoyl-CoA and palmitoylcarnitine oxidation

Rates of palmitoyl-group oxidation by purified non-synaptic mitochondria were extremely low, i.e. final additions of either 80 μM -DL-palmitoyl-carnitine or 0.4 mM-L-carnitine (40 μM -palmitoyl-CoA already present) resulted in increments in oxygen uptake of only 1.2 and 1.5 ng-atoms/min per mg of protein respectively. These rates were maximal under the experimental conditions used, which were devised so that the acetyl groups formed would react with oxaloacetate (generated from malate) to yield citrate as the final product (see the Materials and methods section). Under these conditions the complete β -oxidation of 1 nmol of palmitoyl group should require 22 ng-atoms of O. Hence rates of β -oxidation could be estimated as approx. 0.05–0.07 nmol of palmitoyl group oxidized/min per mg of protein, which is considerably lower than the measured activities of CPT₁ or CPT₂, suggesting that the activity of neither of these enzymes is likely to be rate-limiting for β -oxidation in brain.

Neonatal development of CPT

Many key enzymes of intermediary metabolism show characteristic developmental patterns (see

Volpe & Kishimoto, 1972; Booth *et al.*, 1980; Leong & Clark, 1984a,b). Therefore we determined CPT₁ and CPT₂ activities at various ages (Fig. 5). Compared with adult values, CPT activities were elevated by 40–80% during the neonatal period. Maximal activities of both enzymes occurred at 20 days of age, and this peak of activity was more pronounced for latent CPT than for the overt enzyme. Even at this peak it is unlikely that activity was high enough to allow fatty acids to contribute appreciably to energy supply in the brain. However, it should be noted that this peak coincides with the time of greatest myelination (Norton & Poduslo, 1973).

Discussion

Demonstration of the presence of a regulated system in brain for translocating long-chain and medium-chain acyl-CoA groups across the mitochondrial inner membrane raises the question of the function of such a system. Early reports (Vignais *et al.*, 1958; Beattie & Basford, 1965) suggested that some carnitine-dependent oxidation of labelled fatty acids (e.g. [¹⁴C]palmitate) may occur in brain. However, we have shown that in purified non-synaptic mitochondria the rates of oxidation of octanoate or of palmitoylcarnitine, or the carnitine-dependent oxidation of palmitoyl-CoA, are very limited (Clark & Nicklas, 1970; see the Results section). Subsequent studies (Kawamura & Kishimoto, 1981) have demonstrated that a large fraction of the label of [1-¹⁴C]palmitate is incorporated into glutamate and aspartate in brain mitochondrial fractions. Other studies have shown that [1-¹⁴C]palmitate may be incorporated into brain slices without being oxidized to acetate, suggesting an acylation-deacylation cycle (Mizobuchi *et al.*, 1982). Although it is well established that the neonatal mammalian brain utilizes both ketone bodies and glucose for energetic and synthetic functions (Page *et al.*, 1971; Cremer & Heath, 1974; Land *et al.*, 1977; Patel & Clark, 1980), and that under conditions of starvation this is also true of the adult brain (Owen *et al.*, 1967), there is little or no evidence to support a major role for fatty acid oxidation in brain energy homeostasis. Even in insulin-induced hypoglycaemia, where endogenous brain carbohydrate and amino acid substrates are depleted after 5 min of coma and endogenous brain non-esterified fatty acids increase 6-fold, it was concluded that there is no simple, direct, quantitative relationship between O₂ consumption and cortical fatty acid oxidation (Agardh *et al.*, 1981). It cannot be excluded, however, that specialized regions of the brain may have a requirement for fatty acid oxidation, as has been suggested for ketone bodies (Hawkins &

Fig. 5. Developmental pattern for brain carnitine palmitoyltransferase

The non-synaptic mitochondrial fraction was isolated from four to twenty brains of rats at various times *post partum*. CPT₁ activity with (●) 40 μM - or (○) 100 μM -palmitoyl-CoA, and CPT₂ activity with 40 μM -palmitoyl-CoA (▲), were measured. Activities at 50 days were taken from Table 1.

Biebuyck, 1979). In this respect, L-carnitine and acylcarnitine concentrations and total CPT activities are known to be higher in some regions of the brain than in others, particularly in the hypothalamic regions, where there is a high energy requirement for polypeptide synthesis (Bresolin *et al.*, 1982). A low capacity for β -oxidation may also simply play a homeostatic role in catabolizing non-esterified fatty acids present in excess of requirements for the synthesis and turnover of structural lipids. High concentrations of long-chain acyl-CoA are known to have deleterious effects on biological membranes (Brecher, 1983). In this respect it may be pertinent that in other tissues, and therefore possibly in brain, increasing the concentration of long-chain acyl-CoA greatly decreases the effectiveness of malonyl-CoA in inhibiting CPT₁, thereby allowing acyl-group transfer to proceed at a greater rate (McGarry *et al.*, 1978; Saggerson & Carpenter, 1981b).

Another possible function for an acyltransferase system in brain is in providing intramitochondrial acyl-CoA, either for elongation by a reversal of the β -oxidation sequence, in which case medium-chain acyl-CoA species may be the favoured substrates, or for the partial or complete degradation of very-long-chain fatty acids (e.g. C₂₂ or C₂₄). In these cases there may be a requirement for both medium-chain and long-chain acyltransferases. For the elongation of long-chain fatty acids, the microsomal system utilizing malonyl-CoA and NADPH appears to be the major pathway (Cook & Spence, 1974; Bourre *et al.*, 1977). However, the latter authors also reported that mitochondrial elongation of behenyl-CoA in mouse brain increases steadily during the neonatal period, in contrast with microsomal elongation. Here we reported that mitochondrial CPT showed a peak of activity at 20 days of age in rat brain. This coincides with the age at which the highest rate of myelination is observed (Norton & Poduslo, 1973). Hence it is possible that, in rat brain, mitochondrial elongation may make a contribution to myelination and that the CPT system is more concerned with this function than with a role in energy homeostasis. In the absence of measurements of malonyl-CoA concentration in brain, it is impossible to assess the physiological role of malonyl-CoA in regulating these processes.

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