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FATTY ACID ACTIVATION IN THE BRAINS OF NEUROLOGICALLY MUTANT MICE

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Abstract—1. Palmitoyl-CoA synthetase activity was assayed in subfractions of control and Quaking, Jimpy, Shiverer and Trembler mouse brain.
2. Mouse brain palmitoyl-CoA synthetase activity is not altered during myelination.
3. Mouse brain enzyme activity (homogenate 1.5 ± 0.3 nmol palmitoyl carnitine/min/per mg protein crude mitochondria 0.6 ± 0.1 nmol/min/per mg protein and microsomes 1.9 ± 0.3 nmol/min/per mg protein) does not differ markedly from rat and rabbit brain activity.
4. The lesions of the above mutants which affect myelination and lipid synthesis do not include the enzyme palmitoyl-CoA synthetase.

The activation of fatty acids to their respective long-chain fatty acyl-CoA derivatives is the primary reaction in the metabolism of fatty acids. In the brain, where rates of fatty and oxidation are low, activated fatty acids are required primarily for the synthesis of membrane constituents. This is particularly true in the synthesis of the myelin membrane in the oligodendrocyte during development. The enzyme responsible for the acylation reaction, acyl-CoA synthetase (acid:-CoA ligase (AMP) EC 6.2.1.3) has been measured in brain tissue from several laboratory animals (Aas, 1971; Brophy & Vance, 1976; Cantrill & Carey, 1975). Furthermore, this enzyme has at least two subcellular locations, the mitochondrial and microsomal fractions. Thusfar no report has described the activity of acyl-CoA synthetase in mouse brain or in dysmyelinating mutants.

Neurological mutants have been used extensively in the investigation of myelination in the mouse (Baumann, 1980). The Jimpy mutant is characterized by a lesion which prevents the increase in axon diameter and maturation of the oligodendrocytes. Lesions in the Quaking and Shiverer mutants are characterized by defects in oligodendrocyte function. The Trembler mutation affects mainly the myelin of the peripheral nervous system. Therefore it is important to determine the precise lesion in these mutants both quantitatively and temporally. Activities of palmitoyl-CoA synthetase vary during myelination in the rabbit brain (Cantrill & Carey, 1975) but not in the rat brain (Brophy & Vance, 1976). The variation in enzyme activity in the rabbit brain was most marked in the microsomal fraction.

This fraction contains the majority of the enzymes of phospholipid and sphingolipid synthesis. The role of acyl-CoA synthetase in brain mitochondria is more obscure since there is little fatty acid oxidation, however, activation for elongation in the acetyl-CoA-dependent system of mitochondria cannot be ruled out.

Fatty acid elongation (Bourre *et al.*, 1977), desaturation (Carreau *et al.*, 1979) and fatty alcohol synthesis (Bourre & Daudu, 1978) from acyl-CoA have been shown to be reduced in the Quaking and Jimpy mutants. The activity of acyl-CoA synthetase was studied in crude mitochondrial and microsomal fractions obtained from the brains of Quaking, Jimpy, Shiverer and Trembler mutants during the period of myelination.

MATERIALS AND METHOD

Quaking mutant mice were obtained on C57-B6 strain, Jimpy and Trembler mutants were on B6-CBA strain and the Shiverer mutation was on C3H-5WV strain as described previously (Bourre *et al.*, 1980). All animals were from the colonies kept at the Laboratoire de Neurochimie, Hôpital de la Sâlpetrière, Paris. Control animals were age-matched and from the appropriate strains and within each age group all animals were littermates.

At least three brains were removed quickly following cervical dislocation, weighed and homogenized together in ice-cold 0.32 M sucrose, pH 7.4 to give a 10% homogenate. This gave sufficient material for each age point. Crude mitochondrial (P_2) and microsomal (P_3) fractions were prepared using a modification (Lapetina *et al.*, 1967) of the centrifugation scheme of Gray & Whittacker (1962). Briefly, the homogenate was initially spun at 800 *g* for 10 min, the resultant supernatant was reserved and the pellet resuspended in sucrose and repelleted twice more at 800 *g* for 10 min. Supernatants were pooled and centrifuged at 800 *g* for 10 min before the final supernatant was

spun at 18,000 *g* for 30 min to prepare the crude mitochondrial fraction (P_2). The microsomal fraction (P_3) was prepared from the supernatant from the mitochondrial separation at 100,000 *g* for 60 min. Both fractions were made up to a known volume with 0.32 μM sucrose and stored at −20°C overnight.

Palmitoyl-CoA synthetase was assayed using the exchange reaction of Farstad *et al.* (1967) and was a modification of that described previously (Cantrill & Carey, 1975). Maximum synthetase activity (measured as palmitoyl [^{3}H]carnitine) was obtained under the following conditions. Duplicate incubations (5 in 10 min at 30°C) contained the following: 100 mM Tris–HCl pH 7.5, 80 μm CoA, 3.0 mM dithiothreitol, 1.5 mM ATP. 0.4 mM potassium palmitate, 3 mg albumin (fat free), 2 mM $MgCl_2$, 5 mM DL [^{3}H]carnitine (0.5 Cl/mol), excess carnitine-acyl-transferase and tissue sample in a total volume of 1 ml. Partially purified carnitine-acyl-transferase was prepared from calf liver mitochondria (Farstad *et al.*, 1969). Carnitine-acyl-transferase and potassium palmitate were suspended in 1% Nonidet-P40 (Shell Chemicals). Reactions were terminated by the addition of 100 μl of 5 M HCl and palmitoyl-carnitine was extracted with 2 ml *n*-butanol saturated with distilled water. Washed samples (200 μl) of the butanol phase were counted directly in scintillation fluid (NEN aquasol) in a Packard 460 liquid scintillation spectrometer with automatic quench correction. In the determination of enzyme activity, linearity of incorporation of radioactivity into palmitoyl-carnitine was established with respect to added tissue samples and time. Protein concentration was determined by the method of Lowry *et al.* (1951).

Radiochemicals were purchased from the Radiochemical Centre, Amersham, U.K. All other chemicals were of AR purity.

RESULTS AND DISCUSSION

Jimpy, Trembler and Shiverer mutants were only detectable when tremor and convulsions appear. This is coincident with myelination which takes place between 10 and 30 days in the mouse. Research into the neurological lesion in the Jimpy mutant is hindered by it short lifespan. Death usually occurs between 25 and 30 days of age. The introduction of genetic markers which are detectable at birth has made the early identification of homozygous Quaking mutants possible and thus allowing the study of early postnatal development in these animals.

Table 1 shows activity of palmitoyl-CoA synthetase in homogenates and subfractions of Quaking and control brain tissue during early postnatal development. Low amounts of enzyme activity were detectable at 5 days after birth which rose to a plateau level for the rest of the study period. At all ages, there was no difference between control and mutant values in any of the fractions studied. Furthermore, there was no rise in enzyme activity during the period of myelination. This is consistent with observations made on the developing rat brain (Brophy & Vance, 1976) but not in line with those made on the developing rabbit which show a distinct increase palmitoyl-CoA synthetase activity in the microsomal fraction during myelination (Cantrill & Carey, 1975).

Replacement of palmitic acid by hydroxypalmitic ($C_{16:OH}$) and lignoceric ($C_{22:0}$) acids yielded very low results (mean value 0.06 nmol/mg protein/min) in all fractions of Quaking and control brain. No differences were seen with respect to age or the strain of mouse used.

Since there is yet no way of differentiating mutants from either littermate homozygous or heterozygous controls for Quaking, Jimpy, Shiverer or Trembler mice, only data from day 15 is available for these animals (Table 2). The lethality of the Jimpy mutation makes it impossible to obtain data from animals older than 25 days of age. However all other mutants investigated survive well past this age thus making it possible to obtain data during the whole of myelination. As with the Quaking mutant no age-dependent differences in palmitoyl-CoA synthetase activity were seen in any of the mutant strains investigated. The large variations in enzyme activity measured between mutant and control animals may indicate a degree of heterogeneity within the different mutant strains.

Thus the dysmyelinating mutations which affect the synthesis of complex lipids and the utilization of acyl-CoA do not appear to involve the enzyme acyl-CoA synthetase.

SUMMARY

The activity of the enzyme, palmitoyl-CoA synthetase was determined in fractions prepared from the brains of dysmyelinating mutant mice (Quaking, Jimpy, Shiverer, Trembler). No age dependent change in enzyme activity was detected during myelination in either mutant or control animal. No differences were seen between mutants and their respective controls at

Table 1. Activity of palmitoyl-CoA synthetase in subfractions of normal and quaking mouse brain

Age in days	5	10	15	20	25	30
Control Mouse						
Homogenate	0.2	1.8 ± 0.4	1.4 ± 0.4	1.5 ± 0.3	1.4 ± 0.3	1.9 ± 0.4
Crude mitochondria	0.5	0.7	0.8	0.5	0.7	0.6
Microsomes	0.3	2.5	1.8	1.7	1.8	2.0
Quaking Mouse						
Homogenate	0.3	2.0 ± 0.4	1.6 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.5 ± 0.4
Crude mitochondria	0.3	0.8	0.9	0.5	0.6	0.7
Microsomes	0.2	2.4	1.6	1.8	2.0	2.0

Activity of palmitoyl-CoA synthetase (nmol palmitoyl-carnitine formed/mg protein per minute). Subfractions were prepared as detailed in the methods section. All values are mean ± SEM derived from duplicate assays of samples obtained by pooling 3 to 6 brains depending on age.

Table 2. Activity of palmitoyl-CoA synthetase in subfractions of normal and mutant mouse brain (Trembler, Shiverer and Jimpy)

Age in days	15	20	25	30
Jimpy control				
Crude mitochondria	0.7 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	ND
Microsomes	1.5 ± 0.3	ND	2.0 ± 0.3	ND
Jimpy mutant				
Crude mitochondria	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	ND
Microsomes	1.6 ± 0.2	1.7 ± 0.3	1.9 ± 0.3	ND
Shiverer control				
Crude mitochondria	0.6 ± 0.1	1.0 ± 0.2	1.3 ± 0.2	0.9 ± 0.2
Microsomes	1.0 ± 0.2	1.2 ± 0.3	1.2 ± 0.3	1.4 ± 0.2
Shiverer mutant				
Crude mitochondria	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	0.8 ± 0.2
Microsomes	1.3 ± 0.2	1.3 ± 0.3	1.4 ± 0.2	1.5 ± 0.3
Trembler control				
Homogenate	1.8 ± 0.3	1.5 ± 0.2	1.2 ± 0.2	1.3 ± 0.3
Crude mitochondria	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.2
Microsomes	1.2 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.3
Trembler mutant				
Homogenate	2.0 ± 0.3	1.5 ± 0.2	1.0 ± 0.1	1.0 ± 0.2
Crude mitochondria	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.1
Microsomes	1.5 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	1.2 ± 0.2

ND = Not determined.

any age. The enzyme palmitoyl-CoA synthetase is not affected by the lesion in any of the above mutants.

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REFERENCES

Aas M. (1971) Organ and subcellular distribution of fatty acid activating enzymes in the rat. *Biochim. biophys. Acta* **231,** 32–47.

Baumann N. A. (1980) Mutations affecting myelination in the central nervous system: research tools in neurobiology. *Trends Neurosci.* **3,** 82–85.

Bourre J. M. & Daudu O. (1978) Stearyl-alcohol biosynthesis from stearyl-CoA in mouse brain microsomes in normal and dysmyelinating mutants (Quaking and Jimpy). *Neurosci. Lett.* **7,** 225–230.

Bourre J. M., Paturneau-Jouas M. Y., Daudu O. L. & Baumann N. A. (1977) Lignoceric acid biosynthesis in the developing brain. Activities of mitrochondrial acetyl-CoA dependent synthesis and microsomal malonyl-CoA chain-elongating system in relation to myelination. *Eur. J. Biochem.* **72,** 41–47.

Bourre J. M., Jacque C., Delassalle A., Nguyen-Legros J., Dumont O., Lachapelle F., Raoul M., Alvarez C. & Baummann N. (1980) Density profile and basic protein measurement in the myelin range of particulate material from normal developing mouse brain and neurological mutants (Jimpy:Quaking:Trembler:Shiverer and its MLD allele) obtained by zonal centrifugation. *J. Neurochem.* **35,** 458–464.

Brophy P. J. & Vance V. E. (1976) The synthesis and hydrolysis of long-chain fatty acyl-coenzyme A thioesters by soluble and microsomal fractions from the brain of the developing rat. *Biochem. J.* **160,** 247–251.

Cantrill R. C. & Carey E. M. (1975) Changes in the activities of *de novo* fatty acid synthesis and palmitoyl-CoA synthetase in relation to myelination in rabbit brain. *Biochim. biophys. Acta* **380,** 165–175.

Carreau J. P., Daudu O., Mazliac P. & Bourre J. M. (1979) Palmitoyl-CoA and Stearyl-CoA desaturase in mouse brain microsomes during development in normal and neurological mutants (Quaking and Jimpy). *J. Neurochem.* **32,** 659–660.

Farstad M., Bremer J. & Norum K. R. (1967) Long-chain acyl-CoA synthetase in rat liver. A new assay procedure for the enzyme and studies on its localisation. *Biochim. biophys. Acta* **132,** 492–502.

Gray E. G. & Whittacker V. P. (1962) The isolation of nerve endings from brain: an electron microscopic study of the cell fragments of homogenisation and centrifugation. *J. Anat.* **96,** 79–88.

Lapetina E. G., Soto E. F. G. & De Robertis E. (1967) Gangliosides and acetylcholinesterase in isolated membranes of the rat brain cortex. *Biochim. biophys. Acta* **135,** 33–43.

Lowry O. H., Rosebrough N. J., Farr A. L. & Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265–275.