Journal of Neurochemistry Vol. 32, pp. 659–660
Pergamon Press Ltd 1979. Printed in Great Britain

0022-3042/79/0201-0659$02.00/0

44

SHORT COMMUNICATION

Palmityl-CoA and Stearyl-CoA desaturase in mouse brain microsomes during development in normal and neurological mutants (Quaking and Jimpy)

(*Received* 25 *July* 1978. *Revised* 22 *September* 1978. *Accepted* 27 *September* 1978)

DUE TO their role in the structure of the myelin membrane, the monounsaturated long chain fatty acids are very important in brain. They are synthesized by a microsomal elongation system using oleyl-CoA as a primer (BOURRE *et al.*, 1977*a*). However the origin of C_{18} monoenoic components of brain is not elucidated and it remains to be found whether or not the brain by itself is able to introduce double bonds into saturated fatty acids. Indeed, brain slices as well as homogenate provide oleic acid (COOK & SPENCE, 1973; SENG & DEBUCH, 1975), but desaturase activity was not detected in brain microsomes (COOK & SPENCE, 1973; SENG & DEBUCH, 1975; SENG *et al.*, 1976). In contrast, PULLARKAT & REHA (1975) measured a brain microsomal stearyl-CoA desaturase activity. The aim of the present experiment was therefore to measure the desaturase activity in brain microsomes using palmityl-CoA and stearyl-CoA as substrates. We tried also to determine the alteration of this activity during postnatal brain development and to compare the desaturase activities of normal animals with those of neurological mutants with defective myelination (Quaking and Jimpy).

MATERIAL AND METHODS

[1-^{14}C]Stearic acid and [1-^{14}C]palmitic acid were purchased from CEA (France) and acyl-CoAs were synthesized as previously described (BOURRE & DAUDU, 1978). All animals were killed between 9 and 11 a.m., the brains were removed and homogenized in 0.25 M-sucrose (2 ml/g). The homogenate was centrifuged at 14,500 *g* for 30 min and the supernatant was spun at 104,000 *g* for 60 min in order to pellet the microsomal fraction. The standard incubation system was based on the method of SENG *et al.* (1976) and consisted of 20 mM-glucose, 2.5 mM-$MgCl_2$, 4.6 mM KCl, 120 mM-NaCl, 4.4 mM-Na_2PO_4H, 28.2 mM-Tris–HCl pH 7.4, BSA (0.14%), 1 mM-NADH, [1-^{14}C]stearyl-CoA or [1-^{14}C]palmityl-CoA (51 nM). O_2 was bubbled through the incubation medium for 20 s; 2 mg of microsomal proteins were then added and the tubes stoppered. Incubations were carried out at 37°C in a shaker. The reaction were stopped after 20 min by 1 ml KOH 10% (in methanol–water 1:1, v/v). After 20 min at 80°C, 1 ml 7 N-HCl was added, and again after another period of 20 min at 80°C. The fatty acids were extracted by hexane–chloroform (7:3, v/v) and the extracts were dried. To these 0.2 ml anhydrous methanol–HCl (1.1 N) was added and methylation was carried out at 60°C for 1 min (CARREAU & DUBACQ, 1978). The fatty acid methyl esters were separated on $AgNO_3$–SiO_2 thin-layer chromatography (Merck G 60 commercial plates immersed in a solution of 8% $AgNO_3$ in 90% methanol for 10 s.; plates were re-activated at 105°C for 10 min after drying). The solvent was *n*-hexane-benzene (7:3, v/v). The plates were sprayed with 2′-7′-dichlorofluorescein in methanol and bands were scraped into vials containing liquid scintillation counting.

Eventually, the methyl-esters were analysed by radio-gas–liquid chromatography (Barber-Colman apparatus).

RESULTS AND DISCUSSION

Brain microsomes, as well as microsomes from other sources, catalysed the desaturation of palmityl-CoA and stearyl-CoA. Both substrates were desaturated at a similar rate in the presence of NADH. According to the age of the animal, between 7 and 10% of the added substrate were desaturated by brain microsomes. The reaction rate was constant for 1 h and proportional to the quantity of microsomal protein (up to 2 mg). In control adult animals on a standard diet the activity in liver was about 5 times higher than in brain. The difficulty that COOK & SPENCE (1973), SENG & DEBUCH (1975) and SENG *et al.* (1976) had in obtaining active microsomal preparations remains unexplained, and our results confirm those of PULLARKAT & REHA (1975) in demonstrating the existence of a microsomal stearyl-CoA desaturase in brain. The brain stearyl-CoA desaturase specific activity which we measured was much higher than the specific activity reported by COOK & SPENCE (1974) in brain homogenate, but lower than the values reported by PULLARKAT & REHA (1975) for rat brain microsomes. Even though the specific activity of palmityl-CoA and stearyl-CoA desaturases significantly decreased with development (Fig. 1 and 2), the total activity per brain

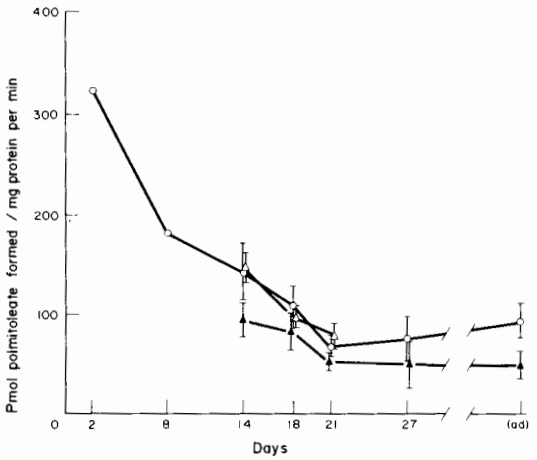

FIG. 1. Palmityl-CoA desaturase activity expressed as pmol of palmitoleic acid formed per min per mg of protein: normal ○—○; Quaking ▲—▲; Jimpy △—△; (Ad) adult. Each point is the mean of five experiments.

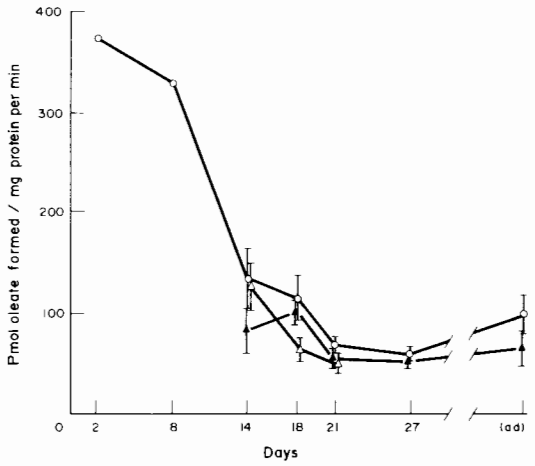

FIG. 2. Stearyl-CoA desaturase activity expressed as pmol of oleic acid formed per min per mg of protein: normal ○——○; Quaking ▲——▲; Jimpy △——△; (Ad) adult. Each point is the mean of five experiments.

remained nearly constant. However the specific activity and the activity per gram of fresh brain followed a similar pattern. In contrast with the microsomal fatty acid synthesizing systems (BOURRE *et al.*, 1976, 1977*b*; MURAD & KISHIMOTO, 1978), there is no peak at the myelination period.

In mutants, the desaturase activity is not linked to myelination. This absence of correlation between these two processes is illustrated by the fact that in Jimpy and Quaking mice the decrease in desaturase activity was only 10%, whilst the lowering activity of the myelin synthesizing enzymes was about 60%.

J. P. CARREAU[1]
O. DAUDU[2]
P. MAZLIAK[1]
J. M. BOURRE[2]

[1] *Laboratoire de Physiologie Cellulaire, Université Pierre et Marie Curie 12, Rue Cuvier, 75005 Paris, France*
[2] *Laboratoire de Neurochimie, INSERM U 134 Hôpital de la Salpêtrière, 47 Boulevard de l'Hôpital, 75013 Paris France.*

REFERENCES

BOURRE J. M., DAUDU O. & BAUMANN N. (1976) Ontogènèse des trois systèmes de biosynthèse des acides gras dans les microsomes cérébraux: relation avec la myélinisation. *Biochimie* **58,** 1077–1079.

BOURRE J. M., POLLET S., PATURNEAU-JOUAS M. & BAUMANN N. (1977*a*) Saturated and mono-unsaturated fatty acid biosynthesis in brain relation to development in normal and dysmyelinating mutant mice. *Int. Symp. on Function and Biosynthesis of Lipids* (BAZAN N. G., BRENNER R. R. & GIUSTO N. M., eds), pp. 103–109. Plenum Press, New York.

BOURRE J. M., PATURNEAU-JOUAS M., DAUDU O. & BAUMANN N. (1977*b*) Lignoceric acid biosynthesis in the developing brain. Activities of mitochondrial acetyl-CoA dependent synthesis and microsomal malonyl-CoA chain elongation systems in relation to myelination. Comparison between normal and dysmyelinating mutants (Quaking and Jimpy). *Eur. J. Biochem.* **72,** 41–47.

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

CARREAU J. P. & DUBACQ J. P. (1978) Adaptation of a macro-scale method to the micro-scale for fatty acid methyl transesterification of biological lipid extracts. *J. Chromatog.* **151,** 384–390.

COOK H. W. & SPENCE M. W. (1973) Formation of monoenoic fatty acids by desaturation in rat brain homogenate. *J. biol. Chem.* **248,** 1793–1796.

COOK H. W. & SPENCE M. W. (1974) Biosynthesis of fatty acids in vitro by homogenate of developing rat brain: desaturation and chain-elongation. *Biochim. Biophys. Acta* **369,** 129–141.

MURAD S. & KISHIMOTO Y. (1978) Chain-elongation of fatty acids in brain: a comparison of mitochondrial and microsomal enzyme activitie. *Archs Biochem. Biophys.* **185,** 300–306.

PULLARKAT R. K. & REHA H. (1975) Stearyl coenzyme A desaturase activities in rat brain microsomes. *J. Neurochem.* **25,** 607–610.

SENG P. N. & DEBUCH H. (1975) Untersuchungen zur Stearinsäure-Dehydrogenase in Leber und Gehirn von Ratten verschiedenen Alters. *Z. Physiol. Chem.* **356,** 1043–1053.

SENG P. N., DEBUCH H. & STROSZNAJDER J. (1976) Stearate desaturase in rat brain and liver. *J. Neurochem.* **27,** 825–826.