Journal of Neurochemistry, 1975, Vol. 24, pp. 1095–1097. Pergamon Press. Printed in Great Britain

SHORT COMMUNICATION

Fatty acid biosynthesis in mice brain and kidney microsomes: comparison between Quaking mutant and control

(*Received* 3 *December* 1974; *Accepted* 23 *December* 1974)

THE QUAKING mouse is a neurological mutant characterized by a disturbance in myelin deposition (SIDMAN *et al.*, 1964). In the brain several enzymes are deficient, especially those involved in the elaboration of myelin lipids (NESKOVIC *et al.*, 1970, 1972; BOURRE *et al.*, 1971, 1973; COSTANTINO-CECCARINI & MORELL, 1971; SARLIEVE *et al.*, 1971; KANDUTSCH & SAUCIER, 1972; GOLDBERG *et al.*, 1973). It is essentially the CNS which is damaged but the peripheral system is also affected (MEIER & MAC PIKE, 1972; KISHIMOTO, 1971; FRIEDRICH & HAUSER, 1973). Galactolipids, especially those built up with long chain fatty acids, are characteristic of myelin. However they are found elsewhere, for instance sulphatides are found in the kidney. In the mutant, the analysis of sulphatides with long chain fatty acids shows that they are normal in the kidney (BAUMANN & HARPIN, 1972) but not in the brain (JACQUE *et al.*, 1971). Skin glycolipids are normal (KISHIMOTO, 1971).

We have found that the different microsomal systems responsible for the biosynthesis of myelin fatty acids, especially those involved in the synthesis of C24:0, are impaired in the mutant (BOURRE *et al.*, 1971, 1973). These results have been confirmed (GOLDBERG *et al.*, 1973), and could explain, by lack of substrate, the deficiency in glycolipid formation. To establish whether the deficiency in long chain fatty acids is due to a mutation in a structural gene or to a brain-specific regulatory mechanism, it seemed appropriate to compare fatty acid biosynthesis in microsomes of brain and kidney. It is noteworthy that the biosynthesis of fatty acids has not yet been studied in the latter.

MATERIAL AND METHODS

Microsomal preparation. The brains and kidneys of 30 20-day-old mice were removed and washed in 0·1 M-phosphate buffer containing 0·32 M-sucrose and 0·9% NaCl, pH 6·9. Then the organs were homogenized in a Potter–Elvehjem homogenizer with 5 ml of the same buffer per g of tissue. Microsome isolation and protein determination were carried out as previously described (BOURRE *et al.*, 1971).

Incubation media and fatty acid analysis. Enzyme assays were performed as previously described (POLLET *et al.*, 1973). The *de novo* system from the supernatant fraction was studied in an incubation medium of 1 ml made up of 1 mg enzyme protein, 0·5 μmol NADPH, 0·025 μmol acetyl-CoA and 0·05 μmol malonyl-CoA. Either acetyl-CoA or malonyl-CoA was radioactive (4 mCi per mmol). The elongating microsomal systems were studied under the same conditions except for acetyl-CoA which was replaced by 0·05 mM-acyl-CoA: palmityl-CoA or stearyl-CoA, unlabelled or radioactive (4 mCi per mmol). Fatty acids were extracted by petroleum ether after saponification and acidification. Then they were methylated by methanolic boron trifluoride (MORRISON & SMITH, 1964) and analysed by radiochromatography using a 3% S.E. 30 column with automatic counting of eluate (Beckman GLC and Panax counter). The separation of unsaturated and saturated fatty acids was sufficient on this phase, as the activity of unsaturated fatty acids was only between 0 and 3% of the total activity and located in one peak. The presence of large concentration of sucrose inhibits the biosynthesis of α-hydroxylated fatty acids (HOSHI & KISHIMOTO, 1973), therefore hydroxylated and non-hydroxylated fatty acids were not separated prior to analysis.

RESULTS

Table 1 shows that the synthetase from brain or kidney of normal or mutant mice produced the same acids, in

TABLE 1. SOLUBLE *de novo* SYSTEM IN BRAIN AND KIDNEY

Chain length			12:0	14:0	16:0	18:0	20:0
Main substrates							
Acetyl-CoA + 1-^{14}C Malonyl-CoA	Brain	control		4 ± 0·8	86 ± 2	10 ± 1	0·5 ± 0·2
		quaking		4 ± 1	86 ± 2	10 ± 1·2	0·5 ± 0·2
	Kidney	control	0·2	3 ± 0·5	87 ± 2	9 ± 1	0·2 ± 0·1
		quaking	0·3	4 ± 0·7	87 ± 2	8 ± 0·9	0·3 ± 0·1
1-^{14}C-Acetyl-CoA + Malonyl-CoA	Brain	control	tr.	4 ± 0·7	87 ± 1		
		quaking	tr.	5 ± 0·9	85 ± 2		
	Kidney	control	tr.	5 ± 1	86 ± 2		
		quaking	tr.	4 ± 0·9	88 ± 2		

At least 3 experiments have been made. Two to 4 chromatograms were run for each experiment. The values given here are means ± S.E.M. (expressed in percentage) for at least 9 chromatographies. tr.: Trace amount of the fatty acid; — non detectable.

TABLE 2. MICROSOMAL ELONGATION OF PALMITYL-COA AND STEARYL-COA IN BRAIN AND KIDNEY MICROSOMES

Chain length			16:0	18:0	20:0	22:6	22:0	24:1	24:0
Substrates									
Palmityl-CoA + 1-^{14}C Malonyl-CoA	Brain	control	3 ± 0·7	90 ± 2	3·2 ± 0·9	0·4 ± 0·1	0·6 ± 0·2	—	1·7 ± 0·1
		quaking	3 ± 0·5	94 ± 1	1·6 ± 0·5	0·4 ± 0·2	0·3 ± 0·1	—	0·3 ± 0·1
	Kidney	control	2 ± 0·2	95 ± 2	3 ± 0·4	—	tr.	—	tr.
		quaking	2 ± 0·3	95 ± 2	3 ± 0·5	—	tr.	—	tr.
Stearyl-CoA + 1-^{14}C Malonyl-CoA	Brain	control	14 ± 0·7	23 ± 1	29 ± 2	3·4 ± 0·6	10 ± 2	1 ± 0·4	17 ± 3
		quaking	18 ± 0·9	17 ± 2	36 ± 1	2 ± 0·5	8 ± 2	1 ± 0·5	5 ± 3
	Kidney	control	5 ± 0·3	37 ± 2	45 ± 1	tr.	8 ± 2	—	4 ± 2
		quaking	4 ± 0·4	38 ± 2	45 ± 2	—	9 ± 2	—	5 ± 2

Same remarks as in Table 1.

identical relative percentages. Therefore this enzyme synthesizes the same fatty acids in both organs. Table 2 shows the elongation of palmityl-CoA and stearyl-CoA in the presence of radioactive malonyl-CoA. Normal and quaking kidney microsomes synthesize the same products in the presence of palmityl-CoA; this substrate is mainly elongated by 2 carbon atoms, and slightly by 4–8 carbon atoms. The Quaking brain shows a pattern which is slightly different fron normal brain, as there are many fewer 24-carbon atom fatty acids being formed. The elongation of stearyl-CoA is disturbed in the mutant brain, as opposed to the kidney. The activity found in C_{16} and C_{18} may be the result of the *de novo* microsomal system (POLLET *et al.*, 1973). It must be noted that the brain forms, under our conditions, a small amount of a compound which has the same R_F as $C_{22:6}$, whereas the kidney does not. In Table 3 the results are confirmed by a study of the biosynthesis with radioactive acyl-CoA in the presence of non-radioactive malonyl-CoA. The yield of reaction expresses conversion rate of the acyl-CoA into longer chains.

DISCUSSION

The values given here for the brain are somewhat different from those published elsewhere (BOURRE *et al.*, 1973). In fact the mice are of a different age and the accuracy of the radiochromatography has been increased, especially for calculation of the small peaks. The different distribution of radioactivity in the fatty acids of various chain lengths, using palmityl-CoA or stearyl-CoA as substrate, has been explained by two different systems of elongation in the microsomes of brain (BOURRE *et al.*, 1970, 1973*a*; POLLET *et al.*, 1973). These results have been confirmed (GOLDBERG *et al.*, 1973). The presence of radioactive $C_{22:6}$ in brain incubate may be due to elongation and subsequent desaturation of C_{18} precursors of $C_{22:6}$.

TABLE 3. MICROSOMAL ELONGATION OF RADIOACTIVE ACYL-COA

Chain length			18:0	20:0	22:0	24:0	Yield
Substrates							
1-^{14}C Palmityl-CoA + Malonyl-CoA	Brain	control	96 ± 1	2·5 ± 0·4	0·5 ± 0·2	1 ± 0·2	36 ± 5
		quaking	98 ± 1	1·7 ± 0·3	0·2 ± 0·1	0·3 ± 0·2	27 ± 4
	Kidney	control	97 ± 1	3 ± 1	—	—	13 ± 3
		quaking	97 ± 1	3 ± 1	—	—	17 ± 3
1-^{14}C Stearyl-CoA + Malonyl-CoA	Brain	control		75 ± 1	9 ± 1	15 ± 2	2·2 ± 0·3
		quaking		85 ± 1	8 ± 1	6 ± 2	1·2 ± 0·2
	Kidney	control		92 ± 1	6 ± 1	2 ± 1	3·5 ± 0·4
		quaking		93 ± 1	6 ± 1	2 ± 1	3·4 ± 0·4

Same remarks as in Table 1. The incubation is run in the presence of radioactive acyl-CoA. This compound is extracted by petroleum ether and found in the radiochromatogram. Thus the activity found in the corresponding peak is the activity of the substrate which has not been used. Under these conditions the yield is the activity found in C_{18} to C_{24} (for palmityl-CoA) or C_{20} to C_{24} (for stearyl-CoA) divided by the sum of activity found in all peaks.

which are contained in high concentration in brain and not in kidney.

Mitochondria also contain a fatty acid elongation system similar in some respects to the microsomal systems, but acetyl-CoA serves as the condensing unit, and both NADH and NADPH appear to be required (HARLAN & WAKIL, 1963; BOONE & WAKIL, 1970; YATSU & MOSS, 1971). However we have demonstrated that very long chain fatty acids which are components of myelin galactolipids are synthesized in microsomes (BOURRE *et al.*, 1973*b*) and that there is a precursor–product relationship between microsomes and myelin for long chain galactolipids. Fatty acid elongation is impaired in the Quaking brain; but not in the kidney. The same behaviour has been observed for 3′-phospho-adenosine 5′-phosphosulphate cerebroside sulphotransferase (SARLIEVE *et al.*, 1971), thus confirming that the mutation seems to be localized to the nervous system, and eliminating a mutation in a structural gene.

The ultrastructural studies during ontogenesis show a block in maturation of myelin (BERGER, 1971; WISNIEWSKI & MORELL, 1971). Thus the oligoglial cell seems to be affected in one of the stages of its differentiation. The appearance of long chain fatty acids in cerebrosides seems to be correlated to this period (BAUMANN *et al.*, 1971). Our results could be explained by the deficiency in microsomes from oligoglial cells, as there may be a different genetic control of fatty acid biosynthesis according to the cellular type; in this respect the chain length specificity of the elongating systems could be related to the origin of the microsomes. Gangliosides, which are mostly found in neurons, do not contain fatty acids over 20 carbon atoms, cerebrosides which contain longer chain fatty acids are essentially constituents of glial cells (afterwards they are found in myelin and only those are impaired). It could be deduced that long chain fatty acid regulation is not the same in glial and neuronal cells.

In the same way the genetic control of an enzyme can be different according to the organ. In the kidney and in the brain there is a production of long chain fatty acids, but their genetic control must be different; it could involve different isoenzymes or it is possible that the enzyme from kidney is constitutive whilst the one from brain is inducible. Indeed it has been demonstrated that the same enzyme from two different organs can be under the regulation of an inducer in one organ, whereas it is not in another (OHNO *et al.*, 1970). The inducer should be thyroid hormone: this hormone is strictly necessary for the well-proportioned development of the brain (EAYRS, 1955) by its action on various enzymes. Moreover some myelin glycolipid enzymes have been shown to be induced by thyroid hormone (WISOCKI & SEGAL, 1972). On the other hand thyroidectomy seems to affect cerebroside fatty acids (MANTZOS *et al.*, 1973).

Acknowledgement—This research was supported by I.N.S.E.R.M. and D.G.R.S.T.

Laboratoire de Neurochimie J. M. BOURRE[1]
INSERM U 134, O. L. DAUDU
Clinique des Maladies du Système Nerveux, N. A. BAUMANN[2]
Hôpital de la Salpêtrière,
47 *Boulevard de l'Hôpital*,
75013 *Paris, France*

REFERENCES

BAUMANN N., BOURRE J. M., JACQUE C. & POLLET S. (1971) *Lipids, Malnutrition and Developing Brain*. Ciba Foundation Symposium pp. 91–100.

BAUMANN N. & HARPIN M. L. (1971) *C.r. hebd. Séanc. Acad. Sci. Paris* **275**, 937–940.

BERGER B. (1971) *Brain Res.* **25**, 35–53.

BOONE S. C. & WAKIL S. J. (1970) *Biochemistry, Easton* **9**, 1470–1479.

BOURRE J. M., POLLET S. A., DUBOIS G. & BAUMANN N. A. (1970) *C.r. hebd. Séanc. Acad. Sci. Paris* **271**, 1221–1223.

BOURRE J. M., POLLET S. A., DAUDU O. & BAUMANN N. A. (1971) *C.r. hebd. Séanc. Acad. Sci. Paris* **273**, 1534–1537.

BOURRE J. M., POLLET S., CHAIX G., DAUDU O. & BAUMANN N. A. (1973*a*) *Biochimie* **55**, 1473–1479.

BOURRE J. M., POLLET S. A., DAUDU O. L. & BAUMANN N. A. (1973*b*) *Brain Res.* **51**, 225–239.

COSTANTINO-CECCARINI E. & MORELL P. (1971) *Brain Res.* **29**, 75–84.

EAYRS J. T. (1955) *Acta anat.* **25**, 160–183.

FRIEDRICH V. L. & HAUSER F. (1973) *J. Neurochem.* **20**, 1131–1141.

GOLDBERG I., SHELTER I. & BLOCH K. (1973) *Science, N.Y.* **182**, 497–499.

HARLAN W. R. & WAKIL S. J. (1963) *J. biol. Chem.* **238**, 3216–3221.

HOSHI M. & KISHIMOTO Y. (1973) *J. biol. Chem.* **248**, 4123–4130.

JACQUE C., BOURRE J. M., MORENO P. & BAUMANN N. A. (1971) *Biochimie* **53**, 1121–1124.

KANDUTSCH A. A. & SAUCIER S. E. (1972) *Biochim. biophys. Acta* **260**, 26–34.

KISHIMOTO Y. (1971) *J. Neurochem.* **18**, 1365–1368.

MANTZOS J. D., CHIOTAKI L. & LEVIS G. M. (1973) *J. Neurochem.* **21**, 1207–1213.

MEIER H. & MACPIKE A. D. (1972) *Expl. Neurol.* **37**, 643–646.

MORRISON W. P. & SMITH L. M. (1964) *J. Lipid Res.* **5**, 600–608.

NESKOVIC N., NUSSBAUM J. L. & MANDEL P. (1970) *Brain Res.* **21**, 39–53.

NESKOVIC N., SARLIEVE L. L. & MANDEL P. (1972) *Brain Res.* **42**, 147–157.

OHNO S., STENIUS C. & CHRISTIAN L. C. (1970) *Clinical Genetics* **1**, 40–50.

POLLET S., BOURRE J. M., CHAIX G., DAUDU O. & BAUMANN N. A. (1973) *Biochimie* **55**, 333–341.

SARLIEVE L. L., NESKOVIC N. M. & MANDEL P. (1971) *FEBS Lett.* **19**, 91–95.

SIDMAN R. L., DICKIE M. M. & APPEL S. H. (1964) *Science, N.Y.* **144**, 309–311.

WISNIEWSKI H. & MORELL P. (1971) *Brain Res.* **29**, 63–73.

WISOCKI S. J. & SEGAL W. (1972) *Eur. J. Biochem.* **28**, 183–189.

YATSU F. & MOSS S. (1971) *J. Neurochem.* **18**, 1895–1901.

[1] Attaché de Recherches I.N.S.E.R.M.

[2] Maître de Recherches I.N.S.E.R.M.