Δ^5 -Desaturase activity in liver and brain microsomes during development of the pig

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Microsomes isolated from liver and brain tissue were assayed to examine transitions in metabolic capability to synthesize tetraenes and pentaenes by chain elongation–desaturation of $C_{20:3(8,11,14)}$ during the perinatal development of the pig. Rates of synthesis of tetraenes and pentaenes by chain elongation–desaturation of $C_{20:3(8,11,14)}$ were greatest in liver. During the latter half of gestation, the capability to synthesize tetraenes increased 7- or 23-fold on a per mg of microsomal protein basis for brain and liver respectively. Increase in the capacity to synthesize tetraenes from $C_{20:3(8,11,14)}$ suggests a significant transition in the activity of the Δ^5 -desaturase during the last half of gestation. These observations indicate that in liver and brain the capability to chain elongate–desaturate $C_{18:2(9,12)}$ to longer chain homologues increases significantly during early development as a function of transitions in the activity of the Δ^5 -desaturase.

$C_{20:4(5,8,11,14)}$, $C_{22:4(7,10,14,16)}$, $C_{22:5(4,7,10,13,16)}$, $C_{22:5(7,10,13,16,19)}$ and $C_{22:6(4,7,10,13,16,19)}$ are significant constituents of membranes in brain as these fatty acids impart specific structural characteristics to the membrane (reviewed by Clandinin *et al.*, 1985a,b). Biosynthesis of these fatty acids during brain development is not well established. Whether the mechanism for fetal accretion of long chain metabolites involves fetal synthesis, or maternal–placental synthesis and transfer, is also not clear. There is evidence for a maternal source (Naughton, 1981); however, recent studies indicate some fetal metabolic capability to chain-elongate–desaturate $C_{18:2(9,12)}$ (Purvis *et al.*, 1982, 1983) and to chain-elongate $C_{16:0}$ to fatty acids of 20, 22 or 24 carbon atoms in chain length (Clandinin *et al.*, 1985c).

During brain development, transitions in relative activity of Δ^6 , Δ^5 and Δ^4 -desaturases for chain elongation–desaturation of essential fatty acids have been assessed (Clandinin *et al.*, 1985a). These observations suggest decreased activity of the Δ^5 -desaturase as a potentially rate-limiting biosynthetic step in the synthesis of building blocks of membranes integral to the ‘brain growth spurt’ known to occur during early brain development (Dickerson & Dobbing, 1966; Dobbing & Sands, 1972). Thus, the present study was designed to determine the rate of microsomal synthesis of

$C_{20:4(5,8,11,14)}$ by Δ^5 -desaturation of $C_{20:3(8,11,14)}$ in liver and brain tissue during the perinatal period in an appropriate animal model, the piglet.

Materials and methods

Materials

$C_{20:3(8,11,14)}$ was obtained from New England Nuclear, Montreal, Quebec, Canada. Radiochemical purity determined by argentation t.l.c. was >98%. Fatty acid standards and chemicals were purchased and prepared as previously described (Clandinin *et al.*, 1985a). Emulsions of fatty acid substrate were prepared by using twice the molar amounts of 0.1 M-NH₄OH, then suspending the mixture in a 1% (w/v) aqueous solution of Triton WR-1339 (Ruger Chemical Co., Irvington, NJ, U.S.A.).

Source of microsomal membranes

Male Yorkshire piglets at 60 days of gestation and at full-term (116 days of gestation) were killed, brains and livers were immediately removed and used for isolation of microsomal membranes at 0–4°C (Purvis *et al.*, 1983). Microsomal purity was assessed (Purvis *et al.*, 1983) and the absence of mitochondrial membrane verified. Microsomal protein was measured by a colorimetric method (Lowry *et al.*, 1951).

Synthesis of chain elongation-desaturation products of $C_{20:3(8,11,14)}$

Incubations were carried out at 37°C for 30 min in a metabolic shaker (Purvis *et al.*, 1983). [^{14}C] $C_{20:3(8,11,14)}$ (50 nmol; 200 000 d.p.m.) in NH_4OH solution was added to each incubation and the assay was conducted (Purvis *et al.*, 1983). Lipids were extracted in the presence of butylated hydroxytoluene as an antioxidant; the fatty acids were methylated with 10% (w/w) methanolic HCl at 95°C for 45 min (Purvis *et al.*, 1983).

Lipid analysis

Fatty acid methyl esters were separated according to degree of unsaturation on silica gel G t.l.c. plates containing 6% (w/w) $AgNO_3$ (Clandinin *et al.*, 1985a) visualized under u.v. light and bands containing trienoic, tetraenoic or pentaenoic fatty acids were identified by comparison with reference standards.

The identity of labelled fatty acid products was confirmed by checking argentation t.l.c. bands for cross-contamination by g.l.c. Chromatography was conducted essentially as described previously (Innis & Clandinin, 1981) with the injector splitter switched off and labelled fatty acid products collected at the appropriate retention time (Clandinin *et al.*, 1985a). Both sample and control reaction mixtures were carried through the entire assay procedure before the sample minus control value was determined.

Liquid-scintillation counting

^{14}C -labelled fatty acid methyl esters were separated, and radioactivity was counted and expressed as d.p.m. before molar equivalents were calculated (Clandinin *et al.*, 1985a).

Results and discussion

For the piglets examined, it is apparent that liver and brain microsomes were capable of desaturating $C_{20:3(8,11,14)}$ to synthesize homologous tetraenes and pentaenes (Table 1). For liver, rates of synthesis of Δ^5 -desaturase products were higher than observed in the brain. A marked increase in rate of synthesis was observed from preterm to term periods. Liver synthesis of tetraenes increased approx. 23-fold over rates observed midway through gestation. Synthesis of tetraenes by brain microsomal Δ^5 -desaturase increased from preterm to term by some 6-fold. Comparison of the relative desaturase activities during this fetal-neonatal period suggests that chain elongation-desaturation of trienes to tetraenes to pentaenes occurs (Clandinin *et al.*, 1985b) and is markedly stimulated by transitions in microsomal Δ^5 -desaturase activity (Table 1).

Table 1. Δ^5 -Desaturase activity in liver and brain microsomes

Microsomes were isolated from piglets at 63 days of gestation (preterm) and 3 days post partum. Microsomal assays used $C_{20:3(8,11,14)}$ as the substrate for chain elongation-desaturation. All assays were conducted in duplicate and corrected for corresponding control assays. Values for each age group represent the mean $\pm$ s.d. for four individual animals. The value for each animal was determined in duplicate.

Fatty acids synthesized by Δ^5 -desaturase (pmol/20 min per mg of protein)		
	Tetraenes	Pentaenes
Liver		
Preterm	8.1 $\pm$ 0	2.8 $\pm$ 0.45
Term	188.0 $\pm$ 2	8.3 $\pm$ 0
Brain		
Preterm	27.7 $\pm$ 23.8	0.81 $\pm$ 0
Term	177.3 $\pm$ 0	4.86 $\pm$ 2.88

Results from this laboratory indicate that liver and brain microsomes from fetal pigs, a model analogous to the human with respect to fatty acid accretion in brain (Purvis *et al.*, 1982), are capable of synthesizing trienes, tetraenes and pentaenes from $C_{18:2(9,12)}$ (Clandinin *et al.*, 1985a); however, synthesis of C_{20} and C_{22} homologues of $C_{18:2(9,12)}$ is apparently limited during development by the activity of the Δ^5 -desaturase. Developmental, hormonal and nutritional mechanisms for stimulation of this function remain to be determined but are clearly integral to mechanisms controlling tissue growth.

Whether or not relative rates of formation of individual chain-elongated fatty acids support the developing neonate's entire whole-body utilization of these membrane components, or only a portion thereof, with the balance supplied by the placenta during development *in utero*, and whether longer chain essential fatty acid homologues can be transported into brain for tissue synthesis remain questions to be examined from both qualitative and quantitative perspectives.

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