Acetoacetate and Brain Lipogenesis: Developmental Pattern of Acetoacetyl-Coenzyme A Synthetase in the Soluble Fraction of Rat Brain

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The existence of acetoacetyl-CoA synthetase in rat brain cytosol is reported. The coupling of this enzyme with cytosolic acetoacetyl-CoA thiolase can provide acetyl-CoA for lipogenesis and cholesterol synthesis without the need for mitochondrial participation. This new route for acetoacetate utilization may be important in developing brain.

Measurements of arteriovenous differences for acetoacetate and 3-hydroxybutyrate across brain of human beings (Owen *et al.*, 1967) and rats (Hawkins *et al.*, 1971) and of the activities of the enzymes that use these substrates in rat and human brain (Page *et al.*, 1971; Tildon *et al.*, 1971; Page & Williamson, 1971) suggest that this tissue derives an appreciable proportion of its energy requirements from ketone bodies when they are available in the blood. It has been postulated that some of the acetyl-CoA derived from ketone bodies may be directed to lipogenesis and cholesterol synthesis (Klee & Sokoloff, 1967; Page *et al.*, 1971), especially during the neonatal period. Incorporation of radioactivity from infused DL-3-hydroxy[3-¹⁴C]butyrate into phospholipids and free cholesterol of brains of newborn puppies (Spitzer & Weng, 1972) provides experimental support for this suggestion.

Two of the enzymes considered to be involved in ketone-body utilization, namely D-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) and 3-oxo acid CoA-transferase (EC 2.8.3.5), are specifically located in the mitochondria of brain (Williamson, 1967; Tildon & Svedalian, 1972). The third enzyme of the ketone-body utilization pathway, acetoacetyl-CoA thiolase (EC 2.3.1.9), is present in both the mitochondrial and the cytosolic compartment of rat brain, and activity in the soluble fraction is the major component in the early neonatal period (Middleton, 1971; Page, 1971). Stern *et al.* (1956) have briefly described in pigeon brain cytosol another enzyme, acetoacetyl-CoA synthetase (acetoacetate-CoA ligase), which catalyses the reaction:

The existence of this enzyme in rat brain has been questioned (Williamson & Hems, 1970; Tildon & Svedalian, 1972), but it has been shown to be active in rat liver cytosol (Stern, 1971).

Consideration of the fact that the synthesis of fatty

acids and of cholesterol are cytoplasmic processes, and that direct utilization of acetoacetate in the cytosol would avoid the necessity for transport of C₂ units from the mitochondria, led us to test for the presence of acetoacetyl-CoA synthetase in rat brain cytosol during development. The results were positive. This means that there is in rat brain cytosol a pathway for acetoacetate utilization involving acetoacetyl-CoA synthetase and acetoacetyl-CoA thiolase. For comparison, measurements were made of the activities of ATP citrate lyase (EC 4.1.3.8) and acetyl-CoA synthetase (EC 6.2.1.1), which may likewise be concerned in the provision of C₂ units in brain cytosol.

Methods

Rats of the Wistar strain fed *ad libitum* on Oxoid breeding diet for laboratory rats and mice (Oxoid Ltd., London S.E.1, U.K.) were used. Pregnant females weighed 300–375 g. Determination of enzyme activities in adult brain were performed on tissue from young males, about 250 g in weight. Litter size varied from eight to twelve, and no attempt was made to keep litter size constant. Litters were born naturally, but foetuses were delivered by caesarean section. Foetal age was estimated by weighing and referring to the values reported by Thaler (1972). Suckling rats were killed by decapitation and adults by cervical fracture, and brains were rapidly excised and placed on ice. Brains were homogenized by hand with 15 passes of a Potter–Elvehjem all-glass homogenizer in 4 vol. of ice-cold 10 mM-Tris–HCl buffer, pH 7.4, containing 0.25 M-sucrose and 1 mM-2-mercaptoethanol. The homogenates were centrifuged at 35000g for 45 min, and the supernatant was taken for enzyme assays. Acetoacetyl-CoA synthetase activity was determined by measuring the citrate and NADH formed on incubation of brain extract with 1 mM-ATP, 0.025 mM-CoA, 0.25 mM-acetoacetate, 5 mM-MgCl₂, 5 mM-L-malate, 15 mM-NAD⁺, 14 units

of malate dehydrogenase and 0.35 unit of citrate synthase in 75 mM-Tris-HCl buffer, pH 8.5. This method depends on the presence of excess of acetoacetyl-CoA thiolase in the brain extract (see Table 1). Results were corrected for formation of citrate and NADH in the absence of acetoacetate. Acetyl-CoA synthetase activity was determined by measurement of citrate and NADH formation on the incubation of brain extract with 2.5 mM-ATP, 0.25 mM-CoA, 2.5 mM-sodium acetate, 10 mM-GSH, 5 mM-MgCl₂, 5 mM-L-malate, 15 mM-NAD⁺, 14 units of malate dehydrogenase and 0.35 unit of citrate synthase in 75 mM-Tris-HCl buffer, pH 8.5. Results were corrected for formation of citrate and NADH in the absence of acetate. Citrate was determined enzymically (Dagley, 1970) after deproteinization. ATP citrate lyase activity was determined by a modification of the method of Inoue *et al.* (1966), by measurement of NADH oxidation on incubation of brain extract with 0.19 mM-NADH, 5 mM-MgCl₂, 2.5 mM-ATP, 20 mM-citrate, 1 mM-CoA and 7 units of malate dehydrogenase in 75 mM-Tris-HCl buffer, pH 8.5. Results were corrected for NADH oxidation in the absence of added CoA. Acetoacetyl-CoA thiolase activity was measured by determining the decrease in E_{303} due to cleavage of acetoacetyl-CoA (Williamson *et al.*, 1971). Concentrations of reactants were 0.1 mM-CoA, 10 mM-MgCl₂ and 0.05 mM-acetoacetyl-CoA in 75 mM-Tris-HCl buffer, pH 8.5. Results were corrected for non-enzymic removal of acetoacetyl-CoA. Brain cytosolic fractions were examined for contamination by mitochondrial enzymes by measuring glutamate dehydrogenase activity (Schmidt, 1963). All enzyme assays were performed at 30°C.

Results and discussion

In the acetoacetyl-CoA synthetase assay formation of citrate or NADH was dependent on the presence of acetoacetate, ATP and CoA. Succinate and GTP did not stimulate citrate formation, indicating that the activity was not due to succinyl-CoA (3-carboxypropionyl-CoA) synthetase and 3-oxoacyl-CoA transferase contamination from the mitochondria. The nature of the nucleotide product of the ligase reaction catalysed by the enzyme has not yet been determined. Acetoacetyl-CoA synthetase had a high affinity for acetoacetate, with an apparent K_m less than 50 μ M.

The activities of acetoacetyl-CoA synthetase and acetoacetyl-CoA thiolase in rat brain cytosol at various stages of development are presented in Table 1. The activities of both enzymes varied in a similar manner after birth, falling from their high postnatal activity throughout the suckling period, to decrease sharply on weaning with a further decline to the low adult activity. The pattern found for acetoacetyl-CoA thiolase is similar to that reported by

Table 1. Comparison of the developmental patterns in the cytosol of rat brain of enzymes concerned in provision and utilization of acetyl-CoA

Enzyme	Age of rats ...	Enzyme activity (μ mol of acetyl-CoA equivalents/h per g)					
		-5 days to term	0-5 days	6-10 days	11-15 days	16-21 days	Weanlings
Acetoacetyl-CoA synthetase	...	1.58 $\pm$ 0.07 (13)	2.78 $\pm$ 0.10 (13)	2.41 $\pm$ 0.16 (10)	1.91 $\pm$ 0.05 (6)	2.02 $\pm$ 0.08 (10)	1.46 $\pm$ 0.11 (7)
Acetoacetyl-CoA thiolase	...	393 $\pm$ 19 (11)	291 $\pm$ 9.6 (9)	264 $\pm$ 20.8 (6)	198 $\pm$ 8.6 (4)	216 $\pm$ 11.4 (8)	143 $\pm$ 13.8 (7)
Acetyl-CoA synthetase	...	1.87 $\pm$ 0.06 (11)	3.20 $\pm$ 0.20 (10)	3.16 $\pm$ 0.11 (8)	4.44 $\pm$ 0.32 (9)	7.26 $\pm$ 0.51 (6)	5.74 $\pm$ 0.41 (4)
ATP citrate lyase	...	5.69 $\pm$ 0.29 (12)	6.30 $\pm$ 0.49 (10)	6.50 $\pm$ 0.29 (7)	5.10 $\pm$ 0.26 (10)	5.13 $\pm$ 0.49 (9)	4.11 $\pm$ 0.48 (6)
Fatty acid synthetase*	...	6.0	5.0	4.8	4.2	3.8	3.4
							2.4

* The values for fatty acid synthetase are taken from Volpe & Kishimoto (1972).

The enzyme activities are mean values ($\pm$ s.e.m.) expressed as μ mol of acetyl-CoA equivalents formed or removed/h per g fresh wt. of tissue at 30°C. The numbers of observations are in parentheses. For other details see the text under 'Methods'.

Scheme 1. *Metabolism of ketone bodies in rat brain*

Key to enzymes: (1) 3-hydroxybutyrate dehydrogenase; (2) 3-oxo acid CoA-transferase; (3) acetoacetyl-CoA thiolase; (4) acetoacetyl-CoA synthetase; (5) ATP citrate lyase; (6) acetyl-CoA synthetase.

Middleton (1971) and Page (1971). The activity of acetoacetyl-CoA thiolase was about 100-fold higher than that of the synthetase, as might be expected from the role of acetoacetyl-CoA thiolase in maintaining equilibrium between its substrates. Comparison of these developmental patterns with that for fatty acid synthetase (Volpe & Kishimoto, 1972) reveals a close parallelism (Table 1). The relative activities of fatty acid synthetase and acetoacetyl-CoA synthetase indicate that by coupling with acetoacetyl-CoA thiolase the latter could play a significant role in the supply of C₂ units for lipid synthesis. The neonatal period in brains is also associated with active cholesterol synthesis (Sreere *et al.*, 1950), and thus the combination of the synthetase and thiolase could equally well provide C₂ units for this process.

The developmental patterns of ATP citrate lyase and acetyl-CoA synthetase in rat brain cytosol did not resemble those of the three enzymes already discussed. The activity of ATP citrate lyase did not change greatly over the entire developmental period.

D'Adamo & D'Adamo (1968) found a decline in activity from birth onwards, but their absolute activities are lower than those reported in the present paper. The activity of acetyl-CoA synthetase rose markedly over the suckling period to a maximum just before weaning, and then fell somewhat in the weaning and adult. The existence of acetoacetyl-CoA synthetase in rat brain cytosol necessitates a reappraisal of the metabolism of ketone bodies by brain during development (Scheme 1).

The developmental patterns of the mitochondrial route for ketone-body utilization (Page *et al.*, 1971) and the cytosol route for acetoacetate (Table 1) suggest that the major role of the former is in supplying energy, whereas that of the latter is specifically concerned with biosynthesis, especially during early development. It is perhaps relevant in this regard that acetoacetate is the predominant ketone body in the circulation during the early neonatal period, whereas 3-hydroxybutyrate assumes this role in later life. The fact that the activity of the cytosol

route for provision of acetyl-CoA from acetoacetate is similar to the activities of alternative routes requiring mitochondrial participation supports the idea that acetoacetyl-CoA synthetase may make an appreciable contribution to the furnishing of acetyl-CoA in the cytosol. The advantages of this route for acetoacetate conversion into acetyl-CoA are as follows: (1) it directs acetoacetate to biosynthesis owing to the absence of any competing degradative pathway from the cytosol; (2) it avoids the need for translocation of acetyl-CoA units across the mitochondrial membrane; (3) it is energetically economical, requiring half the expenditure of ATP incurred by alternative mitochondrial routes.

The main conclusion, then, is that acetoacetyl-CoA synthetase together with acetoacetyl-CoA thiolase can play an important role in the provision of acetyl-CoA in rat brain cytoplasm, and contribute to the direction of fatty acids, in the form of ketone bodies, from the liver to the neonatal brain.

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