ACETOACETYL-CoA SYNTHETASE; A LIPOGENIC ENZYME IN RAT TISSUES

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1. Introduction

Ketone bodies are generally considered to be energy-yielding substrates in rat tissues and their potential for providing acetyl-CoA for the biosynthesis of fatty acids and cholesterol has not been emphasized. Two of the enzymes involved in the utilization of ketone bodies, 3-D-hydroxybutyrate dehydrogenase (EC 1.1.1.30) and 3-oxoacid-CoA transferase (EC 2.8.3.5) are specifically located in the mitochondrial fractions of rat tissues in which they occur [1]. A third enzyme, acetoacetyl-CoA thiolase (EC 2.3.1.9) has been shown to be present in both cytosolic and mitochondrial fractions of rat tissues [2]. The presence of another enzyme, acetoacetyl-CoA synthetase (Reaction I) has been reported in various tissues [3–5], but its existence remained in doubt until the report by Stern [6] that it was active in rat liver.

$$\text{Acetoacetate} + \text{ATP} + \text{CoASH} \rightleftharpoons \text{Acetoacetyl-CoA} + \text{AMP} + \text{PP}_i \quad (1)$$

The enzyme has subsequently been shown to be present in other tissues of various species [7–9].

We have found that the developmental pattern of acetoacetyl-CoA synthetase parallels that of the fatty acid synthesising enzyme in cytosolic fractions of neonatal rat brain [9], and have suggested that this enzyme, together with cytosolic acetoacetyl-CoA thiolase, might play an important part in the provision of acetyl-CoA for lipogenesis in developing rat brain. This paper reports the results of a survey of the activity of acetoacetyl-CoA synthetase in other lipogenic tissues of the rat (liver, mammary gland, adipose tissue).

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2. Materials and methods

Wistar rats were used. Unless otherwise indicated they were fed ad libitum on Oxoid breeding diet for rats and mice (Oxoid Ltd., London, UK). Adult rats used weighed about 250 g; pregnant females weighed 300–375 g. Litter sizes varied from about 8–12. Litters were born naturally, and foetuses were obtained at laparotomy, foetal age being determined using the weight data of Thaler [10]. Young rats were killed by decapitation, adults by cervical dislocation. Liver was placed on ice on excision, mammary tissue was pressed free of milk on excision and placed on ice, and adipose tissue was maintained at room temperature before homogenisation to prevent cell damage by solidification of fat. Tissues were minced, and homogenised using a Potter–Elvehjem homogeniser in 4 vols of ice-cold 0.25 M sucrose in 10 mM Tris–Cl buffer containing 1 mM 2-mercaptoethanol. The soluble fraction (cytosol) was routinely obtained by centrifugation for 1 h at 35 000 *g*, the supernatant being taken. Absence of appreciable contamination by enzymes of the mitochondrial matrix was confirmed for each extract by assay for glutamate dehydrogenase (EC 1.4.1.3) [11]. Supernatant fractions derived by centrifugation for 90 min at 105 000 *g* possessed identical activity to those obtained by the routine procedure indicating the cytosolic location of the enzyme. Acetoacetyl-CoA synthetase was measured as previously described [9].

3. Results

3.1. *Liver*

The activities of acetoacetyl-CoA synthetase

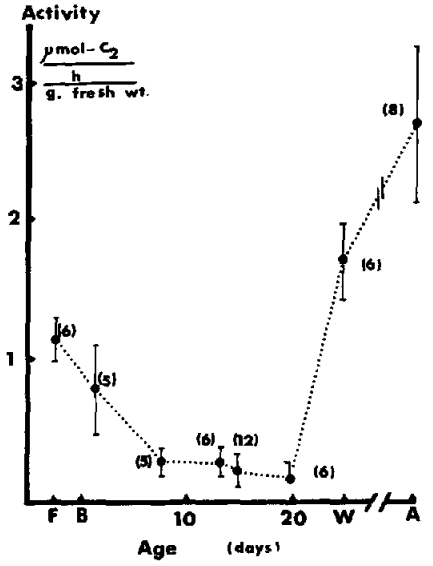

Fig.1. Changes in activity of acetoacetyl-CoA synthetase in rat liver cytosolic fraction during postnatal development. Values are expressed as means ± SEM, with the number of observations in parentheses. F denotes 18–19 day foetuses, B denotes birth, W denotes animals weaned 4 days previously at 21 days of age, and A denotes fed adult males.

measured in cytosolic preparations of liver from foetal, suckling and adult rats are shown in fig.1. Enzyme activity in foetal liver falls to a low level at birth, and continues to be depressed throughout the suckling period. On weaning, the activity rapidly rises to attain fed adult values. This developmental pattern is similar to those of enzymes and processes associated with fatty acid and cholesterol synthesis in rat liver [12,13], and contrasts with the higher capacity of the suckling livers to oxidise fatty acids and produce ketone bodies compared to those from foetal and adult rats [14].

The similarity between the response of the acetoacetyl-CoA synthetase and the responses of lipogenic processes in liver cytosol is also found in adult liver (table 1). Like other enzymes of lipogenesis [15], the activity of acetoacetyl-CoA synthetase increases in pregnancy to give a level in liver cytosol during lactation of more than twice the normal adult activity. The decrease in activity of acetoacetyl-CoA synthetase in starved rat liver is in agreement with observations of Stern [6], and parallels in extent the decrease in activity of most of the enzymes of lipogenesis in rat liver after 48 h starvation [16].

Table 1
Activities of acetoacetyl-CoA synthetase in adult rat liver and adipose tissue

Tissue	Physiological state	Activity[a]
Liver	Fed	2.7 ± 0.6 (8)
	24 h-fasted	2.4 ± 0.4 (4)
	48 h-fasted	1.0 ± 0.1 (5)
	18 days pregnant	6.3 ± 0.7 (3)
	Lactating	7.0 ± 1.0 (9)
	5 days post-lactating	4.1 ± 1.0 (4)
Adipose tissue	Fed	1.0 ± 0.1 (6)
	48 h-fasted	0.4 ± 0.1 (5)

[a] Activities are expressed as μmol-C_2 equivalents/h/g fresh wt, and are means ± SEM, with the number of observations in parentheses.

3.2. *Adipose tissue*

White adipose tissue has not been previously reported to possess acetoacetyl-CoA synthetase activity. Data in table 1 show it to be present at low activity in epididymal fat pad in the fed rat, and to decrease on starvation.

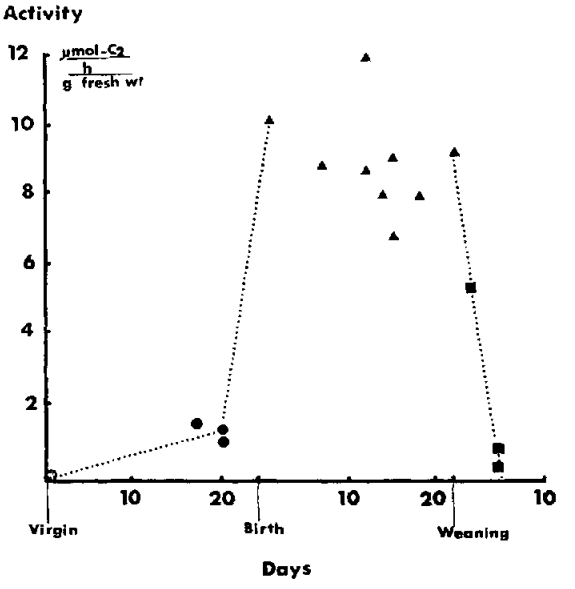

Fig.2. Changes in acetoacetyl-CoA synthetase activity in rat mammary gland cytosolic fraction before, during, and after lactation. Values represent individual observations on (○) virgin, (●) pregnant, (▲) lactating, (■) post-lactational animals.

3.3. *Mammary gland*

The lactating mammary gland of the rat is a major site of fat synthesis. Activities of acetoacetyl-CoA synthetase measured in cytosolic fractions of mammary gland from virgin, pregnant, lactating, and post-lactational rats are shown in fig.2. The enzyme was not detectable in the tissue from virgin rats, but appeared during pregnancy. At birth, activity rose rapidly to the lactating level and declined sharply on removal of the litter. This pattern is typical of that shown by the enzymes of lipogenesis in mammary gland [17].

4. Discussion

The patterns of acetoacetyl-CoA synthetase activity reported here suggest an association between the presence of this enzyme and active lipogenesis in rat liver, adipose tissue and mammary gland. It has been suggested that acetoacetyl-CoA synthetase in rat brain cytosol can act together with acetoacetyl-CoA thiolase to form acetyl-CoA from acetoacetate for fatty acid synthesis [9]. Some experimental support for this postulate has recently been provided by use of [^{14}C] acetoacetate [18]. A major advantage of this pathway is that it eliminates the need to transport acetyl-CoA as citrate from the mitochondrial matrix. However, the low activity of such a pathway for the acetyl-CoA supply in liver cytosol, about 3 μmol C_2-units formed/h/g (table 1), compared with the rat of fatty acid synthesis in the tissue of about 20 μmol C_2-units formed/h/g [19], would appear to argue against this role for acetoacetyl-CoA synthetase in liver. Furthermore, there is a marked difference between the developmental pattern of cytosolic acetoacetyl-CoA thiolase [20] and those of the synthetase and the enzymes of fat synthesis in liver. In addition, thiolase in liver cytosol does not change in activity with starvation [21]. The low activity of acetoacetyl-CoA synthetase in mammary gland and adipose tissue cytosolic fractions compared with the rates of fatty acid synthesis observed in these tissues [22,23] would also seem to preclude a major role of acetoacetyl-CoA synthetase in providing C_2-units for fatty acid synthesis in these tissues.

An alternative explanation for the correlation between the activities of acetoacetyl-CoA synthetase and the enzymes of lipogenesis stems from the suggestion that butyryl moieties bound to fatty acid synthetase are more effective as acetyl-acceptors for malonyl-CoA than are acetyl-groups in preparations from rat liver and mammary gland [24]. Enzymes have been demonstrated in cytosolic preparations of these tissues which can catalyse the reduction of acetoacetyl-CoA to butyryl-CoA [24]. The possibility exists therefore that acetoacetyl-CoA synthetase might act in liver and mammary gland cytosol in the provision of butyryl-CoA for the 'priming' step of the fatty acid synthetase sequence. Given that one equivalent of butyryl-CoA is required for every seven C_2-unit equivalents incorporated into a C_{18}-fatty acid, the activity of acetoacetyl-CoA synthetase is such that it could make a significant contribution to provision of primer in liver and mammary gland. Data on the primer preference of fatty acid synthetase in adipose tissue suggest that acetyl-CoA might be preferred to butyryl-CoA in this tissue [24].

Another possible role of acetoacetyl-CoA synthetase is to provide an alternative source of precursor for cholesterol synthesis which is also a cytosolic process.

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