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Activities of enzymes of acetoacetate metabolism in rat brown adipose tissue during development

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The activities of two mitochondrial enzymes concerned in the utilization of acetoacetate, namely 3-oxoacid CoA-transferase and acetoacetyl-CoA thiolase, were high throughout the suckling and weanling period in brown adipose tissue of the rat. In contrast, 3-hydroxybutyrate dehydrogenase activity was comparatively low during this period. The activity of cytosolic acetoacetyl-CoA synthetase (involved in lipogenesis) declined after birth and remained low until the pups were weaned. Experiments with brown-adipose-tissue slices from weanling rats indicated that 70% of the [3-^{14}C]acetoacetate utilized was oxidized to $^{14}CO_2$, and this value was not altered appreciably by the addition of glucose and insulin.

INTRODUCTION

The activities of two mitochondrial enzymes concerned in the utilization of acetoacetate, 3-oxoacid CoA-transferase (EC 2.8.3.5) and acetoacetyl-CoA thiolase (EC 2.3.1.9), are high in the interscapular brown adipose tissue of the adult rat (Agius & Williamson, 1981). This implies that acetoacetate may be an important substrate for this tissue in the mature rat whenever it is readily available in the circulation. The acetyl-CoA derived from acetoacetate can be used for lipogenesis (McCormack, 1982) or alternatively oxidized in the tricarboxylic acid cycle; in either case a fuel is provided for the thermogenic process (Nicholls, 1979).

The suckling period in the rat is associated with increased concentrations of acetoacetate and 3-hydroxybutyrate in the blood (Page *et al.*, 1971; Dahlquist *et al.*, 1972) and it has been suggested that they may be substrates for thermogenesis in brown adipose tissue during development (Hahn & Skala, 1972). To examine this hypothesis we have measured the activities of 3-oxoacid CoA-transferase, acetoacetyl-CoA thiolase, 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) and acetoacetyl-CoA synthetase (EC 6.2.1.16) in rat brown adipose tissue during development. Acetoacetyl-CoA synthetase is involved in the direct conversion of acetoacetate into fatty acids or cholesterol in the cytosol of lipogenic tissues (Stern, 1971; Buckley & Williamson, 1975; Endemann *et al.*, 1982). In addition, the metabolism of [3-^{14}C]acetoacetate was studied in slices of brown adipose tissue from weanling (2–5 days post-weaning) rats to obtain information on its possible fate.

EXPERIMENTAL

Rats of the Wistar strain were used. The adult rats were females weighing 210–250 g and were fed *ad libitum* on a commercial rat diet PRM (E. Dixon and Sons, Ware, Herts., U.K.). They were maintained in a room with lights on at 08:00 h and off at 20:30 h and at a room temperature of 21–23 °C. The suckling and weanling rats were a mixture of males and females. For measurements of enzyme activities the suckling pups were killed by decapitation, the adult and weanling rats were anaesthetized with Nembutal (60 mg/kg body wt.), and the brown adipose tissue was carefully dissected free from the surrounding white adipose tissue and muscle. It was sliced manually with a scalpel blade, weighed and homogenized in an all-glass homogenizer in 10 vol. of 0.25 M-sucrose containing 1 mM-mercaptoethanol/10 mM-Tris/HCl buffer, pH 7.4. The homogenate was then centrifuged for 20 min at 30000 *g* and 4 °C. The supernatant fluid (cytosol) was used for the assay of acetoacetyl-CoA synthetase (Buckley & Williamson, 1973). The particulate fraction (mitochondria plus nuclei) was resuspended in the original volume of the 0.25 M-sucrose homogenizing medium, and Triton X-100 (10%, w/v) was added to give a final concentration of 0.1%. The suspension of disrupted particles was used directly for the measurement of 3-hydroxybutyrate dehydrogenase (Lehninger *et al.*, 1960); the acetoacetate formed was measured by the colorimetric method of Walker (1954). For the measurements of acetoacetyl-CoA thiolase and 3-oxoacid CoA-transferase (Williamson *et al.*, 1971), the suspension was centrifuged for 20 min at 30000 *g* and 4 °C and the supernatant was used. The activity of citrate synthase (EC 4.1.3.7) was measured (Alp *et al.*, 1976) in the cytosolic and particulate fractions to test for the integrity of the mitochondria. The cytosolic activity over the development period studied was $14.4 \pm 6.0\%$ ($n = 12$; S.D.) of the total activity (cytosol plus particulate fraction).

The metabolic studies *in vitro* with [3-^{14}C]acetoacetate and brown adipose tissue slices were carried out essentially as described by Agius & Williamson (1981). The final concentration of [3-^{14}C]acetoacetate in the incubation medium [2 ml; Krebs–Henseleit (1932) saline] was 2 mM, and glucose (5 mM) and insulin (50 m-units/ml) were added where indicated. The incubations were in duplicate for 30 and 60 min at 37 °C. The rates of acetoacetate metabolism were linear over this period. [3-^{14}C]Acetoacetate was prepared by hydrolysis of ethyl [3-^{14}C]acetoacetate (Amersham International, Amersham, Bucks., U.K.) by the method of Krebs *et al.* (1966). The purity (98%) was checked by enzymic determination of acetoacetate (Williamson *et al.*, 1962) and by

Table 1. Activities of 3-oxoacid CoA-transferase, acetoacetyl-CoA thiolase and acetoacetyl-CoA synthetase in brown adipose tissue of the rat during development

For experimental details see the text. The results are mean values ± S.D. with the numbers of observations in parentheses. The enzyme activities are expressed as μmol of substrate transformed/min per g fresh wt. of tissue. Results that are significantly different by Student's *t* test from the corresponding adult values are shown: $*P < 0.05$; $**P < 0.005$.

Enzyme	Activity: Time after birth (days)... 1	2–4	5–10	11–15	16–20	21–24	25–30	Adult
3-Oxoacid CoA-transferase	12.7 ± 4.8 (6)	10.5 ± 2.4* (6)	7.7 ± 6.9* (9)	10.8 ± 3.5 (12)	16.5 ± 7.0* (9)	8.8 ± 2.3** (5)	10.5 ± 6.6 (7)	13.9 ± 2.6 (7)
Acetoacetyl-CoA thiolase	17.2 ± 3.0 (6)	20.7 ± 12.0 (6)	9.8 ± 5.7* (9)	22.8 ± 11.4 (12)	34.6 ± 5.8** (9)	20.3 ± 6.4 (5)	18.9 ± 11.9 (6)	17.8 ± 8.6 (7)
Acetoacetyl-CoA synthetase	0.043 ± 0.021 (5)	0.014 ± 0.003** (5)	0.021 ± 0.007** (8)	0.010 ± 0.005** (10)	0.017 ± 0.006** (8)	0.037 ± 0.10 (3)	0.055 ± 0.029 (7)	0.045 ± 0.013 (5)

measurement of residual radioactivity after conversion into CO_2 and acetone by heating for 30 min at 100 °C in 1 M-HCl and then evaporation to dryness *in vacuo* at 40 °C.

RESULTS AND DISCUSSION

The activities of 3-oxoacid CoA-transferase and acetoacetyl-CoA thiolase in the particulate fraction of intrascapular brown adipose tissue were similar immediately after birth (day 1) to those in adult rats (Table 1). The developmental profile of the two enzymes was also similar, with the lowest activity at 5–10 days and highest activity just before weaning (16–20 days) (Table 1). Lipoprotein lipase activity in brown adipose tissue has a nadir at 7 days and peaks at 2 and 12 days *post partum* (Cryer & Jones, 1978), whereas the activities of certain enzymes concerned in hepatic fatty catabolism exhibit peaks at 5–10 days and 15–20 days (Foster & Bailey, 1976).

In contrast with the high activities of particulate 3-oxoacid CoA-transferase and acetoacetyl-CoA thiolase in brown adipose tissue, that of 3-hydroxybutyrate dehydrogenase was comparatively low. The mean activity in suckling rats (8–10 days) was 0.24 ± 0.056 (3) μmol of substrate oxidized/min per g fresh wt. of tissue and that in weanling rats (25–28 days) was 0.25 ± 0.16 (3) μmol/min per g. Mixing experiments with a rat liver particulate preparation revealed no evidence for the presence of an inhibitor of the enzyme in brown adipose tissue. This low activity would suggest that 3-hydroxybutyrate is not such an effective substrate for brown adipose tissue as acetoacetate (see below).

The activity of cytosolic acetoacetyl-CoA synthetase was approx. 300–400-fold lower than that of the two mitochondrial enzymes in the brown adipose tissue of the adult rat (Table 1). The activity at birth was similar to that of the adult, but it decreased rapidly, remained low throughout the suckling period, but increased on weaning (Table 1). A similar developmental pattern has been demonstrated for the hepatic activity of this enzyme (Buckley & Williamson, 1975). Further support for the view that this enzyme is concerned with lipogenesis and/or cholesterol synthesis is the parallelism between its activity in brown adipose tissue (Table 1) and the rate of lipogenesis in this tissue *in vivo* (Pillay & Bailey, 1983).

To obtain information on the fate of acetoacetate in intrascapular brown adipose tissue, the metabolism of [3-^{14}C]acetoacetate was studied in slices of the tissue from weanling rats (Table 2). The rate of acetoacetate utilization when present as sole substrate was at least 10-fold higher than that reported for glucose under similar conditions (McCormack, 1982). Addition of glucose or insulin did not significantly alter the rate of acetoacetate removal (Table 2). About 70% of the [3-^{14}C]acetoacetate removed was converted into $^{14}CO_2$, 10% was reduced to D-3-hydroxybutyrate and 6% converted into ^{14}C-labelled lipid. The rate of oxidation of acetoacetate was approx. 10 times that previously found for D-3-hydroxy[3-^{14}C]butyrate (2 mM; Agius & Williamson, 1981). This difference in rates of oxidation would be expected from the relative activities of 3-oxoacid CoA-transferase and 3-hydroxybutyrate dehydrogenase reported here. Addition of glucose decreased the accumulation of 3-hydroxybutyrate, whereas addition of glucose plus insulin significantly ($P < 0.05$) stimulated

Table 2. Metabolism of acetoacetate in brown-adipose-tissue slices from weanling rats

For experimental details see the text. The rats were 23–26 days old and were weaned on day 21 *post partum*. The rates of [3-^{14}C]acetoacetate removal and its conversion into $^{14}CO_2$, ^{14}C-labelled lipid and 3-hydroxybutyrate are expressed as μmol of acetoacetate/h per g fresh wt. of tissue, and are mean values ± S.D. with the numbers of observations in parentheses. Values that are significantly different by Student's *t* test from those with acetoacetate alone are shown: $*P < 0.05$.

Experimental conditions	Rate			
	Acetoacetate removed	3-Hydroxybutyrate formed	$^{14}CO_2$ formed	^{14}C-lipid formed
[3-^{14}C]Acetoacetate (2mM)	15.1 ± 4.0 (5)	1.57 ± 0.24 (5)	11.1 ± 2.1 (5)	0.93 ± 0.45 (5)
[3-^{14}C]Acetoacetate (2mM) + glucose (5 mM)	13.6 ± 5.9 (8)	0.95 ± 0.45 (8)*	9.1 ± 3.4 (8)	1.72 ± 1.3 (8)
[3-^{14}C]Acetoacetate (2mM) + glucose (5 mM) + insulin (50 munits/ml)	17.7 ± 7.2 (4)	1.25 ± 0.93 (4)	10.7 ± 3.7 (4)	2.88 ± 1.9 (4)*

lipogenesis from [3-^{14}C]acetoacetate (Table 2). Similar results were obtained with slices from suckling rats (10–12 days; results not shown). Use of preparations of brown adipose tissue *in vitro* has been criticized, in part because of loss of ATP and possible lack of oxygenation (McCormack, 1982). In our experiments the rate of acetoacetate utilization and oxidation over 60 min was appreciable and linear; nevertheless, in view of the above-mentioned reservations about the viability of the slice preparation, the results should not necessarily be viewed as comparable with those obtained *in vivo*.

It is concluded that acetoacetate is an important potential substrate of respiration for brown adipose tissue during development and that 3-hydroxybutyrate is less effective. The question arises why this tissue has the capacity to utilize acetoacetate during the suckling period when adequate supplies of triacylglycerol and non-esterified fatty acids are available and ketone bodies are likely to be vital substrates for developing brain (Hawkins *et al.*, 1971). The answer may lie in the ready reversibility of the 3-oxoacid CoA-transferase and acetoacetyl-CoA thiolase reactions (Weidemann & Krebs, 1969; for review see Robinson & Williamson, 1980), which means that acetoacetate can act as a buffer for the intramitochondrial pool of acetyl-CoA, utilization occurring when the production of acetyl-CoA from other sources decreases (Robinson & Williamson, 1980). Thus the major determinant of the rate of acetoacetate utilization *in vivo* is likely to be the rate of acetyl-CoA removal rather than the activity of the two enzymes. The comparatively low activity of 3-hydroxybutyrate dehydrogenase in brown adipose tissue can be seen as an adaptation to conserve ketone bodies in the suckling period, when this is the major form (57–72%) of ketone body in the circulation (Page *et al.*, 1971; Dahlquist *et al.*, 1972).

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