Carnitine and Brown Adipose Tissue Metabolism in the Rat during Development

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1. The content of carnitine, acylcarnitine and total acid soluble carnitine in brown adipose tissue of rats increases rapidly after birth, attaining a peak on about day 10 and then decreases. Similar changes with age were found for carnitine acetyltransferase activity in mitochondria from brown adipose tissue and heart. The activity of this enzyme in brain and in liver is much smaller, but also increases postnatally. 2. The activity of carnitine palmitoyltransferase in brown adipose tissue, however, decreases after birth then increases later in life. 3. Exposure of 18-day-old rats to the cold for 20 days leads to an increase in carnitine content in brown adipose tissue and raises the activity of carnitine acetyltransferase. The activity of carnitine palmitoyltransferase is not affected by cold adaptation.

In infant mammals brown adipose tissue plays an important role in heat production (Dawkins & Hull, 1963). It is assumed that on exposure to cold nor-adrenaline is released. This activates the adenylate cyclase of brown adipose tissue (Skala *et al.*, 1970b), leading to the formation of cyclic AMP (adenosine 3':5'-cyclic monophosphate), which activates a hormone-sensitive lipase. Fatty acids released from triglycerides are then oxidized in the mitochondria (Drahota *et al.*, 1969) and may perhaps uncouple respiration from phosphorylation. This uncoupling effect of fatty acids may be one of the mechanisms for the extra heat production in brown adipose tissue on cold exposure (Hittelman *et al.*, 1969; Prusiner *et al.*, 1968).

The rate at which fatty acids are oxidized by mitochondria from brown adipose tissue is almost completely dependent on the presence of carnitine and ATP (Drahota *et al.*, 1968).

In rat brown adipose tissue the number of mitochondria and the activities of some mitochondrial enzymes increase postnatally, reaching a peak on about day 10 (Barnard & Skala, 1970; Skala & Lindberg, 1970). No information, however, is available on changes in the content of carnitine and the enzymes carnitine acetyl- and carnitine palmitoyltransferase in brown adipose tissue during the postnatal period. In the present paper we describe age-dependent changes in the content of carnitine, acylcarnitine and acylcarnitine and the activities of carnitine acetyl- and carnitine palmitoyltransferase in brown adipose tissue of the rat. Some

characteristics of mitochondria isolated from brown adipose tissue of infant rats are also described.

Experimental

Materials

Animals. Wistar rats aged from 1 to 50 days and foetuses weighing 3.5–5 g were used. Litters were always decreased to eight animals on the 2nd. day after delivery. The young were removed from the mother rat on the 30th. postnatal day. They were fed on a Purina Chow (Ralston, St. Louis, Mo., U.S.A.) pellet diet. Animals exposed to the cold were kept at 4°C for 20 days in the presence of the mother animal, from the 18th. postnatal day.

Tissue preparations. Animals were killed by decapitation. Interscapular brown adipose tissue, liver, heart and brain were rapidly removed in that order and immediately placed in ice-cold glass beakers. For carnitine determinations in brown adipose tissue, the tissue was immediately homogenized in 2.5% perchloric acid (1–2 ml/g of tissue). For enzyme determinations tissues were homogenized in a glass homogenizer with a Teflon pestle in 10 vol. of 0.25 M-sucrose containing 5 mM-ethanedioxybis(ethylamine)-tetraacetic acid (EGTA).

Mitochondria. Mitochondria were isolated by centrifuging the homogenate at 600g for 10 min and recentrifuging the supernatant at 10000g for 20 min. The mitochondria were resuspended in a 0.1%

aqueous solution of Triton X-100 (10× the volume of the original tissue weight) and frozen overnight. All operations were performed at 1–3°C.

Methods

All assays were performed at 30°C with a Unicam SP.800 spectrophotometer equipped with a Unicam SP.20 recorder.

Carnitine was determined by the method of Marquis & Fritz (1964). Total acid-soluble carnitine was determined by the same method after incubating the extract with 0.1 M-KOH at room temperature for 45 min.

Long-chain acylcarnitine was determined in the precipitate obtained by centrifuging the perchloric acid homogenate. The precipitate was dissolved in water with KOH added to give about 0.2 M excess of KOH. The solution was incubated at 55°C for 2 h, then a few drops of 72% perchloric acid were added, and the precipitate was removed by centrifugation and carnitine was then determined in the neutralized supernatant by the method of Marquis & Fritz (1964).

Carnitine acetyltransferase was determined by the method of Barker *et al.* (1968), with the dithiobis-(2-nitrobenzoic acid) assay. Activity was negligible in the postmitochondrial supernatant and hence only Triton X-100-treated mitochondria were examined.

Carnitine palmitoyltransferase was determined by the method of Norum (1964, 1965) under the conditions described by Fritz & Marquis (1965). Again only isolated Triton X-100-treated mitochondria were examined. Oxygen consumption of isolated mitochondria resuspended in 0.25 M-sucrose in 5 mM-EGTA (1:1 original tissue weight) was measured with a Clark-type oxygen electrode (Yellow Springs Instruments, Yellow Springs, Ohio, U.S.A.) connected to a modified Unicam SP.20 recorder. The incubation medium contained in 3 ml: KCl, 80 mM; tris-HCl (pH 7.4), 20 mM; MgCl₂, 6 mM; KH₂PO₄ (pH 7.4), 14 mM; potassium malate, 3.3 mM; 0.1 ml of the mitochondrial suspension (0.5–1 mg of mitochondrial protein). When added, the final concentrations of other substances were: carnitine, 1 mM; ATP, 0.5 mM; acetylcarnitine, 0.1 mM; palmitoylcarnitine, 0.1 mM; albumin, 40 mg/ml. Measurements were made at 25°C. The [1-¹⁴C]palmitate oxidation was examined in the same medium. The CO₂ was trapped in suspended vessels containing 0.2 ml of Hyamine hydrochloride-ethanol (1:1, v/v). After incubation for 30 min at 30°C, 0.4 ml of 0.05 M-H₂SO₄ was added to the mitochondria through a rubber stopper and CO₂ was collected for another 45 min. Radioactivity was determined in a Picker Nuclear scintillation counter (Montreal, Que., Canada). The scintillation fluid consisted of 10 ml of toluene, containing 80 mg of 2,5-diphenyloxazole and 1.0 mg of *p*-bis-(*o*-methylstyryl)benzene and 5 ml of ethanol.

Fig. 1. Developmental changes in the content of carnitine, acylcarnitine and total acid-soluble carnitine in brown adipose tissue of the rat

F, foetuses weighing 3.5 to 4.5 g; A, adult. □—□, Total acid-soluble-carnitine; □---□, free carnitine; ●, acylcarnitine. Vertical bars, $\pm$ S.E.M. There are four to six determinations in each age group.

Table 1. *Effect of cold exposure for 20 days on the activities of carnitine acetyl- and carnitine acyl-transferase in brown adipose tissue*

For experimental details see the text. Enzyme activities are expressed as $\mu\text{mol}/\text{min}$ per mg of mitochondrial protein. The numbers of animals used are given in parentheses.

	Rats living at ...	25°C	4°C	
Carnitine acyltransferase		0.41 ± 0.02 (4)	0.39 ± 0.03 (4)	
Carnitine acetyltransferase		0.27 ± 0.013 (4)	0.38 ± 0.02 (4)	$P < 0.01$
Total acid-soluble carnitine ($\mu\text{mol}/\text{g}$ of wet tissue)		0.36 ± 0.011 (4)	0.62 ± 0.015 (4)	$P < 0.01$

Fig. 2. *Developmental changes in carnitine acetyltransferase activity in different organs of the rat*

F, foetus; A, adult; ○, brown adipose tissue; ■, heart; □, brain; ▲, liver. Vertical bars: $\pm$ S.E.M. There are four to six determinations in each age group.

Protein was determined by the method of Lowry *et al.* (1951).

Chemicals. These were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., except bovine serum albumin (fraction V), which was from Calbiochem, Los Angeles, Calif., U.S.A., and palmitoyl-L-carnitine, a gift from Dr. I. B. Fritz.

Results

The content of carnitine in brown adipose tissue increases rapidly after birth, reaching a peak on the 10th. day. Significantly lower values were obtained in adult rats. Since the tissue was not immediately frozen in liquid air after removal from the animals, the values for short- and long-chain acylcarnitine may not be accurate (Pearson & Tubbs, 1967). Nevertheless the content of both substances and the total carnitine content in brown adipose tissue again attain a peak on the 10th. postnatal day (Fig. 1). Exposure to cold increases the carnitine content in brown adipose tissue (Table 1).

Age changes in the activity of carnitine acetyltransferase closely follow those of carnitine content (Fig. 2). Similar changes are observed in the heart and to some extent also in liver and brain, although in the latter two organs these changes are much less pronounced. Exposure to the cold increases activity of the enzyme in brown adipose tissue (Table 1). Since the amount of mitochondrial protein increases to a peak between days 10 and 17 in brown fat and also on exposure to the cold (Skala *et al.*, 1970a) these changes in enzyme activity would appear even more pronounced if expressed per unit wet weight.

In contrast with the similar developmental pattern of carnitine acetyltransferase found in all organs examined, the activity of carnitine palmitoyltransferase decreases in brown adipose tissue after birth and increases again only after weaning (Fig. 3) as opposed to the rapid postnatal increase in activity found in the liver by Augenfeld & Fritz (1970).

Exposure to the cold has no effect on this enzyme in brown adipose tissue (Table 1).

Discussion

Carnitine acetyltransferase activity and carnitine content in brown adipose tissue are highest in the suckling period when brown fat mitochondrial oxidative metabolism reaches its peak (for review see Barnard & Skala, 1970). Moreover at this period the body is geared to fatty acid oxidation (Hahn &

Koldovsky, 1966); fatty acid synthesis in liver (Carrol, 1964) and adipose tissue (Hahn, 1970) is suppressed and ketone-body production by the liver and blood concentrations of ketones and fatty acids are considerably elevated (Drahota *et al.*, 1964; Hahn & Koldovsky, 1966).

There has been some discussion as to the role of carnitine acetyltransferase. It has been suggested that this enzyme aids the transfer of acetyl-CoA out of (Bressler & Katz, 1965) or into mitochondria (Fritz & Yue, 1964; Bode & Klingenberg, 1964). It has also been suggested that the enzyme transfers acetyl-CoA between inner and outer mitochondrial compartments (Brdiczka *et al.*, 1969).

Our results suggest that acetylcarnitine may serve as a substrate for mitochondria from brown adipose tissue, as it does in other tissues (Bode & Klingenberg,

Fig. 3. Developmental changes in carnitine palmitoyltransferase activity in brown adipose tissue and liver

Values for liver are taken from Table 1 of the paper by Augenfeld & Fritz (1970). F, foetus; A, adult. □, brown adipose tissue; ■, liver. Vertical bars: $\pm$ S.E.M.

1964). Addition of acetylcarnitine in both the presence and the absence of albumin increases the rate of respiration (Table 2) of isolated mitochondria. The rate of oxidation of radioactive palmitate in the absence of albumin is enhanced by added acetylcarnitine to the same extent as by carnitine itself, indicating that under these conditions acetylcarnitine probably is a source of carnitine. In the presence of albumin, however, acetylcarnitine is much less effective than carnitine in enhancing the rate of palmitate oxidation and since under identical conditions it raises the respiratory rate, we may conclude that it is being oxidized (Table 2), and does not act by supplying the initial investment of energy needed to start fatty acid oxidation (Siliprandi *et al.*, 1965).

Since the postnatal rise and fall in carnitine acetyltransferase activity and carnitine content in brown fat follows the rise and fall in blood ketone concentrations it is tempting to suggest that brown adipose tissue utilizes ketone bodies under resting conditions and that carnitine acetyltransferase is involved. Few results are available comparing the rates of oxidation of β -hydroxybutyrate or acetoacetate with those of their carnitine derivatives by mitochondria from brown adipose tissue. Christiansen *et al.* (1969) suggested that such mitochondria can utilize ketone bodies, and showed that the rate of oxidation of DL- β -hydroxybutyrylcarnitine was similar to that of malate plus pyruvate. Our own preliminary experiments with isolated mitochondria from brown adipose tissue have shown that the respiratory rate in the presence of β -hydroxybutyrate was only 7 ng-atoms/min per mg of protein and was raised to 127 ng-atoms/min per mg if carnitine was added to the usual mitochondrial buffer containing albumin, ATP and CoA. None of the above results, however, are conclusive proof that carnitine and a carnitine acyltransferase are required for the optimum rate of oxidation of ketone bodies by mitochondria from brown adipose tissue.

The rate of fatty acid synthesis in brown adipose

Table 2. Oxygen consumption and $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ palmitate by mitochondria from brown adipose tissue of 10-day-old rats

For details see the text.

Addition	Without albumin		With albumin (40mg/ml)	
	(ng-atoms/min per mg)	Radioactivity*	(ng-atoms/min per mg)	Radioactivity (c.p.m.)
None	16.8	2500	17.5	2000
DL-Carnitine	270	42620	35	12520
Acetyl-DL-carnitine	270	43000	400	4500
Palmitoyl-L-carnitine	84	16028	300	2500

* The radioactivity is expressed as counts/20min per mg of mitochondrial protein; 0.1 ml of a 2.5 mM- $[1-^{14}\text{C}]$ palmitate solution (1 μCi) was added to 2 ml of buffer as described in the Experimental section.

tissue from suckling rats is much greater from acetate than from citrate (Hahn, 1970) and there might be recycling of fatty acids, i.e. oxidation of acyl-CoA to acetyl-CoA in the mitochondria and synthesis of fatty acids from transported acetyl-CoA in the cytoplasm. It is also possible that carnitine acetyltransferase is mainly involved in biological acetylation as suggested by Bressler & Brendel (1966) or acts as an acetyl buffer (Pearson & Tubbs, 1967). Whatever the explanation, there seems little doubt that when the amount of heat produced in brown adipose tissue is large (infant rats, cold-adapted rats) carnitine and carnitine acetyltransferase play an important role in that tissue.

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