Hepatic ketogenesis in newborn pigs is limited by low mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity

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In newborn-pig hepatocytes, the rate of oleate oxidation is extremely low, despite a very low malonyl-CoA concentration. By contrast, the sensitivity of carnitine palmitoyltransferase (CPT) I to malonyl-CoA inhibition is high, as suggested by the very low concentration of malonyl-CoA required for 50% inhibition of CPT I (IC_{50}). The rates of oleate oxidation and ketogenesis are respectively 70 and 80% lower in mitochondria isolated from newborn-pig liver than from starved-adult-rat liver mitochondria. Using polarographic measurements, we showed that the oxidation of oleoyl-CoA and palmitoyl-L-carnitine is very low when the acetyl-CoA produced is channelled into the hydroxymethylglutaryl-CoA (HMG-CoA) pathway by addition of malonate. In contrast, the oxidation of the same substrates is high when the acetyl-CoA produced is directed towards the citric acid cycle by addition of malate. We demonstrate that the limitation of ketogenesis in newborn-pig liver is due to a very low amount and activity of mitochondrial HMG-CoA synthase as compared with rat liver mitochondria, and suggest that this could promote the accumulation of acetyl-CoA and/or β-oxidation products that in turn would decrease the overall rate of fatty acid oxidation in newborn- and adult-pig livers.

INTRODUCTION

The neonatal period is characterized by increased fatty acid availability. Indeed, newborn mammals are fed with milk, which in most species is a high-fat low-carbohydrate diet (Jenness, 1974). Moreover, in some species (rabbit, guinea-pig, human), fatty acids also originate from adipose triacylglycerol stores that are mobilized immediately after birth (Girard et al., 1992). Then fatty acid oxidation develops rapidly after birth in many peripheral tissues and in the liver, where fatty acids are used as precursors for ketone-body synthesis (Williamson, 1982). As a direct consequence, the concentration of blood ketone bodies increases during day 1 after birth in many newborn mammals (Williamson, 1982; Girard et al., 1992). This physiological hyperketonaemia persists during all the suckling period and decreases progressively when young mammals begin to nibble the high-carbohydrate diet of the adult (Williamson, 1982; Girard et al., 1992). These co-ordinated changes are essential for the newborn to cover its energy needs, as demonstrated by the dramatic consequences of inborn errors of mitochondrial fatty acid oxidation (Turnbull et al., 1988).

However, previous studies have shown that blood ketone-body levels remain very low in suckling newborn pigs, despite high concentrations of plasma non-esterified fatty acids (Bengtsson et al., 1969; Gentz et al., 1970; Pégorier et al., 1981). This is due to a limited capacity for hepatic fatty acid oxidation, since most of the oleate taken up by isolated pig hepatocytes is converted into esterified fats, whatever the age of the animal or its nutritional state (Pégorier et al., 1983). As the rate of lipogenesis is very low in isolated hepatocytes from fed or starved newborn pigs (Pégorier et al., 1983), it was suggested that the limitation of long-chain fatty acid oxidation was not due to inhibition of carnitine palmitoyltransferase (CPT) I. However, the malonyl-CoA concentration and the activity and sensitivity of CPT I to malonyl-CoA inhibition were not determined in these experiments. It is noteworthy that the limitation of fatty acid oxidation was only restricted to the liver of newborn pigs, because fatty acid oxidation develops normally after birth in extra-hepatic tissues (Bieber et al., 1973; Mersmann and Phinney, 1973; Wolfe et al., 1978; Ascuitto et al., 1989; Werner et al., 1989).

The aim of the present work was to investigate the possible limiting step(s) of hepatic fatty acid oxidation and ketogenesis in the newborn pig.

MATERIALS AND METHODS

Animals

Pigs from the Large White strain, farrowed in the Institut National de la Recherche Agronomique (Jouy-en-Josas, France), were used. Pregnant sows were fed daily with 2.5 kg of cereals/soya-bean-meal diet. Non-pregnant adult pigs were fed *ad libitum* on the same diet. Normal delivery occurs during the night of day 114–115 of pregnancy. As precise timing of birth was desired, parturition was introduced by injecting pregnant sows on day 113 of gestation with a prostaglandin analogue (cloprostenol, 10 μg/kg body wt.; Bellon, Neuilly, France). As the hepatic metabolism of long-chain fatty acid was similar in 2-day-old fed or starved newborn pigs (Pégorier et al., 1983), experiments were conducted in starved animals. Newborn pigs were separated from the mother immediately after birth and starved for 48 h at 35 °C. Adult pigs were also used after a 48 h starvation.

Male Wistar rats weighing 200–300 g were used for comparison. They were housed in individual cages (24 °C; light from 06:00 to 20:00 h) and fed *ad libitum* on pelleted laboratory chow (65% carbohydrate, 11% fat and 24% protein of total energy). Rats were starved for 24 h before the experiments.

Abbreviations used: CPT, carnitine palmitoyltransferase; HMG, 3-hydroxy-3-methylglutaryl-CoA; RCR, respiratory control ratio.

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Isolation and incubation of hepatocytes

Hepatocytes from newborn pigs or adult rats were isolated as described previously (Pégorier et al., 1982, 1988). The tissue-dissociating solution was Hepes buffer (NaCl 137 mM; KCl 2.7 mM; Na_2HPO_4,12 H_2O 0.7 mM; Hepes 10 mM; pH 7.6) containing 0.025% collagenase and 5 mM $CaCl_2$. Cell viability, estimated by Trypan Blue exclusion, was always greater than 90%.

Hepatocytes from newborn pigs and adult rats were incubated as described previously (Pégorier et al., 1988); (3–5) $\times 10^6$ hepatocytes were incubated at 37 °C in 2 ml of Krebs–Henseleit buffer (pH 7.4), except for malonyl-CoA determinations, where the cell density was increased to (20–25) $\times 10^6$ hepatocytes. Experiments were performed in duplicate for each condition. Fatty acid oxidation and ketogenesis from [1-^{14}C]oleate (0.3 mM, 0.5 μCi/μmol) bound to defatted BSA (2% final concn.) were measured in the presence of carnitine (1 mM). After 30 min, the incubation was ended by adding 0.25 ml of $HClO_4$ (40%, v/v), and CO_2 and acid-soluble products were determined as previously described (Pégorier et al., 1988).

Malonyl-CoA concentration was determined after incubation for 30 min in the presence of oleate (0.3 mM) and carnitine (1 mM). The incubations were ended by adding 0.2 ml of $HClO_4$ (30%, v/v) and malonyl-CoA were assayed as described by McGarry et al. (1978). Rat liver fatty acid synthase was prepared as described by Stoops et al. (1979).

Isolation of liver mitochondria and measurements of respiration

Pig and rat liver mitochondria were isolated as described by Mersmann et al. (1972). Livers were rapidly sampled and rinsed in a medium containing 220 mM mannitol, 70 mM sucrose, 2 mM Hepes and 0.1 mM EDTA (pH 7.4). All processing steps were conducted at 4 °C. After mincing, liver slices were homogenized by two up/down strokes of a loose-fitting motor-driven pestle (Heidolph, RGI, Germany) with a constant speed of 50–60 rev./min. After centrifugation (successively at 700 ***g*** and 10000 ***g***, each for 10 min), the final pellet was resuspended in the isolation medium at a concentration of 10 mg of protein/ml. Protein was determined by the method of Lowry et al. (1951), with BSA as standard.

Mitochondrial incubations

Incubations were performed as described previously (Escriva et al., 1986). Briefly, mitochondria (1 mg of mitochondrial protein/flask) were incubated for 15 min at 30 °C in the modified Krebs–Henseleit buffer described by McGarry et al., (1977), containing 4.9 mM NaCl, 25.5 mM $NaHCO_3$, 121 mM KCl, 2.4 mM $MgSO_4$ and 1.2 mM KH_2PO_4. The incubations were performed in the presence of 4 mM ATP, 1 mM ADP, 50 μM CoA, 250 μM GSH, 200 μM L-carnitine and 100 μM [1-^{14}C]oleate (0.025 μCi/nmol) or 200 μM [1-^{14}C]octanoate (0.04 μCi/nmol) bound to dialysed BSA (fatty acid/BSA ratio = 3.5). The flasks were gassed with O_2/CO_2 (19:1), and the incubation was ended by addition of 0.4 ml of $HClO_4$ (5%, v/v). Labelled CO_2 and acid-soluble products were determined as described above. The net rate of ketone-body production in the presence of oleate was obtained after subtraction of the ketone-body concentration present at the end of the incubation period in the absence of added substrate. In some experiments, ketone-body production was determined in adult-rat and newborn-pig liver mitochondria incubated for 30 min at 30 °C in a sucrose/phosphate buffer (28 mM KH_2PO_4, 7.2 mM $MgCl_2$, 250 mM sucrose, pH 7.4) either in the absence or in the presence of 2.8 mM 4-methyl-2-oxopentanoate, as described by Noda and Ichihara (1974).

Mitochondrial respiration and fatty acid oxidation

Mitochondrial respiration was measured at 30 °C by using an oxygraph (Gilson, model 5/6 H) equipped with a 2 ml water-jacketed chamber and with a Clark oxygen electrode. For the measurement of coupled and uncoupled respiratory capacity, mitochondria (1 mg of mitochondrial protein) were added to the respiratory medium of Aprille and Asimakis (1980) as previously reported in detail (Escriva et al., 1986). The mitochondrial respiration was measured from succinate (10 mM) or glutamate (5 mM) plus malate (5 mM) in the presence of 90 μM ADP (State 3) and after exhaustion of ADP (State 4). Uncoupled rates of oxygen consumption were determined in the presence of 40 μM 2,4-dinitrophenol.

The rates of oleoyl-CoA (10 μM) or palmitoyl-L-carnitine (10 μM) oxidation were measured in the respiratory medium of Osmundsen and Sherratt (1975) in the presence of carnitine (2 mM) and either malonate (10 mM) or malate (2.5 mM), both under uncoupled conditions (0.1 mM 2,4-dinitrophenol).

Measurement of enzyme activities in isolated mitochondria

CPT I

CPT I activity was assayed at 30 °C as the formation of palmitoyl-L-[*methyl*-^{3}H]carnitine from L-[*methyl*-^{3}H]carnitine and palmitoyl-CoA as described previously (Herbin et al., 1987). The sensitivity of CPT I to malonyl-CoA inhibition has been estimated by measuring the concentration of malonyl-CoA required for 50% inhibition of the enzyme activity (IC_{50}). For this purpose, the palmitoyl-CoA concentration was 80 μM and the malonyl-CoA concentration varied between 0.01 to 150 μM. In all experiments ($n = 15$), the formation of palmitoylcarnitine from L-[*methyl*-^{3}H]carnitine (1 mM; 1.6 nCi/nmol) was almost completely suppressed (98 ± 1%) by the highest malonyl-CoA concentration (150 μM). This suggested that membrane integrity of the mitochondrial preparations was good, and that only CPT I activity was measured, without any significant contribution of CPT II.

Enzymes of ketone-body synthesis

Acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA (HMG-CoA) synthase and HMG-CoA lyase activities were measured at 25 °C as described by Williamson et al. (1968). Briefly, the 'forward' and 'backward' assays were used to measure respectively the activities of HMG-CoA lyase and acetoacetyl-CoA thiolase. The activity of HMG-CoA synthase was assayed by the measurement of acetoacetate production from acetyl-CoA in the presence of an acetyl-CoA-generating system. The mitochondrial HMG-CoA synthase activity and the degree of succinylation were determined directly as described by Quant et al. (1989, 1990).

Immunoblotting of mitochondrial proteins

HMG-CoA synthase

Mitochondrial proteins from 2-day-old or adult pig and adult rat were submitted to SDS/PAGE (15% gel) in reducing conditions (100 mM dithiothreitol) as described by Laemmli (1970). After electrotransfer to a nitrocellulose membrane (0.45 μm pore; Schleicher and Schuell, Germany), the membrane was blocked

Table 1 Oleate oxidation, malonyl-CoA concentration, CPT I activity and sensitivity to malonyl-CoA inhibition in hepatocytes and mitochondria isolated from liver of fasting newborn pigs and adult rats

Hepatocytes and liver mitochondria were isolated from 24 h-fasted rats or 48 h-starved newborn pigs. Hepatocytes were incubated for 30 min in the presence of [1-^{14}C]oleate (0.3 mM) bound to fat-free BSA (2%, final concn.) plus carnitine (1 mM). Malonyl-CoA concentration was determined under the same conditions of incubation. CPT I activity was determined in isolated mitochondria in the presence of 80 μM palmitoyl-CoA and 1 mM carnitine. IC_{50} refers to the concentration of malonyl-CoA required for 50% inhibition of CPT I activity. Values are means ± S.E.M. of the numbers of experiments shown in parentheses: * $P < 0.01$ compared with 2-day-old pigs.

Source of hepatocytes	CO_2 (nmol/30 min per 10^6 cells)	Acid-soluble products (nmol/30 min per 10^6 cells)	Malonyl-CoA (pmol/10^6 cells)	CPT I activity (nmol/min per mg of mitochondrial protein)	IC_{50} (μM)
2-day-old pig	0.40 ± 0.07 (4)	0.35 ± 0.14 (4)	2 ± 1 (9)	1.0 ± 0.1 (4)	0.025–0.050
Adult rat	2.45 ± 0.30 (6)*	24.6 ± 2.2 (6)*	34 ± 9 (9)*	1.5 ± 0.2 (6)	1.27 ± 0.19 (6)*

with 3% (w/v) BSA in Tris-buffered saline (pH 7.6) and incubated in the same solution with the diluted antibody (1/500, v/v). Immune complexes were revealed as described previously (Quant et al., 1991). The polyclonal antibody was raised in the rabbit against the purified ox liver mitochondrial HMG-CoA synthase and shown to cross-react with the rat enzyme (Quant et al., 1991).

CPT I and II

Mitochondrial proteins were solubilized in 0.3 mM KCl/10 mM Tris buffer (pH 7.4) containing 0.2% octyl glucoside as described by Woeltje et al. (1987). After centrifugation (1 h at 100000 *g*), solubilized proteins were submitted to SDS/PAGE (8.5% gel) and, after electrotransfer to nitrocellulose membrane (see above), the blot was probed with the anti-CPT I antiserum (1/150, v/v) as described by Esser et al. (1993). Then the membrane was incubated for 2 min in 50 mM glycine/24 mM HCl (pH 2.6) buffer containing 0.2% SDS and blotted with the anti-CPT II serum (1/100, v/v) as described by Woeltje et al. (1987). Immune complexes were revealed with ^{125}I-Protein A.

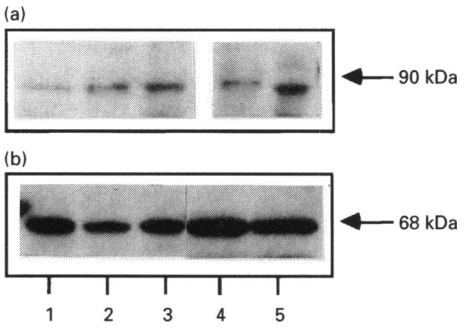

Figure 1 Western-blot analysis of CPT I and II in mitochondria isolated from newborn- or adult-pig liver and adult-rat liver

Mitochondrial proteins were solubilized in octyl glucoside and submitted to SDS/PAGE (8.5% gel). The blot was probed with either an anti-CPT I serum (**a**) or an anti-CPT II serum (**b**). Lane 1, 2-day-old newborn pigs; lanes 2 and 3, adult pigs; lanes 4 and 5, adult rats. For more details see the Materials and methods section.

Chemicals

All substrates and enzymes were obtained from Boehringer-Mannheim (Meylan, France). Fatty-acid-free BSA, L-carnitine, malonyl-CoA, oleoyl-CoA, palmitoyl-L-carnitine, CoA and HMG-CoA were purchased from Sigma (St. Louis, MO, U.S.A.). [1-^{14}C]Oleate, [1-^{14}C]octanoate, L-[*methyl*-^{3}H]carnitine and ^{125}I-Protein A were obtained from Amersham International (Amersham, Bucks., U.K.).

Statistics

Results are expressed as means ± S.E.M. Statistical analyses were performed by Student's unpaired *t* test.

RESULTS AND DISCUSSION

Metabolic characteristics of long-chain fatty acid oxidation pathway in newborn-pig liver

It was previously shown that the low blood ketone-body concentration in fed or fasted 2-day-old pigs was due to a limited capacity for hepatic fatty acid oxidation (Pégorier et al., 1983). The data presented in Table 1 show that the rate of oleate oxidation (CO_2 plus acid-soluble products) is very low in isolated hepatocytes from 48 h-old starved pigs, since it represented only 5% of that found in hepatocytes from starved adult rats. It seems unlikely that such differences between pig and rat liver fatty acid oxidation rates result from a decreased number of mitochondria. In fact, it was shown that mitochondrial mass was increased in newborn-pig liver within the first 24 h after birth (Mersmann et al., 1972). Although there is no study available to compare pig and rat liver mitochondrial mass, it is noteworthy that the rates of gluconeogenesis from lactate, which requires mitochondrial steps, are similar in isolated hepatocytes from suckling newborn pigs (Pégorier et al., 1982), rats (Ferré et al., 1981) and rabbits (El Manoubi et al., 1983).

Similarly, it seems unlikely that this low rate of oleate oxidation results exclusively from a limitation in the entry of oleate into mitochondrial matrix. Firstly, the concentration of CPT II is as high in pig liver as in rat liver mitochondria (Figure 1). Secondly, despite a 40% lower CPT I protein amount (Figure 1) and activity (Table 1) in newborn-pig mitochondria than in those from rat liver, the rate of oleate oxidation is 95% lower in isolated newborn-pig hepatocytes than in rat adult hepatocytes (Table 1). It is noteworthy that decreased rates of oleate oxidation occurred, despite a very low malonyl-CoA concentration (Table 1), reflecting an impaired hepatic lipogenesis (Pégorier et al., 1983) due to low lipogenic enzyme activities in the newborn-pig liver (Mersmann et al., 1973). In contrast, the limited oleate oxidation in newborn-pig liver could be due to the huge sensitivity of CPT I to malonyl-CoA inhibition, as suggested by the very

Table 2 Oleate oxidation and ketone-body production in isolated liver mitochondria from fasting newborn and adult pigs and starved adult rats

Incubations were carried out in Krebs–Henseleit buffer (pH 7.4) containing 4 mM ATP, 1 mM ADP, 50 μM CoA, 250 μM GSH, 200 μM carnitine and 0.1 mM [1-^{14}C]oleate bound to fatty-acid-free BSA (molar ratio oleate/BSA = 3.5). Rates of oleate oxidation (CO_2 + acid-soluble products) were determined over a 15 min incubation period at 30 °C and expressed as nmol of oleate utilized/min per mg of mitochondrial protein. The rate of ketone-body production was expressed as nmol of ketone body formed from oleate/min per mg of mitochondrial protein. Values are means ± S.E.M. of the numbers of experiments shown in parentheses: * $P < 0.01$ when compared with adult rats.

Mitochondrial source	Oleate oxidation	Total ketone-body production
2-day-old pig	2.5 ± 0.2 (6)*	2.1 ± 0.5 (6)*
Adult pig	2.1 ± 0.3 (4)*	2.7 ± 0.1 (4)*
Adult rat	7.3 ± 0.8 (6)	12.5 ± 2.0 (6)

low IC_{50} value (Table 1). However, if the great sensitivity of CPT I to malonyl-CoA inhibition represented the main regulatory mechanism involved in the limitation of long-chain fatty acid oxidation in the newborn-pig liver, one would expect that the oxidation of oleate might not be limited in a malonyl-CoA-free system such as isolated mitochondria.

Oleate oxidation and ketogenesis in isolated newborn-pig mitochondria

The rate of oleate oxidation was 70 % lower in liver mitochondria isolated from 48 h-old starved pigs than in those from adult-rat livers (Table 2). This decreased long-chain fatty acid oxidation did not result from an eventual difference in the degree of contamination of mitochondrial preparations by other cellular fractions, since the activity of succinate dehydrogenase (used as a mitochondrial marker) was similar in both species (results not shown). This low rate of oleate oxidation seems to be a characteristic of the pig species, rather than being due to a precise stage of development, as suggested by the low capacity of adult-pig liver mitochondria (Table 2). For the reasons discussed above, it was unlikely that this low rate of oleate oxidation results from a limitation in the entry of oleate into the mitochondria. On the basis of CPT activity, similar conclusions were previously suggested (Bieber et al., 1973). Moreover, the oxidation of 0.2 mM octanoate, the entry of which into the mitochondria is independent of the CPT system (McGarry and Foster, 1980), was also very low in newborn-pig liver mitochondria (3.1 ± 0.5 nmol/min per mg of protein; $n = 6$). The rate of ketone-body synthesis is also 80 % lower in pig than in rat liver mitochondria, whatever the stage of development (Table 2).

In order to investigate the controlling steps of fatty acid oxidation and ketogenesis in newborn-pig liver mitochondria, we performed experiments using polarographic techniques. The intactness of the mitochondrial preparation was assessed by calculating the respiratory control ratio (RCR), i.e. the ratio of oxygen consumption due to substrates in the absence (State 4) or in the presence of ADP (State 3). As shown in Table 3, mitochondria isolated from 2-day-old fasting pigs exhibited RCR values with succinate or glutamate + malate greater than 5, indicating that mitochondrial membranes are intact.

Since acetyl-CoA produced from β-oxidation can enter both the HMG-CoA pathway and the tricarboxylic acid cycle, the oxidation of oleoyl-CoA and palmitoyl-L-carnitine was measured in the presence of either malate or malonate. In the presence of malonate, an inhibitor of succinate dehydrogenase (Garland et al., 1965), acetyl-CoA is channelled into the HMG-CoA pathway and acetoacetate is the main final product. In these conditions the oxidation rates of oleoyl-CoA and palmitoyl-L-carnitine (in the presence of 2,4-dinitrophenol) are similar (Table 3), but markedly lower than that found in adult-rat liver mitochondria (Table 3). In contrast, when mitochondria were incubated in the presence of malate (a donor of oxaloacetate), acetyl-CoA is directed into the citric acid cycle and citrate is the main final product (Shepherd et al., 1965). In the presence of malate, the rates of oxygen consumption due to the oxidation of oleoyl-CoA or palmitoyl-L-carnitine were markedly enhanced compared with those measured in the presence of malonate (Table 3). Similar observations have been reported in newborn-rat (Escriva et al., 1986) or -rabbit (Herbin et al., 1987) mitochondria. These results suggest that the formation of ketone body from acetyl-CoA might be limiting as compared with fatty acid oxidation, as previously reported (Bremer and Wojtczak, 1972). Moreover, there was no difference between oleoyl-CoA and palmitoyl-L-carnitine oxidation rates in the presence of malate (Table 3), suggesting that CPT I activity is not rate-limiting for long-chain fatty acid oxidation in newborn-pig liver mitochondria. These results showed that the utilization of acetyl-CoA in the HMG-CoA pathway is markedly decreased in newborn-pig liver mitochondria. Such a limitation could induce the accumulation of acetyl-CoA and/or β-oxidation intermediates that are thought to

Table 3 Respiratory function and polarographic measurements of oleoyl-CoA and palmitoyl-L-carnitine oxidation in liver mitochondria from 2-day-old fasting newborn pigs or adult rats

Mitochondrial respiration (1 mg of protein) was measured from succinate (10 mM) or glutamate (5 mM) + malate (5 mM) in the presence of 90 μM ADP (State 3) or after ADP exhaustion (State 4). RCR was the State-3/State-4 ratio. Uncoupled rates of respiration were determined in the presence of 40 μM 2,4-dinitrophenol. The oxidation of oleoyl-CoA (10 μM) plus carnitine (2 mM) or palmitoyl-L-carnitine (10 μM) was performed in the presence of 2,4-dinitrophenol and either malonate (10 mM) or malate (2.5 mM). Results are expressed as ng-atoms of O/min per mg of protein. Values are means ± S.E.M. of the numbers of experiments shown in parentheses: * $P < 0.01$ when compared with oxygen consumption measured in the presence of malate.

Substrate added	State 3 Newborn pigs	State 4 Newborn pigs	RCR Newborn pigs	Uncoupled Newborn pigs	Uncoupled Adult rats
Succinate	223 ± 10 (8)	42 ± 2 (8)	5.4 ± 0.3 (8)	310 ± 24 (7)	314 ± 22 (5)
Glutamate + malate	173 ± 10 (7)	22 ± 1 (7)	8.0 ± 0.5 (7)	238 ± 17 (7)	224 ± 28 (4)
Oleoyl-CoA + malonate	–	–	–	19 ± 2 (6)*	54 ± 5 (4)*
Oleoyl-CoA + malate	–	–	–	72 ± 9 (4)	111 ± 8 (4)
Palmitoyl-L-carnitine + malonate	–	–	–	14 ± 1 (6)*	56 ± 3 (4)*
Palmitoyl-L-carnitine + malate	–	–	–	90 ± 12 (6)	100 ± 9 (4)

Table 4 Activities of enzymes of the HMG-CoA pathway in liver mitochondria from fasting newborn or adult pigs and adult rats

Enzyme activities were determined at 25 °C in mitochondria isolated from 2-day-old newborn pigs, adult pigs and adult rats, after membrane disruption with 0.5% Triton X-100. Results are expressed in m-units/mg of mitochondrial protein. Values are means ± S.E.M. of the numbers of experiments shown in parentheses (N.D., not determined): * $P < 0.05$ and ** $P < 0.01$ compared with the rat.

	Activity		
Enzyme	Newborn pig	Adult pig	Adult rat
Acetoacetyl-CoA thiolase	235 ± 40 (7)*	N.D.	576 ± 86 (7)
HMG-CoA synthase	0.7 ± 0.1 (8)**	0.8 ± 0.3 (4)**	16.4 ± 1.9 (7)
HMG-CoA lyase	12.9 ± 5.0 (5)**	N.D.	47.3 ± 4.5 (6)

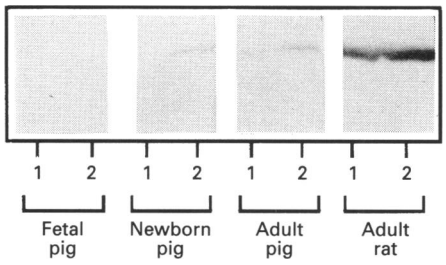

Figure 2 Western-blot analysis of mitochondrial HMG-CoA synthase from newborn- or adult-pig liver and adult-rat liver

Mitochondrial proteins were layered on to a 15% polyacrylamide gel and submitted to SDS/PAGE in reducing conditions. After electrophoresis and blotting on to nitrocellulose, mitochondrial HMG-CoA synthase was detected with a polyclonal antibody raised in the rabbit against the purified ox liver enzyme: lanes 1, 300 μg of mitochondrial protein; lanes 2, 600 μg of mitochondrial protein. This blot is representative of three different experiments.

be strong inhibitors of acyl-CoA dehydrogenase (Bremer and Osmundsen, 1984).

In order to identify possible control sites of the HMG-CoA pathway in newborn pig liver mitochondria, the activities of the three enzymes involved in this pathway have been determined.

Ketogenic-enzyme activities in newborn-pig liver mitochondria

Despite a 60% decrease in the activity of acetoacetyl-CoA thiolase in newborn-pig liver mitochondria as compared with rat liver mitochondria (Table 4), its activity was far higher than the two other enzyme activities, namely HMG-CoA synthase and HMG-CoA lyase, suggesting that it is unlikely to exert significant control over ketone-body synthesis in newborn-pig mitochondria. Similarly, the activity of HMG-CoA lyase was much lower in newborn-pig mitochondria than in those from rat liver (Table 4). However, it seemed unlikely that this enzyme could limit the ketogenic flux, since the rate of ketone-body synthesis from 4-methyl-2-oxopentanoate, a keto acid that enters the pathway at the level of HMG-CoA (Noda and Ichihara, 1974), was similar in mitochondria isolated from newborn-pig liver (2.6 ± 0.6 nmol/min per mg of protein, $n = 9$) and from adult-rat liver (2.2 ± 0.2 nmol/min per mg of protein, $n = 9$). In contrast, the activity of HMG-CoA synthase was markedly depressed (95%) in mitochondria isolated from newborn- and adult-pig liver compared with rat mitochondria (Table 4). The low HMG-CoA synthase activity did not result from a high succinylation state. Indeed, the succinylation state of pig mitochondria (5%) was 4-fold lower than that of suckling rat liver mitochondria (Quant et al., 1991) that possess an active mitochondrial ketogenesis (Escriva et al., 1986). In fact, the low ketogenic capacity of newborn-pig liver mitochondria resulted from the extremely low amount of HMG-CoA synthase protein in newborn- or adult-pig liver mitochondria, as shown by Western-blot analysis (Figure 2).

Concluding remarks

These observations are not restricted to the newborn-pig liver or to the pig strain used for the present work. Firstly, the adult-pig liver also has a decreased capacity for hepatic fatty acid oxidation and ketogenesis (the present work), as confirmed by the absence of hyperketonaemia even after 5 days of starvation (Müller et al., 1982). Secondly, the rates of mitochondrial oleoyl-CoA oxidation (in presence of malonate) and HMG-CoA synthase activity are extremely low in newborn-pig liver of other stains: Pietrain, Chinese and wild boar (results not shown). It remains to determine whether the low amount of mitochondrial HMG-CoA synthase protein results from decreased expression of the gene encoding for this enzyme.

Whatever the cause of the low expression of the mitochondrial HMG-CoA synthase protein, the metabolic consequence is a limited capacity for ketone-body synthesis that in turn could promote the accumulation of acetyl-CoA and/or β-oxidation products, which, through the inhibition of acyl-CoA dehydrogenase activities, would explain the decreased overall rate of fatty acid oxidation in newborn-pig liver mitochondria.

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