Developmental changes in mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene expression in rat liver, intestine and kidney

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The tissue-specific expression of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase gene was studied in 15-day-old suckling rats. The mRNA and protein were present in liver, intestine and kidney, but were absent from brain, heart, skeletal muscles, brown and white adipose tissues. Kidney-cortex mitochondria from suckling rats were able to produce low amounts of ketone bodies from oleate. Hepatic, intestinal and renal HMG-CoA synthase mRNA levels increased slowly during foetal life and markedly after birth. The postnatal increase in liver HMG-CoA synthase mRNA could be due to the increase in plasma glucagon levels, since it rapidly induced the accumulation

of HMG-CoA synthase mRNA in cultured foetal hepatocytes. Hepatic, intestinal and renal HMG-CoA synthase mRNA levels remained elevated throughout the suckling period or in rats weaned on to a high-fat carbohydrate-free diet (HF), but decreased by 50 % in the liver and totally disappeared from the intestine and the kidney of rats weaned on to a high-carbohydrate low-fat diet (HC). When HC-weaned rats were fed on a HF-diet for a week, HMG-CoA synthase mRNA was re-induced in the intestine and the kidney. The role of hormones and nutrients in the regulation of HMG-CoA synthase gene expression is discussed.

INTRODUCTION

Immediately after birth the rat is fed with milk, a high-fat low-carbohydrate diet (Girard et al., 1992). To cover the energy needs of the newborn, fatty acid oxidation develops rapidly after birth in many peripheral tissues (heart, skeletal muscle, lung, kidney cortex, small intestine, brown adipose tissue) and in the liver, where they are used as precursors for ketone-body synthesis (Girard et al., 1992).

The regulation of liver ketone-body synthesis is closely related to the capacity for fatty acid oxidation (Girard et al., 1992), but is also controlled by the activity of mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, a regulatory enzyme of the ketogenic pathway (Quant et al., 1991). The foetal-neonatal transition is associated with an increase in the activity of mitochondrial HMG-CoA synthase (Shah and Bailey, 1977; Ferré et al., 1978; Caswell and Bailey, 1983), but the mechanisms responsible for this increase are still unknown. In the adult rat liver, it has been reported that the increase in mitochondrial HMG-CoA synthase activity during starvation, fat feeding or diabetes (Robinson and Williamson, 1980) was partly due to an increase in the concentration of mRNA encoding this enzyme (Ayté et al., 1990; Casal et al., 1992).

It has also been reported that small-intestinal mucosa of suckling rats had the capacity for ketone-body synthesis (Hahn and Taller, 1987; Hahn et al., 1991), owing to a significant mitochondrial HMG-CoA synthase activity which totally disappears at the time of weaning (Békési and Williamson, 1990). Finally, it has been reported that kidney cortex slices of adult rats possess a low HMG-CoA synthase activity (McGarry and Foster, 1969) and were able to produce acetoacetate from radioactive acetate or oleate (McGarry and Foster, 1969; Weidemann and Krebs, 1969). However, it was suggested that the radioactive acetoacetate produced was due to an exchange of label in the acetyl-CoA pool rather than to a net synthesis (McGarry and Foster, 1969; Robinson and Williamson, 1980).

The aim of this work was to investigate the changes in the hepatic, intestinal and renal mitochondrial HMG-CoA synthase gene expression during the perinatal period and the suckling-weaning transition in the rat.

MATERIALS AND METHODS

Animals

Female Wistar rats bred in our laboratory were housed at 24 °C in individual plastic cages with light from 15:00 to 03:00 h, in order to sample the tissues of the rat between 09:00 and 10:00 h, i.e. during the absorptive period. Newborn rats were allowed to suckle, and then were weaned at 20 days on to a semi-synthetic high-carbohydrate low-fat diet (HC; 72 % carbohydrate, 1 % fat, 27 % protein, in terms of energy) or a high-fat carbohydrate-free diet (HF; < 1 % carbohydrate, 72 % fat, 28 % protein, in terms of energy) as described previously (Coupé et al., 1990).

The studies were performed in 15-, 18-, 19-, 20- and 21-day fetal rats, in 1-, 5-, 10-, 15-, and 20-day-old suckling rats and in 21-, 22-, 25- and 30-day-old weaned rats. The tissue-specific expression of mitochondrial HMG-CoA synthase gene was performed in 15-day-old suckling rats. Intestine was inverted, washed at 37 °C in a saline phosphate buffer (PBS: NaCl 0.13 M; KCl 2.7 mM; Na₂HPO₄ 22 mM; NaH₂PO₄ 1.45 mM; pH 7.6). The renal cortex was roughly separated from the medulla with a razor blade. All the tissues were frozen in liquid nitrogen and stored at –80 °C for subsequent RNA extraction.

Isolation and primary culture of foetal hepatocytes

Hepatocytes from 18-day fetuses were isolated and cultured as described previously (Pégorier et al., 1992). The arginine- and serum-free Minimum Essential medium contained dexamethasone (100 nM) and various concentrations of glucagon. Duplicate dishes (10⁷ cells/75 cm²) were used in all experimental

conditions. The cultures were maintained 2 h at 37 °C in an incubator equilibrated with CO₂/air (1:19).

Isolation of mitochondria and measurement of ketone-body production

Mitochondria were isolated from kidney cortex, liver, scraped jejunum mucosa, white adipose tissue and heart from 15-day-old suckling rats by the differential centrifugation technique previously described (Herbin et al., 1987). The rates of ketone-body production from oleate (0.1 mM) plus carnitine (0.5 mM) by incubated kidney cortex mitochondria have been compared with those found in liver mitochondria incubated as previously described (Escriva et al., 1986).

Extraction and Northern-blot analysis of RNA

Total RNA was extracted either from cultured hepatocytes or from frozen tissues by the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987) and stored at -80 °C. The concentration of RNA was measured from the A_{260} . Northern-blot analysis of total RNA (20 µg) was performed after 1%-agarose-gel electrophoresis in 2.2 M formaldehyde as previously described (Pégorier et al., 1992). Hybridization were performed with the 1.5 kb *KpnI-KpnI* fragment. The full-length cDNA probe for mitochondrial HMG-CoA synthase, pMS1 (Ayté et al., 1990), was provided by Dr. F. G. Hegardt (Barcelona, Spain). The probe was labelled with [³²P]dCTP by using the Multiprime labelling system kit (Amersham International, Amersham, Bucks., UK). Quantifications were performed by scanning densitometry of the autoradiographs.

Western-blot analysis of mitochondrial HMG-CoA synthase

Mitochondrial proteins (75 µg) from kidney cortex, liver, jejunum mucosa, white adipose tissue and heart were submitted to SDS/PAGE (8.5% gel) in reducing conditions (100 mM dithiothreitol). The blot was hybridized with the anti-(ox liver mitochondrial HMG-CoA synthase) antibody (1/500, v/v) as described by Quant et al. (1991). This polyclonal antibody was shown to cross-react with the rat enzyme (Quant et al., 1991).

Analytical methods

Acetoacetate and β-hydroxybutyrate were determined by enzymic methods (Bergmeyer, 1974). Proteins were determined by the method of Lowry et al. (1951) with BSA as standard.

Chemicals

Culture medium and foetal-calf serum were obtained from Eurobio (Paris, France). Dexamethasone was from Merck Sharp and Dohme (Paris, France). Pig glucagon was from Novo-Nordisk (Paris, France). All substrates and enzymes were obtained from Boehringer-Mannheim (Meylan, France). Fatty acid-free BSA, L-carnitine, oleate, antibiotics and reagents for Northern- and Western-blot analysis were purchased from Sigma (St. Louis, MO, U.S.A.).

Statistics

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

RESULTS AND DISCUSSION

Tissue-specific expression of mitochondrial HMG-CoA synthase in 15-day-old suckling rats

In 15-day-old suckling rat mitochondrial HMG-CoA synthase mRNA and protein were found in the liver, in the intestine (with a decreasing mRNA gradient from the duodenum to the colon) and in the kidney (Figure 1). Mitochondrial HMG-CoA synthase mRNA and protein were not found in the brain, heart, hind-limb muscles, brown and white adipose tissues and placenta (Figure 1).

Despite similar levels of HMG-CoA synthase mRNA in the liver and the jejunum of 15-day-old suckling rats, the immunoreactive protein was 70% lower in the jejunum than in the liver (Figure 1b). Similar differences between hepatic and intestinal mRNA and protein levels have been previously reported for phosphoenolpyruvate carboxykinase (Watford and Trato, 1989) and urea-cycle enzymes (Ryall et al., 1985; Wraight et al., 1985). This could be due to the high rate of protein turnover needed for the rapid mucosal cell regeneration. Thus high levels of HMG-CoA synthase mRNA would be required to maintain steady-state levels of HMG-CoA synthase protein. Another possibility would be that the HMG-CoA synthase gene is controlled at a translational level.

A low but significant rate of net ketone-body production was found in kidney cortex mitochondria isolated from 15-day-old suckling rats [2.6 ± 0.3 nmol/min per mg of protein (*n* = 5) versus

Figure 1 Tissue-specific expression of mitochondrial HMG-CoA synthase in 15-day-old suckling rats

(a) Total RNA was extracted from various tissues and analysed by the Northern-blot technique as described in the Materials and methods section. (b) Mitochondrial proteins (75 µg) from liver, jejunum mucosa, kidney cortex, heart and white adipose tissue were size-fractionated and blotted with an anti-(ox liver HMG-CoA synthase) antibody as described in the Materials and methods section. These blots represent three different animals.

Figure 2 Developmental changes in liver mitochondrial HMG-CoA synthase mRNA levels

A representative Northern blot and quantification of liver HMG-CoA synthase mRNA are shown. The reference value (1) is the concentration of mRNA found in the liver of 30-day-old HC-weaned rats. Rats were weaned at 20 days on to either a HC diet or a HF diet. Results, expressed as arbitrary units, are means $\pm$ S.E.M. of five different blots.

14.5 $\pm$ 0.8 for liver mitochondria]. Adult rat kidney mitochondria showed undetectable rates of ketone-body synthesis. These results underlined that, during the suckling period, the kidney cortex was able to perform active ketogenesis through the mitochondrial HMG-CoA pathway. The presence of net ketone-body synthesis in the intestine (Hahn and Taller, 1987; Hahn et al., 1991) and in the kidney (the present work) may supply energy substrates for smooth muscles of intestinal mucosa or for tubular and glomerular cells of developing kidney cortex.

Developmental changes in liver, intestinal and renal mitochondrial HMG-CoA synthase gene expression

The mRNA encoding for mitochondrial HMG-CoA synthase were not detectable in the foetal liver before day 18 of pregnancy. Their concentration increased until term to reach a level similar to that found in fed adult rats (Figure 2). Immediately after birth, the concentration of mitochondrial HMG-CoA synthase mRNA increased markedly in the liver and in the jejunum, and remained elevated during the suckling period (Figures 2 and 3a). In the kidney, HMG-CoA synthase mRNA increased more slowly after birth and reached a maximal level on day 15 (Figure 3b).

The postnatal increase in liver HMG-CoA synthase mRNA was partly controlled by glucagon, as suggested by studies using cultured hepatocytes from 18-day foetal rats. Maximal glucagon concentrations (0.1 μ M) induced in 2 h a 400 $\pm$ 45% (n = 3) increase in HMG-CoA synthase mRNA levels. The concentration of glucagon giving half-maximal effect (75 $\pm$ 18 nM) was close to the concentration of glucagon found in the plasma of newborn

Figure 3 Developmental changes in intestinal and renal mitochondrial HMG-CoA synthase mRNA levels

(a) Representative Northern blot and densitometric quantification of intestinal HMG-CoA synthase mRNA. The reference value (1) is the concentration of mRNA found in the jejunum of 1-day-old suckling rats. For experimental design see Figure 2 legend. In some experiments, newborn rats weaned at 20 days on to a HC diet were refed with a HF diet at 30 days for 1 week. (b) Representative Northern blot and densitometric quantification of renal HMG-CoA synthase mRNA. For experimental design see panel (a). The reference value (1) is the concentration of mRNA found in the kidney of 1-day-old suckling rats. Results, expressed as arbitrary units, are means $\pm$ S.E.M. of four different blots.

rats (Girard et al., 1977). These results are in agreement with studies in which a marked increase in liver mitochondrial HMG-CoA synthase activity (Caswell and Bailey, 1983) was associated with an increase in plasma glucagon and a decrease in plasma insulin in post-mature foetuses (Portha et al., 1976).

When rats are weaned on to a HC diet, mitochondrial HMG-CoA synthase mRNAs decreased by 50% in the liver (Figure 2) and totally disappeared from the jejunum (Figure 3a) and the kidney (Figure 3b), whereas mRNA levels remained elevated in these tissues in HF-weaned rats (Figures 2 and 3). The fact that hepatic, intestinal and renal HMG-CoA synthase mRNA can be maintained in rats weaned on to a HF diet suggests that these

changes are dependent on hormonal and/or nutritional factors, rather than on a precise stage of development. The high plasma glucagon and the low plasma insulin levels that prevailed during the suckling period (Girard et al., 1977) or in rats weaned on to a HF diet (Decaux et al., 1986) could be responsible for the maintenance of high hepatic, intestinal and renal HMG-CoA synthase mRNA levels. It has been reported that injections of insulin or anti-glucagon serum to suckling rats decreased the rates of ketogenesis in the small intestine, whereas anti-insulin or streptozotocin injections have the opposite effects (Hahn et al., 1991). Moreover, intestinal hormones such as vasoactive intestinal peptide that stimulated long-chain fatty acid oxidation in isolated enterocytes of adult rats (Vidal et al., 1989) could be involved in the regulation of intestinal ketogenesis.

The decrease in liver HMG-CoA synthase mRNA when rats were weaned on to a HC diet could be due to the increase in plasma insulin and the fall in plasma glucagon concentrations (Decaux et al., 1986). Indeed, it was demonstrated previously that these hormonal changes were responsible for the rise in the degree of succinylation of the enzyme (Quant et al., 1991) and for the fall in hepatic ketogenesis (Benito et al., 1979).

When 30-day-old rats weaned at 20 days on to the HC diet were fed with a HF diet for 1 week, the mitochondrial HMG-CoA synthase mRNA was re-induced in the jejunum and the kidney (Figure 3). This suggested that HMG-CoA synthase gene expression could also be regulated by the nutrients contained in the diet. For instance, at the time of weaning on to a HC diet, the increased absorption of carbohydrates delivers large amounts of glucose to intestine, liver and kidney. Thus glucose or a metabolite generated by glucose metabolism could decrease the HMG-CoA synthase gene expression and/or enhance the rate of mRNA degradation, as previously reported for L-pyruvate kinase (Decaux et al., 1989), GLUT-2 (Asano et al., 1992) or fatty acid synthase (Foufelle et al., 1992). Conversely, during the suckling period or after weaning on to a HF diet, the absorption of large amounts of fats could promote the transcription and/or the stabilization of hepatic, intestinal or renal HMG-CoA synthase mRNA. Indeed, fatty acids have been shown to stimulate the expression of fatty acid binding protein in adipose cells (Amri et al., 1991) and to inhibit the expression of hepatic fatty acid synthase (Armstrong et al., 1991).

The appearance of HMG-CoA synthase mRNA immediately after birth in three different tissues, the accumulation of HMG-CoA synthase mRNA in response to glucagon in cultured foetal hepatocytes and the re-induction of HMG-CoA synthase mRNA in the intestine and the kidney after refeeding on to a HF diet are rapid phenomena. This suggests that this gene could be controlled at a transcriptional level. However, the mechanisms could be different in the liver and extra-hepatic tissues. For instance, mitochondrial HMG-CoA synthase was expressed earlier in the liver than in extra-hepatic tissues, and increased more slowly in the kidney than in the liver and the intestine. Moreover, mRNAs encoding mitochondrial HMG-CoA synthase were still present in the liver of rats weaned on to a HC-diet, whereas they totally disappeared from extra-hepatic tissues. This suggests that (1) the

stability of mitochondrial HMG-CoA synthase mRNA, (2) the regulatory factors (hormones/substrates) and/or (3) the regulatory region of mitochondrial HMG-CoA synthase gene could be different in these three tissues.

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