

Evidence that the sensitivity of carnitine palmitoyltransferase I to inhibition by malonyl-CoA is an important site of regulation of hepatic fatty acid oxidation in the fetal and newborn rabbit

Perinatal development and effects of pancreatic hormones in cultured rabbit hepatocytes

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The temporal changes in oleate oxidation, lipogenesis, malonyl-CoA concentration and sensitivity of carnitine palmitoyltransferase I (CPT I) to malonyl-CoA inhibition were studied in isolated rabbit hepatocytes and mitochondria as a function of time after birth of the animal or time in culture after exposure to glucagon, cyclic AMP or insulin. (1) Oleate oxidation was very low during the first 6 h after birth, whereas lipogenesis rate and malonyl-CoA concentration decreased rapidly during this period to reach levels as low as those found in 24-h-old newborns that show active oleate oxidation. (2) The changes in the activity of CPT I and the IC_{50} (concn. causing 50% inhibition) for malonyl-CoA paralleled those of oleate oxidation. (3) In cultured fetal hepatocytes, the addition of glucagon or cyclic AMP reproduced the changes that occur spontaneously after birth. A 12 h exposure to glucagon or cyclic AMP was sufficient to inhibit lipogenesis totally and to cause a decrease in malonyl-CoA concentration, but a 24 h exposure was required to induce oleate oxidation. (4) The induction of oleate oxidation by glucagon or cyclic AMP is triggered by the fall in the malonyl-CoA sensitivity of CPT I. (5) In cultured hepatocytes from 24 h-old newborns, the addition of insulin inhibits no more than 30% of the high oleate oxidation, whereas it stimulates lipogenesis and increases malonyl-CoA concentration by 4-fold more than in fetal cells (no oleate oxidation). This poor effect of insulin on oleate oxidation seems to be due to the inability of the hormone to increase the sensitivity of CPT I sufficiently. Altogether, these results suggest that the malonyl-CoA sensitivity of CPT I is the major site of regulation during the induction of fatty acid oxidation in the fetal rabbit liver.

INTRODUCTION

In most mammalian species, a physiological hyperketonaemia develops during the first 24 h after birth [1] as the result of an increased capacity for hepatic fatty acid oxidation [2]. In the newborn rat or rabbit, the development of the capacity for hepatic long-chain fatty acid (LCFA) oxidation seems to be partly regulated by a fall in the malonyl-CoA concentration and by a decrease in the sensitivity of carnitine palmitoyltransferase I (CPT I) to inhibition by malonyl-CoA [3–6]. In the adult rat liver it has been shown that a linear relationship exists between the hepatic malonyl-CoA concentration and the sensitivity of CPT I to malonyl-CoA inhibition in many physiological situations [7]. Although the time course of changes in these parameters is not known, it has been shown that short-term exposure of isolated adult rat hepatocytes to glucagon decreases malonyl-CoA concentration [8–10] and enhances LCFA oxidation [10] in the absence of any change in the sensitivity of CPT I to malonyl-CoA inhibition [11]. This suggests that the variation in the malonyl-CoA concentration is the primary regulatory factor, and that changes in the sensitivity of CPT I to malonyl-CoA may provide a magnifying mechanism in the control of LCFA oxidation in adult rat liver [7,12].

Such a mechanism of regulation can be questioned during the neonatal period or at the suckling–weaning transition in the rat or the rabbit [4–6,13]. In hepatocytes from newborn rats [4,5] and rabbits [6], or from rats weaned on different diets [13], it was shown that LCFA oxidation was mainly regulated through changes in the sensitivity of CPT I to malonyl-CoA inhibition rather than through changes in the malonyl-CoA levels. It has

been shown recently that exposure of cultured fetal rabbit hepatocytes to glucagon or cyclic AMP for 48 h decreased the sensitivity of CPT I to malonyl-CoA inhibition and induced LCFA oxidation [14]. However, the time course of changes in lipogenesis, malonyl-CoA concentration, sensitivity of CPT I to malonyl-CoA inhibition and induction of LCFA oxidation have not previously been determined in either adult or newborn liver.

The aim of the present work was to study the time course of these changes during the first day after birth in the rabbit and to determine, using cultured fetal rabbit hepatocytes, the influence of pancreatic hormones on the development of these changes.

MATERIALS AND METHODS

Animals

Female rabbits of the New Zealand White strain were fed on a pelleted laboratory chow *ad libitum* [33% protein, 55% carbohydrate, 12% fat (of total energy)] and had free access to water. Term in this strain is 32 days *post coitum*. Fetuses were delivered by caesarean section on the morning of day 32 of gestation. Hepatocytes were isolated within 10 min after delivery (term fetus) or at 6, 12, 18 or 24 h after birth in newborns maintained unfed at 36 °C in an humidified incubator (70% relative humidity).

Incubation and monolayer culture of isolated hepatocytes

Hepatocytes from 3–8 animals were isolated simultaneously by the reverse perfusion technique [15] as modified by Pégrier *et al.* [14] for hepatocyte culture.

Abbreviations used: LCFA, long-chain fatty acids; CPT I, carnitine palmitoyltransferase I; MEM, Minimum Essential Hanks Medium; PBS, phosphate-buffered saline; IC_{50} , concn. causing 50% inhibition; Bt₂cAMP, dibutyryl cyclic AMP.

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Incubation. Isolated hepatocytes were incubated in duplicate at 37 °C in 25 ml conical flasks in a final volume of 2 ml of Krebs–Henseleit bicarbonate buffer (pH 7.4) as previously described by Herbin *et al.* [6].

Monolayer culture. Hepatocytes were cultured in 25 cm² flasks (2 × 10⁶ cells) or 75 cm² dishes (10⁷ cells) according to Pégurier *et al.* [14]. After cell attachment (4 h) the medium was replaced by arginine-free and serum-free Minimum Essential Hanks Medium (MEM) containing penicillin (10 units/ml), streptomycin (100 µg/ml), kanamycin (50 µg/ml), amphotericin B (2.5 µg/ml), ornithine (0.4 mM) and dexamethasone (0.1 µM). Duplicate flasks or dishes containing glucagon (1 µM), dibutyryl cyclic AMP (Bt₂cAMP; 0.1 mM) or insulin (0.1 µM), in both the absence and in the presence of theophylline (1 mM) were maintained at 37 °C in an incubator equilibrated with CO₂/air (1:19). The medium was changed every day and the experiments were performed at 12, 24 or 48 h after the beginning of the culture.

Isolation of mitochondria

Whole liver. Liver mitochondria were prepared according to Mersmann *et al.* [16]. Livers from three or four term fetuses or 6, 12, 18 or 24-h-old newborn rabbits were sampled. They were 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, livers were homogenized using two up-and-down strokes of a loose-fitting motor-driven Teflon pestle with a constant speed of 50–60 rev./min. After two centrifugation steps (successively 700 g and 10000 g for 10 min each), the final pellet was resuspended in the isolation buffer at a final concentration of 3 mg of protein/ml and kept at 4 °C.

Hepatocyte culture. Mitochondria were isolated from cultured hepatocytes plated on ten Petri dishes as described previously [14] by using a discontinuous density gradient of iso-osmotic Percoll ($d = 1.044, 1.062$ and 1.095 [17]). The mitochondrial fraction was resuspended in the isolation buffer at a final concentration of 3 mg of protein/ml.

Measurements of fatty acid oxidation, esterification and ketogenesis

In either incubated or cultured hepatocytes, fatty acid metabolism was measured in the presence of carnitine (1 mM) and of a tracer amount of [1-¹⁴C]oleate bound to 2% defatted albumin (Fraction V, fatty-acid free).

Incubation. [1-¹⁴C]Oleate was added to the hepatocyte suspension [(3–5) × 10⁶ cells] after a 30 min preincubation period in the absence or in the presence of lactate (10 mM) plus pyruvate (1 mM), in order to compare the rates of fatty acid oxidation and lipogenesis under the same experimental conditions (see below). Fatty acid metabolism and ketogenesis were determined after an additional incubation of 30 min.

Monolayer culture. After the desired time in culture, [1-¹⁴C]oleate was added to the culture flask. After an additional incubation of 2 h, the culture medium was transferred to 25 ml conical flasks.

For both conditions, i.e. incubation or monolayer culture, the conical flasks were sealed with rubber caps containing plastic centre wells. The incubations were ended by adding 0.25 ml of HClO₄ (40%, v/v) through the rubber cap, and ¹⁴CO₂, acid-soluble products and ketone body production were measured as previously described [18].

The labelled cellular triacylglycerols from incubated hepatocytes [18] or from cultured cells [14] were separated by t.l.c. as described by Duée *et al.* [18].

Measurement of the rate of lipogenesis and malonyl-CoA concentration

Incubation. Hepatocytes [(3–5) × 10⁶] were preincubated for 30 min in the absence or in the presence of lactate (10 mM) plus pyruvate (1 mM). Then, 250 µCi of ³H₂O was added and hepatocytes were incubated for a further 30 min. The incubations were ended by centrifugation for 30 s at 5000 g.

For the determination of malonyl-CoA concentration, hepatocytes [(20–25) × 10⁶] were incubated for 30 min in the presence of lactate (10 mM) plus pyruvate (1 mM). The incubations were ended by adding 25 µl of 70% (v/v) HClO₄.

Monolayer culture. Immediately after hepatocyte isolation or after 12, 24 or 48 h in culture, the monolayer was rinsed twice with phosphate-buffered saline (PBS; 137 mM-NaCl/2.7 mM-KCl/21.8 mM-Na₂HPO₄/1.4 mM-NaH₂PO₄, pH 7.6) and then incubated for 2 h under the same culture conditions as those previously established. Lipogenesis from [U-¹⁴C]acetate (5 mM; 10 µCi/flask) and malonyl-CoA concentration were measured in the absence or in the presence of 5 mM-acetate. Then, the cells from duplicate flasks or dishes were rinsed twice with ice-cold PBS and were scraped off. Malonyl-CoA concentration was determined under the same conditions of culture after the reaction was stopped by adding 200 µl of 70% (v/v) HClO₄.

Labelled fatty acids were extracted from the cell pellet as described by Stansbie *et al.* [19]. Malonyl-CoA was assayed according to McGarry *et al.* [20]. Rat liver fatty acid synthetase was prepared as described by Stoops *et al.* [21].

Determination of the CPT I activity and the sensitivity of CPT I to inhibition by malonyl-CoA

CPT I activity was measured at 30 °C in mitochondria isolated from whole liver or from hepatocyte cultures by a modification of the technique of Bremer [22] as described by Herbin *et al.* [6]. 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 activity of CPT I (IC₅₀). For this purpose, the palmitoyl-CoA concentration was 80 µM and the malonyl-CoA concentration varied between 0.01 and 150 µM. In all experiments ($n = 85$), the formation of palmitoylcarnitine was almost completely suppressed (89 ± 1%) by the highest malonyl-CoA concentration (150 µM). This suggested a good membrane integrity of the mitochondrial preparations and that only CPT I activity was measured without any significant contribution of CPT II.

Analytical methods

Acetoacetate and β-hydroxybutyrate were determined using enzymic methods [23]. Proteins were determined by the method of Lowry *et al.* [24], with bovine serum albumin as standard.

Chemicals

All substrates and cofactors were obtained from Boehringer Corp. (Meylan, France). Fatty-acid-free albumin, L-carnitine, malonyl-CoA, palmitoyl-CoA, antibiotics, dibutyryl cyclic AMP and theophylline were purchased from Sigma (St. Louis, MO, U.S.A.). [1-¹⁴C]oleate, [U-¹⁴C]acetate, ³H₂O, [³H]acetyl-CoA and [³H]carnitine were from Amersham International (Amersham, Bucks., U.K.). Amphotericin B, fetal calf serum and arginine-free MEM were obtained from Eurobio (Paris, France). Dexamethasone was from Merck Sharpe and Dohme (Paris, France); pig glucagon and human insulin were from Novo (Paris, France).

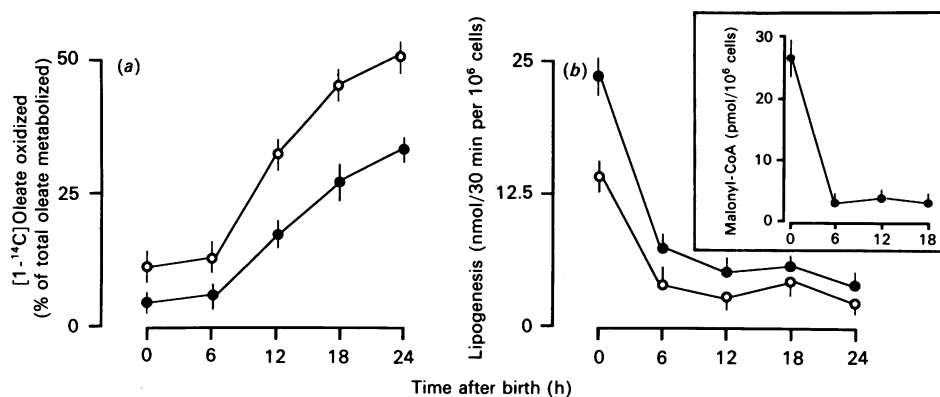


Fig. 1. Time course of oleate oxidation, lipogenesis and malonyl-CoA concentration in isolated hepatocytes during the first day after birth

Isolated hepatocytes were pre-incubated for 30 min in the absence (○) or in the presence (●) of lactate (10 mM) plus pyruvate (1 mM). Then a tracer amount of [1-¹⁴C]oleate, bound to 2% fat-free albumin and in the presence of 1 mM-carnitine, or ³H₂O, was added for 30 min. Oleate oxidation (a), lipogenesis (b) and malonyl-CoA concentration (inset) were determined at the end of this 30 min incubation period. The term 'total oleate metabolized' represents the sum of radioactivity recovered in esterified products and in oxidized products (CO₂ + acid-soluble products). Values are means ± S.E.M. of between five and seven experiments performed in duplicate.

Statistics

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

RESULTS

Experiments were carried out using hepatocytes isolated from term fetal or fasting newborn rabbits. It is noteworthy that in 24-h-old suckling newborns, rates of LCFA oxidation (42 ± 2% of total oleate metabolized) and lipogenesis (3.6 ± 1.1 nmol/30 min per 10⁶ hepatocytes) were similar to those found in 24-h-old fasting newborns (Fig. 1). This suggests that these metabolic changes are not dependent upon the nutritional state of the neonate but are related to the hormonal changes associated with birth (fall in insulin, rise in glucagon) that occurred similarly in suckling or fasted neonates.

Time course of fatty acid metabolism in the rabbit liver *in vivo*

To understand the metabolic changes that occur spontaneously after birth in the liver of the rabbit, oleate oxidation, lipogenesis and malonyl-CoA concentration were measured in isolated hepatocytes during the first 24 h of extra-uterine life.

The amount of [1-¹⁴C]oleate metabolized was similar whatever the conditions of incubation and/or the stage of development [(22.7 ± 1.7) × 10³ d.p.m./30 min per 10⁶ hepatocytes; range (18.7–24.4) × 10³; *n* = 58). This allowed a valid comparison between the different groups studied. As shown in Fig. 1, in both the absence and the presence of lactate plus pyruvate, oleate oxidation, which is very low at birth, increased markedly after a delay of 6 h to reach maximal values at 24 h after birth.

The rates of lipogenesis from endogenous precursors or from lactate/pyruvate decreased rapidly during the first 6 h after birth and then remained low until the end of the first day of life (Fig. 1). The malonyl-CoA concentration followed the same pattern as lipogenesis measured in the presence of lactate/pyruvate (Fig. 1).

The activity of CPT I was low during the first 6 h after birth and then increased to reach a value at 24 h which was twice that observed at birth (Fig. 2). The IC₅₀ followed the same developmental pattern (Fig. 2), except that the IC₅₀ was 17-fold higher in mitochondria isolated 24 h after birth than in mitochondria isolated at birth. Thus there was a marked fall in the

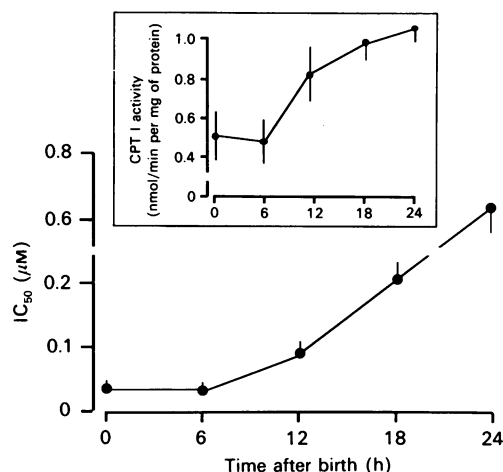


Fig. 2. Temporal changes in the activity and sensitivity of CPT I to malonyl-CoA inhibition during the first 24 h after birth

Rabbit liver mitochondria were isolated at the indicated time after birth. CPT I activity was measured in the presence of 80 μM-palmitoyl-CoA and 1 mM-carnitine. The term IC₅₀ refers to the concentration of malonyl-CoA required for 50% inhibition of CPT I activity. Values are means ± S.E.M. of between four and six determinations.

sensitivity of CPT I to malonyl-CoA inhibition during the first 24 h of life.

Effects of glucagon and cyclic AMP on the metabolic changes occurring during the induction of LCFA oxidation in cultured fetal hepatocytes

Despite the various conditions studied (stage of development, duration of the culture, hormonal treatment, presence or not of acetate), the total amount of [1-¹⁴C]oleate metabolized (oxidized and esterified) was always similar [(31.7 ± 0.4) × 10³ d.p.m./2 h per mg of cell protein; *n* = 137; range (29–33) × 10³]. Moreover, the presence of acetate in the medium did not change the percentage of oleate oxidized or the metabolic fate of oleate between Krebs' cycle and ketone body synthesis (results not shown).

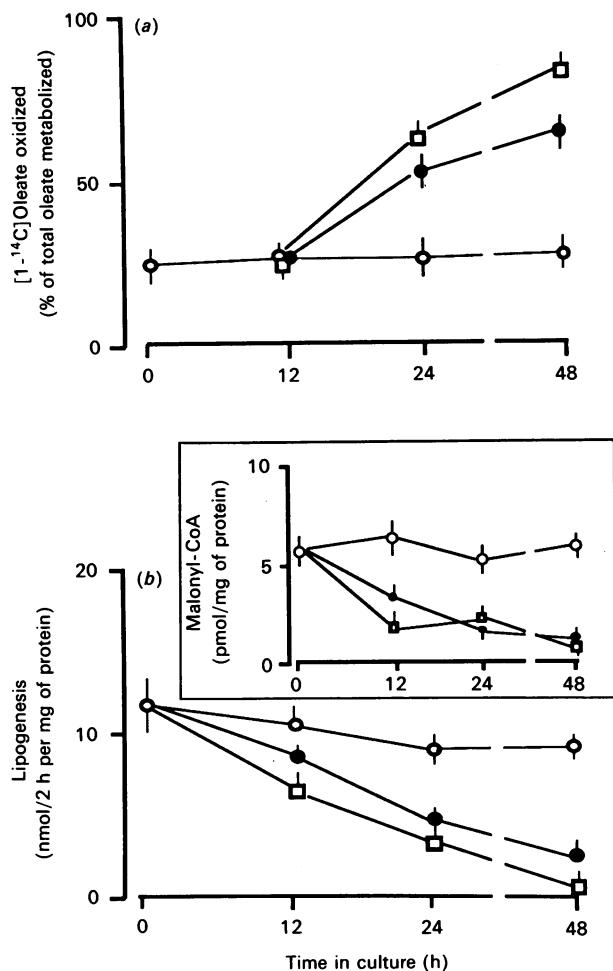


Fig. 3. Effects of glucagon or Bt_2cAMP on oleate oxidation, lipogenesis and malonyl-CoA concentration in fetal rabbit hepatocytes after various times in culture

After cell attachment, hepatocytes from term fetuses were cultured in a serum-free medium for the period of time indicated in the absence (○) or in the presence of glucagon (●, $1 \mu\text{M}$) or Bt_2cAMP (□, 0.1 mM). Oleate oxidation (from tracer amount of $[1-^{14}\text{C}]$ oleate) (a), lipogenesis (from $[U-^{14}\text{C}]$ acetate) (b) and malonyl-CoA concentration (inset) were determined after an additional incubation of 2 h in the presence of sodium acetate (5 mM). Results are means \pm S.E.M. of duplicate flasks from four to six cultures.

In term fetal hepatocytes cultured in basal medium, the percentage of oleate oxidized remained very low during the first 48 h (Fig. 3). Addition of glucagon or cyclic AMP increased oleate oxidation after a delay of at least 12 h. A maximal value was reached after 48 h of exposure to glucagon or cyclic AMP (Fig. 3). In contrast, a 12 h exposure to glucagon or cyclic AMP induced a 20–30% decrease in the rates of lipogenesis (respectively $P < 0.05$ and $P < 0.01$; Fig. 3) which further decreased after 48 h of exposure, whereas the rate of lipogenesis remained high and constant under basal conditions of culture (Fig. 3). The malonyl-CoA concentration followed the same pattern. After 12 h of culture, the inhibitory effects of glucagon or cyclic AMP on malonyl-CoA concentration were more marked than those on the rate of lipogenesis (Fig. 3).

As shown in Fig. 4, the activity of CPT I remained unchanged, whatever the duration and/or the conditions of culture in cultured term fetal hepatocytes. The IC_{50} for malonyl-CoA was extremely low in mitochondria isolated from term fetal hepatocytes cultured under basal conditions (Fig. 4). The ad-

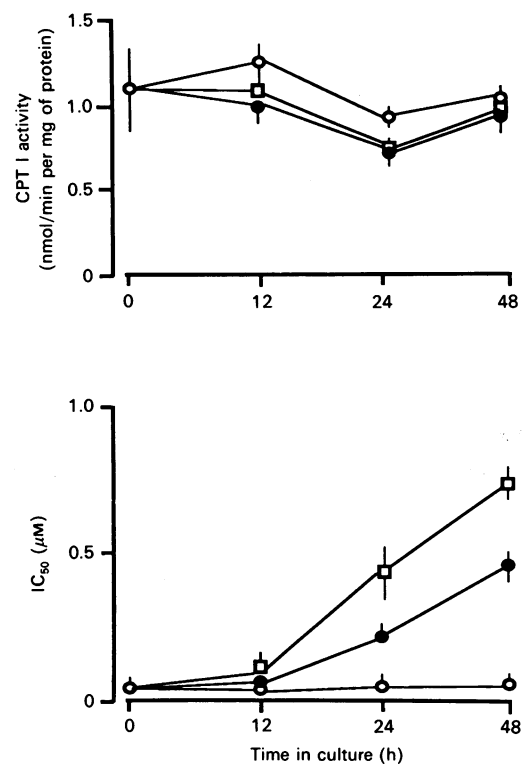


Fig. 4. Effects of glucagon and cyclic AMP on the temporal changes in the activity and sensitivity of CPT I to malonyl-CoA inhibition in cultured fetal hepatocytes

For hormonal concentrations used, see the legend to Fig. 3. After the indicated time in culture in the absence (○) or in the presence of glucagon (●) or Bt_2cAMP (□), the hepatocytes from ten Petri dishes were collected and mitochondria were isolated as described in the Material and methods section. CPT I activity and sensitivity were determined as described in the legend to Fig. 2. Results are means \pm S.E.M. of four to six different cultures.

dition of glucagon or cyclic AMP increased the IC_{50} , after a delay of at least 12 h, before reaching maximal values after 48 h of exposure to each agent (Fig. 4). Thus glucagon or cyclic AMP markedly decreased the sensitivity of CPT I to malonyl-CoA inhibition. It is also noteworthy that the temporal changes in the sensitivity of CPT I to malonyl-CoA inhibition paralleled those of oleate oxidation. Lastly, it must be emphasized that all the metabolic changes induced by glucagon were totally abolished when fetal cells were cultured in the presence of $0.1 \mu\text{M}$ -insulin (results not shown).

Effects of insulin on fatty acid metabolism in cultured hepatocytes from 1-day-old newborns

In hepatocytes from newborn rabbits cultured under basal conditions, the high level of oleate oxidation was maintained during the first 2 days despite 700% increases in the rate of lipogenesis and in the malonyl-CoA concentration (Fig. 5). It is noteworthy that after 24 or 48 h of culture under basal conditions, the rates of lipogenesis and the malonyl-CoA concentration in hepatocytes from newborn rabbits were similar to those observed in hepatocytes from term fetuses, whereas the rate of oleate oxidation was 3–4-fold higher in cultured newborn hepatocytes than in fetal hepatocytes (Fig. 5). This spontaneous increase in the basal rate of lipogenesis in newborn hepatocytes is not completely explained. It could result from a decrease in the cyclic AMP concentration during the 48 h of culture that could be sufficient to affect lipogenesis but not the sensitivity of CPT I

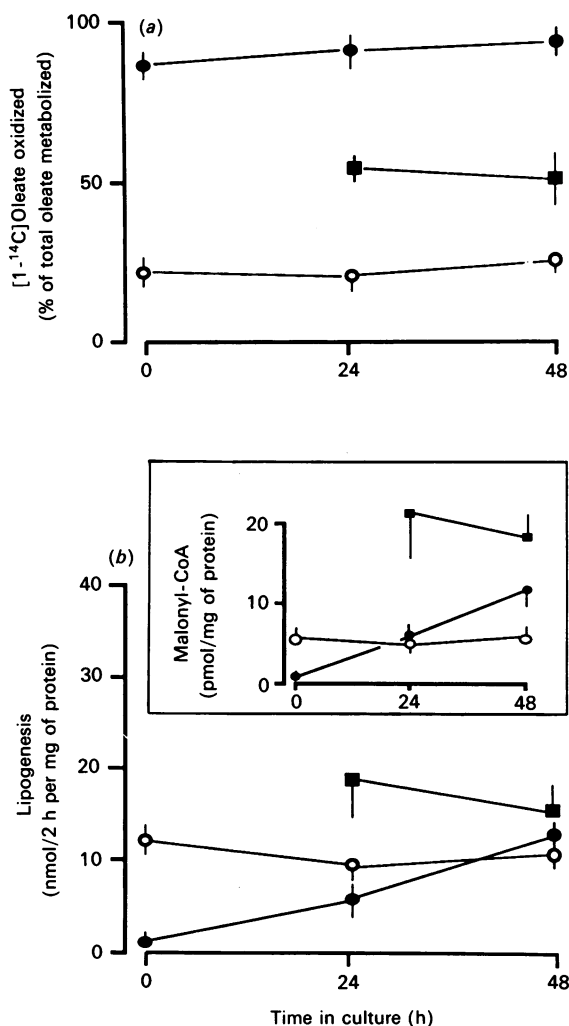


Fig. 5. Effects of insulin on oleate oxidation, lipogenesis and malonyl-CoA concentration in hepatocytes from 24-h-old newborns after various times in culture

For experimental design, see the legend to Fig. 3. The hepatocytes from 24-h-old newborns were cultured in the absence (●) or in the presence of insulin (■, 0.1 μ M). The results were compared with those found in the absence of insulin in cultured fetal hepatocytes (○). Oleate oxidation (a), lipogenesis (b) and malonyl-CoA concentration (inset) were measured as described in the legend to Fig. 3. Values are means \pm S.E.M. of duplicate flasks from four to six different cultures.

or oleate oxidation. Indeed, this increase in lipogenesis was totally blocked when hepatocytes from newborn animals were cultured in the presence of cyclic AMP (results not shown).

Addition of insulin to cultured newborn hepatocytes inhibited no more than 35% of oleate oxidation. The rate of fatty acid oxidation still remained 2-fold higher than in hepatocytes from term fetuses (Fig. 5). This discrete inhibition of oleate oxidation by insulin occurred despite a huge increase in malonyl-CoA concentration and a smaller increase in the rate of lipogenesis (Fig. 5). As previously mentioned for glucagon and cyclic AMP, the presence of insulin did not affect the activity of CPT I in mitochondria isolated from cultured newborn hepatocytes (results not shown). In contrast, insulin decreased the IC_{50} for malonyl-CoA without however reaching the very low level found in cultured fetal cells (Fig. 6). The effects of insulin on the sensitivity of CPT I to malonyl-CoA were similar to those found for oleate oxidation (compare Fig. 5 and 6).

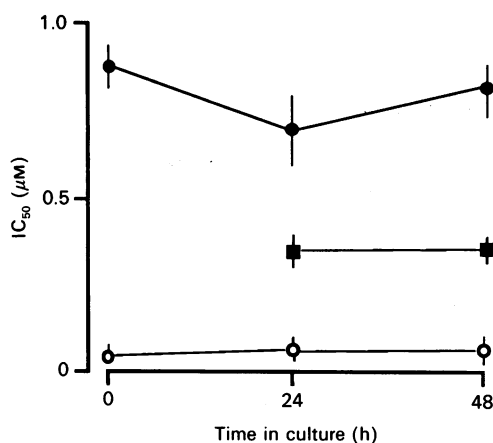


Fig. 6. Effect of insulin on CPT I sensitivity to malonyl-CoA inhibition in cultured newborn hepatocytes

For experimental design, see the legend to Fig. 4. Hepatocytes from newborns were cultured in the absence (●) or in the presence (■) of insulin. Hepatocytes from term fetuses were cultured in the absence of insulin (○). Results are means \pm S.E.M. of four to six different cultures.

DISCUSSION

As pointed out recently by McGarry *et al.* [12], the temporal sequence of changes in LCFA oxidation, malonyl-CoA concentration, lipogenesis, and the kinetic properties of CPT I has never been determined during the short-term fed-starved transition. This work was performed to fill this gap for the fetal-neonatal transition, a physiological situation which is also associated with marked changes in these parameters.

The sequential changes in hepatic fatty acid metabolism that occur spontaneously after birth were first investigated *in vivo*. The emergence of LCFA oxidation occurs after a lag period of 6 h, thus providing an explanation for the low rate of ketone body production observed during the first hours following birth [18]. This delay in the emergence of fatty acid oxidation has been also observed, but to a lesser extent, with octanoate as substrate [25]. Indeed, in term fetal rabbit hepatocytes, 55% of octanoate metabolized was recovered in oxidized products, with the remainder being directly esterified, whereas at 24 h after birth, 90% of octanoate was oxidized [25]. It has been shown that the rates of oxidation of palmitoylcarnitine and octanoylcarnitine were similar in liver rabbit mitochondria isolated at birth or 24 h after birth, whereas the oxidation rates of palmitoyl- and octanoyl-CoA were 50% lower in term fetal mitochondria than in those from newborns [6,25]. This suggests that: (1) the capacity for mitochondrial fatty acid oxidation is not the rate-limiting factor at birth; and (2) the regulation of fatty acyl-CoA oxidation is located at the level of entry into mitochondria, i.e. the carnitine acyltransferase system, which is known to be modulated by malonyl-CoA, at least for long-chain acyl-CoA. The delay in fatty acid oxidation occurs despite rapid falls in the rate of lipogenesis and in the malonyl-CoA concentration that become, 6 h after birth, as low as those found in 24 h-old newborns. This postnatal decrease in the rate of lipogenesis is not due to a reduced availability of lipogenic precursors as a result of a decrease in glycolysis and/or an increase in gluconeogenesis [15,26], because the addition of lactate plus pyruvate to isolated hepatocytes did not prevent the fall in lipogenesis. It is more likely to be the consequence of the decreases in lipogenic enzyme activities that occur after birth [27]. This kinetic study provides evidence showing that the decrease in malonyl-CoA concen-

tration is not sufficient to induce LCFA oxidation during the fetal–neonatal transition.

A similar conclusion has been drawn from data obtained during the suckling–weaning transition [13] or during long-term adaptation to high-fat diets rich in medium-chain triacylglycerol [28]. Other regulatory factors have to be taken into account, as follows. (1) An increase in the activity of CPT I that parallels the increase in LCFA oxidation. Indeed, the 2-fold rise in CPT I activity cannot by itself explain the 8-fold increase in LCFA oxidation. (2) A decrease in the sensitivity of CPT I to malonyl-CoA inhibition. It was recently suggested that the changes in the malonyl-CoA sensitivity of CPT I represents an amplifying system in addition to the effects caused by the decrease in malonyl-CoA concentrations *per se* [7,12]. However, during the fetal–neonatal transition, the fall in the sensitivity of CPT I to malonyl-CoA inhibition seems to be the main regulatory factor in the postnatal development of LCFA oxidation. Whether the fall in malonyl-CoA concentration is a prerequisite for the induction of LCFA oxidation, with the changes in CPT I sensitivity amplifying the fall in malonyl-CoA concentration in newborns, remains to be elucidated.

The role of pancreatic hormones in the induction of hepatic LCFA oxidation in the rabbit was suggested from experiments *in vivo* [29] and recently confirmed in cultured fetal hepatocytes [14]. Indeed, the addition of glucagon or cyclic AMP to cultured fetal hepatocytes reproduces the metabolic changes that occur spontaneously during the first day of extra-uterine life.

There are at least three different conditions of culture that demonstrate the absence of a linear relationship between malonyl-CoA concentration and LCFA oxidation. Firstly, in fetal hepatocytes the induction of LCFA oxidation by glucagon or cyclic AMP occurs after a delay of at least 12 h, at which time the malonyl-CoA concentration is already very low. Secondly, in hepatocytes from 24-h-old newborns cultured for 48 h under basal conditions, a high rate of LCFA oxidation co-exists with a malonyl-CoA concentration as high as that in fetal hepatocytes that have a very low rate of LCFA oxidation. Thirdly, when insulin is added to 24-h-old newborn hepatocytes, it induces a marked increase in lipogenesis and in malonyl-CoA concentration (4 times higher than in fetal cells) but only a 35% decrease in LCFA oxidation, which remains 2-fold higher than the rate observed in term fetal hepatocytes. These results show that: (1) malonyl-CoA concentration *per se* is not the main regulator in the induction of LCFA oxidation, and (2) a low malonyl-CoA concentration is not always required for active LCFA oxidation.

Recent data have shown that short-term exposure (60 min) of hepatocytes to glucagon had marginal [30,31] or no [11] effect on the activity of CPT I. The present work provides evidence that longer exposure (up to 48 h) to glucagon, cyclic AMP or insulin does not affect the CPT I activity. This is in agreement with observations *in vivo* showing the absence of a relationship between CPT I activity and the flux of LCFA oxidation (reviewed in [32]).

In contrast, if short-term exposure to glucagon [30] or cyclic AMP [31] fails to affect the sensitivity of CPT I to malonyl-CoA inhibition, long-term exposure of hepatocytes to pancreatic hormones or cyclic AMP leads to marked changes in kinetic properties of CPT I. This time-dependent effect on the malonyl-CoA sensitivity of CPT I has been observed previously in liver mitochondria isolated from insulin-treated diabetic rats [33,34] or after refeeding starved rats with a high-carbohydrate diet [35].

Although the present work does not provide any molecular explanation of the role of pancreatic hormones in the regulation of the sensitivity of CPT I to malonyl-CoA, the delay required for the induction of these changes probably rules out the possibility that the CPT I is regulated through a phosphorylation–

dephosphorylation mechanism as suggested by Harano *et al.* [36]. Moreover, the fact that pancreatic hormones or cyclic AMP do not affect the activity of CPT I makes unlikely the possibility that the synthesis of a new enzyme occurs, as suggested from experiments with cycloheximide-treated rats during the fed–starved transition [37]. In contrast, if the malonyl-CoA-binding site is located on a protein different from CPT I, as has been suggested [38,39], then it is possible that pancreatic hormones or cyclic AMP could affect the expression of the gene encoding this protein, which in turn could modulate the activity of CPT I. Lastly, the long-term regulation of sensitivity of CPT I to malonyl-CoA by pancreatic hormones could be similar to that described in various physiological situations associated with marked changes in the circulating concentrations of these hormones, such as fed or starved states and diabetic animals. This kind of regulation may include: (1) an alteration in the association of CPT I with the mitochondrial membrane [40,41] and/or with the putative malonyl-CoA-binding regulatory component [42,43], and (2) changes in the composition in the micro-environment of the enzyme [44] and/or in the fluidity of the mitochondrial membrane [45].

In conclusion, this work shows that during the fetal–neonatal transition the hormonal regulation of the induction of LCFA oxidation is tightly associated with changes in the sensitivity of CPT I to malonyl-CoA inhibition, rather than to variations in malonyl-CoA concentration *per se*.

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