Rat Liver Messenger Ribonucleic Acid and Enzyme Activity of 6-Phosphofructo 2-Kinase/Fructose 2,6-Bisphosphatase Impairment during the Late Period of Pregnancy*

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ABSTRACT

Fructose 2,6-bisphosphate concentration, 6-phosphofructo 2-kinase/fructose 2,6-bisphosphatase (PFK-2/FBPase2) activity, and messenger RNA decreased in maternal rat liver during the last days of gestation, and the recovery started after delivery. Phospho(enol)pyruvate carboxykinase activity and messenger RNA increased in contrast to PFK-2 changes. Measurement of the glycolytic capacity in isolated hepatocytes prepared from rats 1 h after parturition showed a low glucose

consumption and an impaired capacity to metabolize glucose. These results stress the relevance of the PFK-2/fructose 2,6-bisphosphate system in the control of the glycolytic flux in liver, and these changes are intended to prevent glucose consumption by maternal liver and contribute to allow gluconeogenesis to proceed at the end of gestation. The physiological basis of this adaptation may lay on the diversion of glucose from maternal to fetal metabolism. (*Endocrinology* 133: 1044–1050, 1993)

DURING the late period of pregnancy important changes occur in liver carbohydrate metabolism manifested in a maternal hypoglycemia, despite the development of different mechanisms devoted to favor glucose production (*i.e.* hyperphagia) and to reduce its consumption (insulin resistance) (1–3). Although the precise hormonal contribution to this adaptive process is not clear, it has been proposed that in this period the high level of circulating catecholamines and PRL, and more precisely the drop in plasma insulin in the last 2 days of gestation, contribute to the increased gluconeogenic capacity of pregnant rats, especially during fasting states (4–6).

One of the control points of the glycolytic/gluconeogenic fluxes is located at the 6-phosphofructo 1-kinase/fructose 1,6-bisphosphatase level and is modulated by the changes in the concentration of fructose 2,6-bisphosphate (Fru-2,6-P₂). In liver, this metabolite is synthesized and degraded by the bifunctional enzyme 6-phosphofructo 2-kinase (PFK-2)/Fru-2,6-P₂, which is under both rapid and long-term dietary and hormonal control (7, 8). Rapid regulation is mediated after activation of cAMP-dependent protein kinase, which through the phosphorylation in a unique Ser residue in the enzyme causes the inhibition of the kinase and the activation of the bisphosphatase activities, resulting in a decrease in Fru-2,6-P₂ concentration (9). Long-term regulation is mediated by the control of PFK-2 gene expression (10), which is decreased in situations such as diabetes, hypothyroidism,

and prolonged starvation (8–11).

In this report we show an important decrease in liver PFK-2 during the late period of pregnancy and discuss the possible physiological relevance of this adaptive response.

Materials and Methods

Chemicals

[α -³²P]deoxycytidine triphosphate, [α -³²P]uridine triphosphate (3000 Ci/mmol), and [U-¹⁴C]glucose (2 mCi/mmol) were from Amersham (Buckinghamshire, UK). Dowex AG1X8 was from Bio-Rad (Richmond, CA). Restriction enzymes were from Boehringer (Mannheim, Germany). Agarose was from Pharmacia (Uppsala, Sweden). Other chemicals and biochemicals were from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany).

Liver preparation

Pregnant albino Wistar rats (300–350 g) fed *ad libitum* on a standard laboratory diet were used. Pregnancy and gestational age were determined by standard criteria (12), and the animals were killed between 0830–0930 h, except for time zero of parturition, in which only animals that delivered between 0800–1030 h were used. To collect the tissue, maternal rat livers were removed and quickly freeze-clamped in liquid N₂. The clamps (30 cm²) were precooled in liquid nitrogen before use. The frozen tissue was crushed with a pestle and stored at –80 C.

Isolation and incubation of hepatocytes

Isolated hepatocytes were prepared by the classic collagenase perfusion method (13). Cell viability was determined by the trypan blue exclusion criteria and was higher than 90%. Hepatocytes (5–7 × 10⁶ cells per flask) were incubated for 15 min at 37 C in 2 ml Krebs-bicarbonate buffer and continuous gassing with carbogen before use. After this incubation period cells were challenged with hormones or incubated with various concentrations of glucose (25 μ Ci/mmol). At the indicated times, aliquots of the cell suspension were collected to measure metabolites and enzyme activities. When the fate of glucose in hepatocyte suspensions was investigated, the cells were preincubated for 5 min

Received November 22, 1992. Accepted February 3, 1993.

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* This work was supported by Grants 91/0229 and 92/0267 from the Fondo de Investigaciones Sanitarias de la Seguridad Social, Spain.

† Supported by a fellowship from the Spanish Ministry of Science and Education.

in the presence of 5 mM glucose and for an additional 10 min in its absence before use.

Preparation of liver homogenates

Livers were homogenized 1/4 (wt/vol) in an Ultra-Turrax homogenizer in a medium containing 20 mM potassium phosphate, 1 mM dithiothreitol, 1 mM EDTA, 50 mM NaF, and 0.5 mM phenylmethanesulfonyl fluoride (pH 7.4 at 4°C). After centrifugation at $100,000 \times g$ for 30 min the supernatant was delipidated through glass-wool filtration and either filtered through Sephadex G-25 (medium) or fractionated with poly(ethyleneglycol) 6000 (PEG), and the 2–15% PEG pellet was resuspended in 0.25 vol homogenization buffer.

Metabolite and enzyme assays

Fru-2,6- P_2 was extracted from cells or liver after homogenization (1/20, wt/vol) with 50 mM NaOH followed by heating at 80°C for 10 min. The metabolite was measured by the activation of the PPi-dependent phosphofructo 1-kinase (14). Lactate was measured enzymically (13) in the hepatocyte suspension after neutralization of perchloric acid extracts (0.5 M final concentration). ^{14}C -Labeled anions were measured in the cell pellets after centrifugation at $80 \times g$ for 2 min and deproteinization with 2 ml ice-cold 0.5 M perchloric acid. After neutralization (pH 8.0) and centrifugation for 10 min in a Eppendorf centrifuge, 1.5 ml of the supernatants were applied to a Dowex AG1X8 (OH^- form) column (2×0.5 cm) equilibrated with 20 mM Tris, pH 8.0, 20 mM NaCl. After washing the column with equilibration buffer until no more radioactivity emerged, the radioactive anions were eluted in 2 ml 0.5 M NaCl, 50 mM HCl, and 1 ml was counted in 10 ml scintillation fluid. PFK-2 was assayed after partial purification with PEG (15). Kinetic changes induced after phosphorylation of the enzyme were assessed by measuring the maximum velocity at pH 8.5 and pH 6.6 as described by Martin-Sanz *et al.* (13) and Bartrons *et al.* (15). Both phosphorylated and dephosphorylated enzymes are active when assayed at pH 8.5 (total activity), whereas at pH 6.6 (active form), only the dephosphorylated enzyme retains its full catalytic activity. The total activity was measured at pH 8.5 in the presence of 5 mM fructose 6-phosphate (Fru 6-phosphate), 5 mM MgATP, and 1 mM potassium phosphate. The active form of PFK-2 was assayed at pH 6.6 in the presence of 1 mM Fru 6-phosphate, 5 mM MgATP and 1 mM potassium phosphate. Fru 6-phosphate was in a 1:3 ratio with glucose 6-phosphate (glc 6-phosphate). One unit of PFK-2 was defined as forming 1 pmol Fru-2,6- P_2 /min at 30°C. Other enzyme activities were measured in homogenate supernatants ($100,000 \times g$ for 30 min) after sephadex G-25 filtration: glucokinase was assayed using 10 mM [U - ^{14}C]glucose (1 μ Ci) in the presence or absence of 1 mM glc 6-phosphate. The reaction was stopped by spotting 25 μ l reaction mixture to a P81 paper filter (Whatman, Clifton, NJ) and was processed as described by Van Schaftingen (16). Pyruvate kinase was assayed at pH 7.4 in the presence of 2 mM MgADP and 2 mM phospho(enol)pyruvate (17). Phosphoenolpyruvate carboxykinase (PEPCK) was measured with 5 mM pyruvate as described by Ballard and Hanson (18). Protein was measured by the method of Bradford (19) using BSA as standard.

RNA analysis and DNA hybridization probes

Total RNA was extracted by the guanidinium thiocyanate method (20). Aliquots of RNA (30 μ g) were denatured at 65°C for 15 min in 5% formaldehyde, 50% formamide, and 8% glycerol, and then were size-separated by electrophoresis in a 0.9% agarose gel containing 2% formaldehyde and 3-(*N*-morpholino)propanesulfonic acid buffering system (21). After transference of the RNA to Nytran membranes (NY 13-N; Schleicher & Schuell, Keene, NH) with $10 \times$ SSC ($10 \times$ SSC is 1.5 M NaCl, 0.3 M sodium citrate, pH 7.4), the membranes were prehybridized for 6 h at 42°C in 50% formamide, 0.25 M NaCl, 0.1 M sodium phosphate, 7% sodium dodecyl sulfate and 0.01% salmon sperm. The PFK-2/FBPase-2 messenger RNA (mRNA) level was detected with a 1.4-kilobase (kb) EcoRI fragment isolated from the complementary DNA (cDNA) of PFK-2/FBPase-2 (8), labeled (37% efficiency) with [α - ^{32}P] deoxycytidine triphosphate using the random-primed labeling kit (Boehringer). Northern blot analysis of PEPCK RNA was carried out using a

2.4-kb PstI probe isolated from a pBR322 vector (kindly supplied by Dr. J. P. Garcia-Ruiz, Autonomous University, Madrid, Spain). The membranes were washed with $0.1 \times$ SSC and 0.1% sodium dodecyl sulfate at room temperature for 10 min and twice at 50°C for 30 min, followed by exposure to x-ray film (Kodak X-OMAT; Eastman Kodak Co., Rochester, NY). Quantification of the films was performed by laser densitometry (Molecular Dynamics, Sunnyvale, CA) using the hybridization with a β -actin probe (0.6-kb EcoRI/HindIII fragment isolated from the VC 18 vector) as an internal standard.

Data analysis

All experiments were carried out by assaying the samples per duplicate or triplicate. Statistical analysis was performed by the Student's *t* test for paired data (parametric test) and by the Wilcoxon test (nonparametric values). The statistical significance of differences between pregnant and virgin rats was evaluated by the U of Mann-Whitney test (22). The significance has been considered at $P < 0.05$.

Results

Maternal liver PFK-2 activity and Fru-2,6- P_2 concentration are decreased around the time of delivery

The profile of maternal liver PFK-2 and Fru-2,6- P_2 revealed important changes during the last days of pregnancy. As Fig. 1A shows, a decrease in the maximal activity of PFK-2 (measured at pH 8.5) is observed. The lowest activity was detected around delivery and represented a 53% of decrease in the specific activity when compared to that of 3-month-old virgin rats (52 ± 8 U/mg protein). The so-called active form of the enzyme (assayed at pH 6.6) was also measured in the same fractions and represents the activity of the dephosphorylated enzyme (15, 13). This activity, measured at pH 6.6, was also minimal (38% of the total activity) during delivery, implying an increase in PFK-2 phosphorylation by cAMP-dependent protein kinase during this period. The activity ratio at pH 8.5/pH 6.6 reflects the phosphorylation state of the enzyme and increases from 1.7 (day 19 of gestation) to 2.7 on the parturition day, returning to basal values at days 1–2 after labor (Fig. 1B). Interesting enough is the profile of liver Fru-2,6- P_2 content (Fig. 1B) that progressively decreases during the last 3 days of gestation (87% decrease at delivery when referred to 3-month-old virgin rats), evolving in parallel with the inactivation of PFK-2 by phosphorylation. The liver Fru-2,6- P_2 content at days 17 and 18 of gestation was similar to that measured at day 19 (not shown).

In addition to PFK-2, the activities of glucokinase, pyruvate kinase, and PEPCK were also measured throughout this period as markers of relevant enzymes controlling the glycolytic/gluconeogenic fluxes in liver (Fig. 2). All these activities were assayed in parallel and were referred to those of 3-month-old virgin rats. Whereas pyruvate kinase remained unchanged along this period, an increased activity of PEPCK (283%) and a decreased activity of glucokinase (50%) were observed before parturition. Indeed, the inactivation of PFK-2 activity and the probable fall in glc 6-phosphate concentration as the result of the decreased glucokinase activity may be related to the low concentration of Fru-2,6- P_2 found in maternal liver during the last 2 days of pregnancy.

FIG. 1. PFK-2 activity and Fru-2,6-P₂ concentration in maternal liver. A, Livers (three or four animals) were obtained at the indicated times, and the activity of PFK-2 was assayed at pH 6.6 (Δ) and pH 8.5 ($\blacktriangle$). B, The ratio of the activities assayed at pH 8.5/6.6 ($\blacktriangle$) and the concentration of Fru-2,6-P₂ ($\circ$) were measured. Data are means $\pm$ SEM of the samples processed. *, $P < 0.05$; **, $P < 0.01$, maternal vs. 90-day-old virgin rats.

FIG. 2. Enzyme activities profile in maternal liver. Livers (three or four animals) were homogenized, and the activities of pyruvate kinase ($\blacktriangle$), glucokinase ($\circ$), and PEPCK (Δ) were measured as described in *Materials and Methods*. Glucokinase was measured in the presence of 1 mM glc 6-phosphate. Results were expressed as the percentage of the activity corresponding to 90-day-old virgin rats fed *ad libitum*, and the enzyme activities in these animals were 1.5, 150, and 31 mU/mg protein for glucokinase, pyruvate kinase, and PEPCK, respectively. Results are the means $\pm$ SEM of at least three different animals. **, $P < 0.01$, maternal vs. 90-day-old virgin rats.

PFK-2/Fru-2,6-P₂ mRNA parallels PFK-2 activity

To determine whether the abundance of PFK-2/FBPase-2 mRNA correlates with the enzyme activity, RNA from maternal liver was prepared for Northern blot analysis using a 1.4-kb cDNA probe. As shown in Fig. 3A, the PFK-2/FBPase-2 mRNA content during the last 3 days of pregnancy decreased to 10% of the content in control animals (90-day-old virgin rats). The recovery of mRNA levels started 1 day after delivery, exhibiting a good parallelism with the PFK-2 activity and Fru-2,6-P₂ concentration profiles (Figs. 1 and 3B). In contrast to the fall in PFK-2 mRNA, an increase in PEPCK

mRNA was observed around delivery, exhibiting a peak value at parturition (8-fold increase over the value of 3-month-old virgin rats). Again, there is a good correlation between the increase in PEPCK activity and in mRNA content along this period. In addition, Northern blot analysis of PEPCK was done in the same membranes used for PFK-2/FBPase-2 analysis and therefore constituted an internal control for the evolution of both mRNA in these animals.

Maternal liver contains the adult hepatic form of PFK-2

The maternal liver profile of PEPCK and PFK-2 observed around parturition strongly resembles the expression pattern of these enzymes in regenerating liver (23, 24), and in this situation the presence of a PFK-2 isoenzyme distinct to that prevailing in adult liver is suggested. One characteristic of this form, shared in common with the enzyme expressed in fetal liver (25, 26), is the loss of inhibition of PFK-2 after phosphorylation by cAMP-dependent protein kinase. To test this possibility, maternal hepatocytes were prepared from animals 1 h after parturition, and the effect of glucagon on both the enzyme activity and the Fru-2,6-P₂ content was assayed. As shown in Fig. 4A, the ratio of the activities of PFK-2 assayed at pH 8.5 and pH 6.6 increased after exposure of the cells to glucagon. The activity assayed at pH 6.6 decreased, whereas the activity at pH 8.5 remained unchanged, suggesting a phosphorylation-dependent inactivation of the enzyme (7, 27). Parallel to this inhibition, an important and rapid decrease in the concentration of Fru-2,6-P₂ was observed after incubation of the cells with 100 nM glucagon (Fig. 4B). This important decrease in Fru-2,6-P₂ is an additional proof reinforcing the presence of the adult hepatic form of PFK-2 in maternal liver. Ancillary information obtained from this experiment is the great relevance of the hormonal situation during pregnancy on the control of Fru-2,6-P₂ levels; the Fru-2,6-P₂ content in animals around parturition is almost negligible (14 pmol/mg protein); however, after isolation and incubation of the hepatocytes in the absence of the maternal hormonal ambience, an important deinhibition of PFK-2 occurs, resulting in an increase in Fru-

FIG. 3. Northern blot analysis of PFK-2 and PEPCK mRNA. Total RNA (30 μ g per lane) was extracted from maternal rat livers (three or four animals) and processed by Northern blot. A, Representative experiment (of three) using a cDNA for PFK-2 or PEPCK. Both blots were performed in the same membrane. B, Densitometric scanning of autoradiograms corresponding to PFK-2 (●) and PEPCK (○) were corrected for the amount of RNA corresponding to β -actin mRNA and were matched for normalization with the values of PFK-2 and PEPCK RNA of virgin rats 90 days old, which were considered 100%.

2,6- P_2 concentration (43 pmol/mg protein) and in a decrease in the ratio of PFK-2 activity assayed at pH 8.5 and pH 6.6 (Figs. 1 and 4).

Glucose sparing in maternal liver

To evaluate the physiological role of the changes in the enzyme activities relevant for the control of the glycolytic/gluconeogenic fluxes, the fate of glucose was investigated in hepatocyte suspensions obtained from animals 1 h after parturition. As Fig. 5 shows, maternal hepatocytes challenged with increasing concentrations of glucose exhibited a very low glycolytic flux (measured as lactate release) when compared with hepatocytes obtained from 90-day-old virgin rats. At physiological glucose concentrations (5–6 mM), the rate of lactate production by maternal hepatocytes was only 30% of that of control cells. Moreover, the analysis of the content of charged molecules after incubation with [U - 14 C]glucose re-

vealed an important fall in charged species that roughly corresponds to a concomitant decrease in glucose consumption (see lactate release under these conditions). This behavior was observed even at glucose concentrations higher than 5 mM, a situation in which important glycolysis occurs (28). Parallel results were observed when the concentration of Fru-2,6- P_2 was measured in these conditions. Taken together, these results stress the physiological relevance of the decrease in Fru-2,6- P_2 concentration and in the total PFK-2 activity in the control of the glycolytic flux in maternal liver. Moreover, the decrease in PFK-2 activity is reinforced by the phosphorylation-induced inactivation of the enzyme in this physiological situation.

Discussion

The studies on the physiological adaptations elicited during the late period of pregnancy in mammals have stressed the relevance of the rapid and specific hormonal changes and the occurrence of an unusual substrate metabolism in this situation (29). A major advance in the understanding of the fetal role in maternal homeostasis was the recognition of the central role played by glucose in the maternal/fetal substrate exchange, higher than for any of the substrates so far analyzed (30). This is the result of the high selectivity of the placental barrier for the exchange of hormones and substrates, allowing a clear cut-off between maternal and fetal circulations. As a consequence of the high glucose requirements for fetal development, a series of changes in the maternal metabolism have been accomplished and are intended to preserve glucose consumption and to favor the use of other alternative fuels, among them triglyceride consumption by liver, enhanced lipolysis, and an increase in the circulating ketone bodies (29, 30). An important issue that summarizes the maternal situation during pregnancy is the concept launched by Freinkel (31) of "accelerated starvation," as depicting the adaptive metabolic changes occurring at the end of pregnancy. The high flux of glucose from the maternal to fetal circulation is due to the high structural and energetic requirements of the fetus for growth, to the absence of an efficient amino acid transport system, and to the placental impermeability to glycerolipids and fatty acid traffic (29–32). Indeed, this central role played by glucose for fetal metabolism is reinforced by the fact that fetal liver lacks the ability to produce glucose from any of the substrates that may cross the placental barrier (33, 34).

In agreement with this preservation of glucose for fetal metabolism, and therefore, a decrease in maternal glycolytic flux, our results clearly show a transient drop in Fru-2,6- P_2 concentration and in mRNA and enzyme activity of PFK-2 in the last 3–4 days of gestation, evolving in parallel. A rapid recovery started immediately after delivery. The fall in Fru-2,6- P_2 during the last day of gestation is impressive. Moreover, due to the low specific activity of fructose 1,6-bisphosphatase and its relatively high affinity for Fru-2,6- P_2 , it may be expected a very low free concentration of this metabolite (less than 14 pmol/mg protein) (28), and therefore, the glycolytic flux through 6-phosphofructo 1-kinase would be minimal (27, 28).

FIG. 4. Effect of glucagon on PFK-2 activity and on Fru-2,6-P₂ concentration in isolated maternal hepatocytes. Hepatocytes were prepared from rats 1 h after parturition and were incubated in the absence (○) or presence of 100 nM glucagon (●). The activity of PFK-2 was assayed at pH 8.5 and 6.6 and expressed as activity ratio (A). The corresponding Fru-2,6-P₂ concentration was measured (B). Results are the means $\pm$ SEM of three independent hepatocyte preparations. *, $P < 0.001$ glucagon vs. none.

FIG. 5. Glycolytic flux in isolated hepatocytes from maternal rats. Hepatocytes were isolated from rats 1 h after delivery (closed symbols) or from control virgin rats aged 3 months (open symbols). The hepatocyte suspension was allowed to incubate for 15 min in the absence of glucose, and cells were challenged with the indicated concentrations of [U-¹⁴C]glucose (25 μ Ci/mmol). Samples were collected after 15 min of incubation for the measurement of Fru-2,6-P₂, and after 30 min of incubation for the determination of the anion content after Dowex AG1X8 (OH⁻ form) chromatography. Samples intended for the measurement of lactate production (1.5 ml cell suspension) were collected after 30 min of incubation and were deproteinized with perchloric acid. Lactate was measured enzymically. Results are means of two independent cell preparations.

To understand the role of the changes in the PFK-2/FBPase-2 system in the pregnant rat, it is of interest to compare this adaptive response with those physiological and pathological situations in which an important decrease in the activity of PFK-2 occurs; prolonged fasting, diabetes, or partial hepatectomy (8, 23, 35, 36) produce an important drop in Fru-2,6-P₂ concentration, PFK-2 activity, and in some cases, a decrease in the mRNA content. Concerning the levels of PFK-2/FBPase-2 mRNA detected in maternal liver, it is noteworthy that a similar pattern is observed after partial liver hepatectomy (23), with a transient (hours) decrease in mRNA concentration and an important drop in Fru-2,6-P₂

concentration (24). However, a major difference exists between regenerating and maternal liver before labor: whereas maternal PFK-2 activity retains its capacity to be inhibited after treatment of the hepatocytes with glucagon, this effect is apparently lost in regenerating liver after hepatectomy (23, 24).

The hormonal contribution to this fall in the maternal hepatic PFK-2/FBPase-2 system and in the increase in PEPCK activity and mRNA remains to be established, but three important components for this behavior may be envisioned: 1) The hepatic insulin resistance described at the end of gestation, probably due to an impaired tyrosine kinase activity of the receptor (29, 30, 37). In fact, the plasma levels of insulin increase 3-fold at day 20 of gestation (38), reaching the value of 3-month-old virgin rats the last day of pregnancy. The development of a hepatic insulin resistance may help both the maintenance of the phosphorylated state of PFK-2 via its stimulatory effects on the serine/threonine protein phosphatase activity (39) and a decrease in the PFK-2/FBPase-2 mRNA levels through a mechanism similar to that described in diabetic animals by other authors, although this aspect of PFK-2 regulation is controversial (35, 36). 2) Hormones increasing cAMP levels such as glucagon may promote a situation similar to that observed during fasting (8, 23). In agreement with this suggestion, treatment of hepatocytes with glucagon produced a rapid decrease in mRNA of PFK-2/FBPase-2 (our unpublished results). In the context of the hormonal situation at the end of the pregnancy, more relevant than glucagon is probably PRL. The levels of PRL are increased during the last days of pregnancy and maintained along lactation. Although in liver a PRL-dependent cAMP increase is well documented, the complexity of the PRL membrane receptor system suggests the existence of a highly regulated action for this hormone (40, 41). 3) Catecholamines are also increased, and they may contribute to the augmented gluconeogenic activity in the late period of pregnancy (30). It is noteworthy that the changes in PEPCK activity occur at the transcriptional level, in contrast with the situation of PFK-2/FBPase-2 (42). Little is known about the proteins involved in the stabilization of mRNA,

although the so-called poly(A)-binding proteins have been proposed as protectors against degradation (43). In addition to this, many mRNAs are stabilized in the presence of protein synthesis inhibitors (as is the case for the fetal hepatic form of PFK-2), suggesting the involvement of short-lived proteins in the mRNA degradation system. In conclusion, it is possible that the specific profile of PFK-2/FBPase-2 changes may be the result of different hormonal and metabolic contributions that converge in a common mechanism intended to promote a decreased glycolytic capacity in liver at the end of gestation.

Concerning PEPCK, our results show a shift between the PEPCK mRNA levels and enzyme activity during the last days of pregnancy that may be attributed to several factors. One of the relevant signals in the control of PEPCK expression is cAMP that also stabilizes the mRNA against degradation (44). Moreover, in these *in vivo* experiments in which multiple hormones affect the expression of PEPCK at different levels, it is difficult to determine the exact contribution of different hormones to the mRNA and enzyme activity profiles observed during the last days of gestation. However, this is not the case with PFK-2, in which an acceptable parallelism between enzyme activity and mRNA levels is observed around delivery.

A further point of interest is the observation that maternal and neonatal changes concerning the expression of glycolytic/gluconeogenic enzymes are quite different. After birth, the neonatal hypoglycemia after glycogen depletion triggers the expression of PEPCK (6) and promotes the switch of PFK-2 from the fetal form to the liver-type isoenzyme following a time course similar to that of PEPCK (25, 26), the process being completed *in vivo* approximately 12 h after birth. In contrast to this situation, maternal PEPCK and PFK-2 exhibit opposite behavior along the perinatal period. Maternal PFK-2/FBPase-2 mRNA decreases by the effect of glucagon, whereas fetal PFK-2 mRNA is increased by this hormone (our unpublished results).

The observation that at delivery the total amount of PFK-2 and mRNA are decreased by about 55 and 90%, respectively, and that the enzyme is inactivated by phosphorylation (activity at pH 6.6), producing an 85% decrease in Fru-2,6-P₂ concentration, reinforces the relevance of this system in the metabolic control to of the glycolytic flux in this period, and in so doing diverts the glucose fate from maternal to fetal metabolism. Accordingly, maternal hepatocytes exhibited a very low glycolytic capacity when compared with the virgin counterparts. Taken together, these results stress the relevance of a drastic drop in the concentration of Fru-2,6-P₂ in the inhibition of glucose consumption, and through this mechanism this metabolite facilitates the process of gluconeogenesis.

Acknowledgments

The authors thank Dr. A. Tauler for generous gift of the cDNA PFK-2 probe and E. Lundin for help in the preparation of the manuscript.

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