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Gene expression of regulatory enzymes of glycolysis/gluconeogenesis in regenerating rat liver

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Levels of mRNA for glucokinase, L-pyruvate kinase, fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase were analysed during liver regeneration. Levels of mRNA for glycolytic enzymes (glucokinase and L-pyruvate kinase) decreased rapidly after partial hepatectomy. Glucokinase mRNA increased at 16–24 h, returning to normal values after this time. L-pyruvate kinase mRNA recovered control levels at 168 h. In contrast, phosphoenolpyruvate carboxykinase mRNA increased rapidly after liver resection and remained high during the regenerative process. However, the levels of fructose-1,6-bisphosphatase mRNA were not modified significantly. These results correlate with the reported increased rate of gluconeogenesis and changes in enzyme levels after partial hepatectomy. The effect of stress on the mRNA levels was also studied. All enzymes showed variations in their mRNA levels after the surgical stress. In general, the differences were more pronounced in regenerating liver than in sham-operated animals, being practically normalized at 24 h.

INTRODUCTION

One of the most important functions of the liver is the maintenance of glucose homoeostasis. This mechanism operates in different physiological conditions [1,2] even after a drastic decrease in the liver mass [3–5]. Indeed, after partial hepatectomy the gluconeogenic capacity of the liver remnant increases. This increase is more acute during the first hours of liver regeneration, and it remains high until the initial cellular mass is restored. It has been reported that there is an increase of over 200% in the gluconeogenic flux from lactate, and a decrease to less than 20% in the glycolytic flux from glucose in hepatocyte suspensions of regenerating liver [3]. In addition, partial hepatectomy produces metabolic changes in the liver remnant, which culminate in coordinate waves of DNA synthesis and mitosis. Therefore, cell proliferation and gluconeogenic flux must take place in a synchronous manner.

The regulation of the hepatic gluconeogenic pathway is brought about by phosphorylation/dephosphorylation and control of gene expression of several key regulatory enzymes. These enzymes control hepatic glucose production and utilization through regulation of three major substrate cycles: glucose/glucose 6-phosphate, fructose 6-phosphate/fructose 1,6-bisphosphate and phosphoenolpyruvate/pyruvate. The fructose 6-phosphate/fructose 1,6-bisphosphate substrate cycle is also regulated by a subcycle in which the amount of the regulatory molecule Fru-2,6-P_2 is controlled by the bifunctional enzyme PFK-2/FBPase-2 [1,2,6,7]. Recently, changes have been reported in Fru-2,6-P_2 concentration, PFK-2 activity [5] and PFK-2/FBPase-2 mRNA levels [8] during liver regeneration. No results have been reported for the levels of mRNA for other glycolytic/gluconeogenic enzymes such as L-PK, GK and FBPase-1, and the results for PEPCK are contradictory [9,10]. The aim of the present paper was to study the changes in the mRNA levels of these enzymes during liver regeneration.

EXPERIMENTAL

Chemicals

[α-^{32}P]dCTP (3000 Ci/mmol) was from Amersham. The random-primer DNA-labelling kit and restriction endonucleases were from Boehringer Mannheim. GeneScreen and N-Hybond membranes were products of Du Pont–New England Nuclear and Amersham respectively. Other biochemical reagents were from either Boehringer Mannheim or Sigma. All chemicals were of analytical grade.

Animals

Fed male Sprague–Dawley rats (180–220 g) were subjected to a 12 h-light/12 h-dark cycle (light periods starting at 08:00 h). To minimize the diurnal variation in liver DNA synthesis [11], partial hepatectomy (comprising laparotomy and removal of two-thirds of the liver) or sham operation (laparotomy) was performed between 08:00 and 10:00 h, under diethyl ether anaesthesia, by the procedure described by Higgins & Anderson [12]. Control rats (0 h) were not subjected to either anaesthesia or surgery. The animals were killed by decapitation and the livers removed and immediately freeze-clamped in liquid nitrogen.

RNA analyses and DNA-hybridization probes

Total RNA was extracted from frozen rat tissues by the LiCl/urea method [13]. Northern-blot analyses was performed by standard procedures [14]. The following fragments were used as probes: a ~ 1.8 kb *Pst*I fragment from cDNA clone (G4) for L-PK [15]; a ~ 0.65 kb *Eco*RI fragment from cDNA for FBPase-1 [16]; a ~ 2.4 kb *Eco*RI fragment from cDNA for GK [17]; a ~ 2.6 kb *Pst*I fragment from cDNA clone (pPCK10) for PEPCK [18]; and a ~ 1.1 kb *Pst*I fragment from cDNA clone (pRSA13) for rat serum albumin [19]. All DNA probes were generated by labelling with [α-^{32}P]dCTP to a specific radioactivity of ~ 1.5×10^9 c.p.m./μg of DNA by random priming with

Abbreviations used: GK, glucokinase; L-PK, liver form of pyruvate kinase; FBPase-1, fructose-1,6-bisphosphatase; PFK-2/FBPase-2, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PEPCK, phosphoenolpyruvate carboxykinase; Fru-2,6-P_2, fructose 2,6-bisphosphate.

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Klenow DNA polymerase. The levels of these mRNAs were measured by densitometric scanning of the autoradiograms with an LKB Ultroscan XL laser densitometer and GelScan XL (2.1) software, and corrected for the amount of albumin mRNA that was used as control [20,21]. Statistical analysis was carried out by Student's t test, with significance level chosen as $P < 0.05$.

RESULTS

In order to determine whether the changes in enzyme activities, previously reported [3,5,22,23], are correlated with mRNA levels, Northern-blot analyses were performed with RNA extracted from regenerating liver. Values were corrected for the amount of albumin mRNA that was used as control [20,21]. As shown in Figs. 1 and 2, the levels of GK and L-PK mRNA decreased transiently to about 20 and 50% of control values respectively within the first 6 h. At 24 h, the levels of GK mRNA returned to approximately normal values. The levels of L-PK mRNA remained low during the first 96 h and returned to the normal values at 168 h. These profiles are concordant with the decrease in total GK and L-PK activities described during liver regeneration [5,22,23]. The L-PK cDNA used as probe showed cross-hybridization with mRNAs of different sizes (3.2, 2.2 and 2.0 kb) [15].

In contrast with the behaviour of glycolytic enzymes described above, the PEPCK mRNA levels increased 13-fold over control at 6 h after hepatectomy. Afterwards, a decrease was observed until 24 h, and other peaks of mRNA accumulation occurred at 36 h and at 72 h after liver resection. These results agree with the changes in transcription rates previously reported for this enzymes [8]. In addition, the increase in the mRNA levels would explain the increase in PEPCK activity observed [3]. The FBPase-1 mRNA levels were not modified, which would also explain the unchanging activity reported [3].

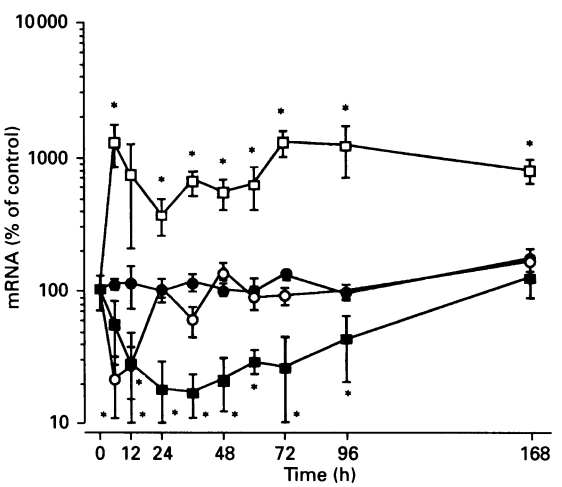

Fig. 1. Gene expression of regulatory enzymes of glycolysis/gluconeogenesis during liver regeneration

Samples of total RNA (20 μg/lane) extracted from normal (0 h) and regenerating rat livers at the indicated times were transferred to nylon membranes after electrophoresis in 1.5% agarose and hybridized with GK (○), L-PK (■), FBPase-1 (●), PEPCK (□) and albumin (control) cDNAs as described in the Experimental section. The levels of these mRNAs were measured by densitometric scanning of the autoradiograms and corrected for the amount of albumin mRNA. The values represent means ± S.E.M. of 3–4 different animals: * significantly different from control rats. Representative Northern blots are shown in Figs. 2 and 3.

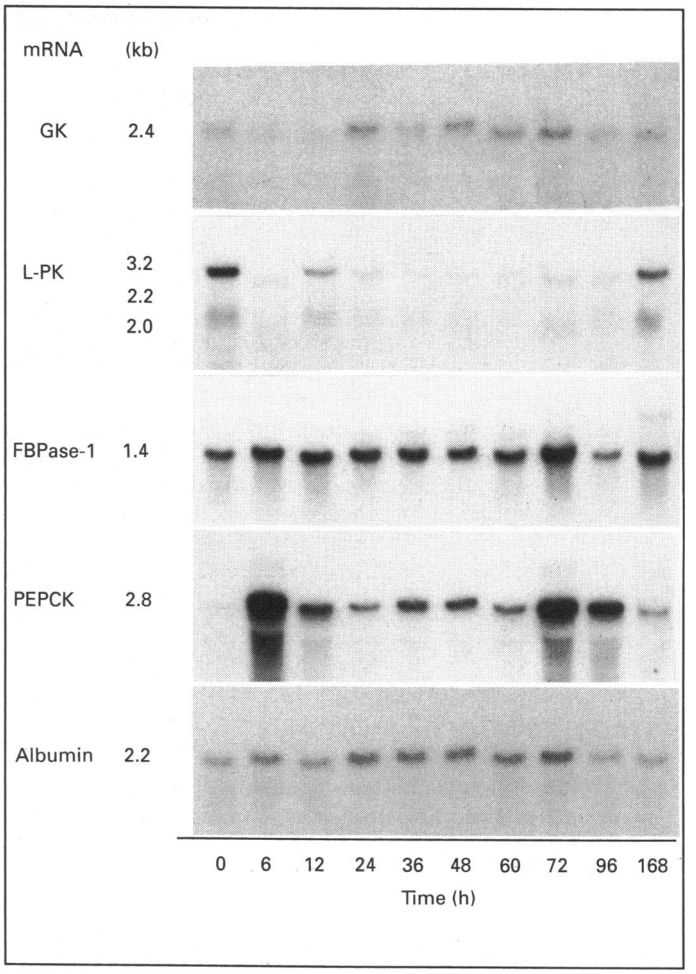

Fig. 2. Northern blots of regulatory enzymes of glycolysis/gluconeogenesis during liver regeneration

Samples of total RNA (20 μg/lane) extracted from normal (0 h) and regenerating rat livers at the indicated times were transferred to nylon membranes after electrophoresis in 1.5% agarose and hybridized with GK, L-PK, FBPase-1, PEPCK and albumin cDNAs as described in the Experimental section. The mRNA size is indicated.

From the results described above, it seems clear that it is during the early phase when most pronounced changes in the mRNA levels occur. To distinguish between the events which are specific for liver regeneration and those which are related to the stress, mRNA from sham-operated controls (animals subjected to a mid-ventral laparotomy without direct manipulation of the liver) was determined. As shown in Fig. 3, the enzymes showed changes in their mRNA levels in sham-operated controls. However, differences were found between the profiles of early partial hepatectomy and sham-operated animals. Although a decrease in the levels of L-PK mRNA was found in sham-operated animals, this was more marked in hepatectomized rats. In both animal groups the minimal levels were found at about 12–16 h, remaining low afterwards only in hepatectomized animals. GK mRNA levels showed a slight decrease during the first 30 min after liver resection, but this was not observed in sham-operated animals, followed by an acute decrease (minimal value at about 8 h) and a large increase at 16 h both in sham-operated and hepatectomized animals. These changes could be related to an effect on the gene expression of this enzyme during the fasting/refeeding cycle, as reported by Iynedjian *et al.* [24]. PEPCK mRNA levels rapidly increased after partial hepatectomy

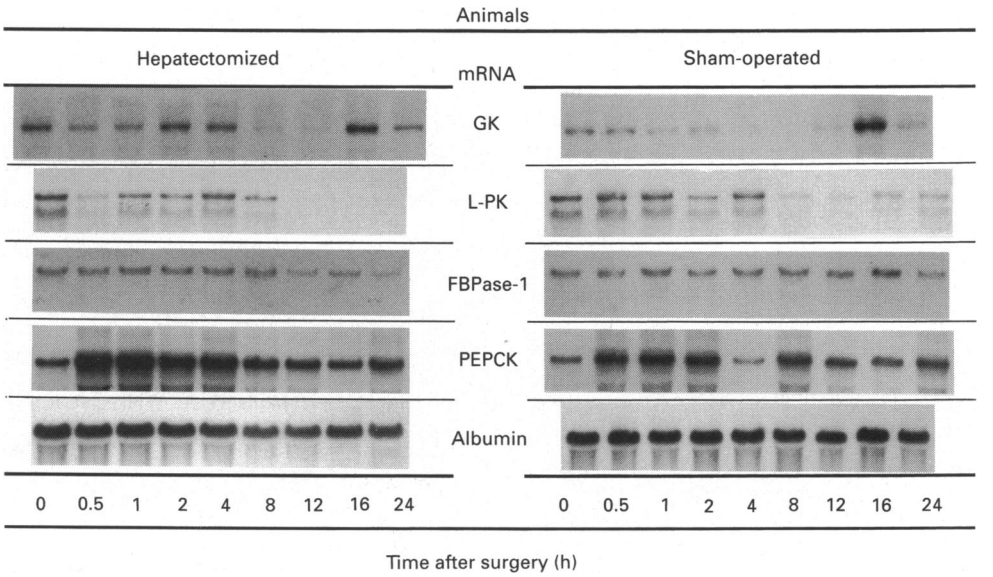

Fig. 3. Effect of stress on the gene expression of regulatory enzymes of glycolysis/gluconeogenesis after partial hepatectomy

Samples of total RNA (20 μg/lane) extracted from normal (0 h), sham-operated and regenerating rat livers at the indicated times were transferred to nylon membranes after electrophoresis in 1.5% agarose and hybridized with GK, L-PK, FBPase-1, PEPCK and albumin cDNAs as described in the Experimental section.

(7-fold at 30 min), remaining high afterwards. A similar profile, but less pronounced, was observed in sham-operated animals. FBPase-1 mRNA levels were unchanged in hepatectomized animals, and a transient increase between 4 and 16 h was observed in sham-operated rats.

DISCUSSION

It is known that after partial hepatectomy the decrease in liver tissue is accompanied by complex hormonal changes which produce an increase in glycogenolysis and gluconeogenesis in the liver remnant, together with a decrease in glycolysis, in order to maintain the blood glucose concentration [3–5]. During this process, the gene expression of glycolytic/gluconeogenic enzymes would also be modified. Various studies have been reported on the regulation of different key enzymes involved in glucose metabolism during hepatic regeneration. High levels of PEPCK activity have been detected at 24 and 48 h after partial hepatectomy [3]. This increase correlates with a decrease in hepatic GK [3,5,22] and L-PK [22,23] activities. Recently [5] we have shown that the amount of Fru-2,6-P_2, the most potent allosteric activator of 6-phosphofructo-1-kinase and inhibitor of FBPase-1 [1,2,6,7], changes after liver resection. This change is a consequence of the modification of the phosphorylation state of PFK-2/FBPase-2 [5] and of the modulation of the gene transcription rate [8].

The results reported herein suggest that the increase in PEPCK activity found during liver regeneration is probably due to an increase in its mRNA levels. This result agrees with the transcriptional activation of this gene reported previously [8] and with the fact that PEPCK was found to be one of the immediate-early genes cloned by differential screening of a subtraction-enriched regenerating-liver cDNA library [9]. However, opposite results were observed by Milland & Schreiber [10], claiming that a certain insulin-like growth factor could de-induce the PEPCK gene expression. Although changes in the mRNA levels have been found for FBPase-1 under different physiological and hormonal conditions [16], we did not find significant changes during liver regeneration. This fact, together with the non-modification of the glucose-6-phosphatase activity [3], points up the major role of PEPCK in the regulation of gluconeogenic flux during this process.

The behaviour of the glycolytic enzymes GK and L-PK was opposed to the gluconeogenic enzyme PEPCK during the pre-replicative phase. The mRNA levels of both glycolytic enzymes, together with that for PFK-2/FBPase-2 [8], showed a similar pattern. Differences were found only in the replicative state (> 12 h after partial hepatectomy) in which the L-PK mRNA were re-established later (at 168 h). This delay in the recovery of the levels of L-PK mRNA and the high levels of PEPCK mRNA found during this time could be related with the suggestion that glycogenesis could be sustained through the indirect pathway, the bulk of liver glycogen being of gluconeogenic origin [5]. In general, the changes in mRNA levels reported in the present paper correlate with the changes in activity previously reported [3,5,22,23], except for GK mRNA during the replicative state. This suggests that some post-transcriptional event could be involved in GK modulation.

Several hormones, growth factors and neuromediators, presumably acting in synergy [25,26], regulate the expression of these genes during liver regeneration. Bearing in mind what is known about the gene expression of these enzymes in other physiological conditions [27], the following tentative model is suggested. The increase in PEPCK mRNA levels found in the earliest phase of liver regeneration could be due to the increase in cyclic AMP [5,28] and the decrease in insulin concentrations [29,30]. By contrast, these hormones would produce repression of gene expression for glycolytic enzymes [27]. The co-ordination between glycolytic and gluconeogenic enzymes would be broken by the effect of increasing glucocorticoid concentrations during the replicative phase [30]. Therefore induction of PEPCK and PFK-2/FBPase-2 genes would take place, whereas L-PK would be inhibited [27].

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