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Transcriptional activity of the phosphoenolpyruvate carboxykinase gene decreases in regenerating rat liver

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Rat cytosolic phosphoenolpyruvate carboxykinase (PEPCK) gene expression and enzyme activity in liver were studied in rats fasted for 12 hours before and after partial hepatectomy, sham operation or no operation. Transcriptional activity and mRNA levels decreased in regenerating liver compared to sham-operated and unoperated controls. In contrast, PEPCK enzyme activity in regenerating liver was similar to that in the livers of sham-operated and unoperated controls. Since all the animals were fasted the decrease in transcription is probably caused by some factor other than insulin, the known repressor of PEPCK gene expression.

Phosphoenolpyruvate carboxykinase; Regenerating liver; Partial hepatectomy; Transcriptional activity; mRNA level; Gluconeogenesis

1. INTRODUCTION

The cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK) is a rate-limiting gluconeogenic enzyme whose gene is regulated at the transcriptional level by insulin [1,2], and, via cAMP, by glucocorticoids and glucagon [3,4]. Changes in PEPCK mRNA levels can usually be correlated with changes in enzyme activity [5,6]. PEPCK mRNA and enzyme activity increase with fasting [6]. The PEPCK enzyme activity in 1 g of regenerating liver is similar to that found in 1 g of the liver of fasted rats, but higher than that in 1 g of the liver of fed rats [7,8]. However, the level of PEPCK mRNA in liver after partial hepatectomy is lower than the level in the liver of fasted rats [9]. To clarify whether changes in PEPCK mRNA are due to changes at the transcriptional level, PEPCK transcriptional activity, mRNA level and enzyme activity were measured in regenerating liver.

2. EXPERIMENTAL

The PEPCK cDNA clone was a gift from Dr G. Yeoh (Perth, Australia). The cDNA was subcloned to the vector pGEM-3Zf (−) from Promega and its identity was confirmed by sequencing of 150 bp of the cDNA using the Sequenase kit from US Biochemical Corporation.

Measurement of transcriptional activities was carried out using the nuclear run-on assay with appropriate controls as described earlier [10]. Nuclei were prepared from pooled livers, collected up to 96 h after partial hepatectomy. Between 5–8 inbred male Buffalo rats weighing between 270 and 320 g were used for each time point. Partial hepatectomies were performed as described by Higgins [11].

In a separate experiment PEPCK enzyme activity and mRNA levels were determined in the livers from rats 12 h after partial hepatectomy. All animals were fasted for 12 h prior to the operation and for the first 12 h after the operation. Approximately 0.2 g of liver tissue was frozen immediately in liquid nitrogen for RNA extraction. The remainder (0.4 g), was assayed immediately for PEPCK activity.

Fresh liver was homogenised and the cytosolic fraction was prepared by centrifuging the homogenate at 100 000 × *g* for 1 h. The supernatant was assayed for PEPCK enzyme activity as described by Ballard and Hanson [12]. Protein content was determined by the Lowry method [13].

Total RNA was purified, separated by electrophoresis, blotted to nitrocellulose, and analysed by hybridisation to the labelled PEPCK cDNA probe. Quantification of bound cDNA was as described [9].

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All procedures involving the handling of animals were performed with the approval of the Animal Experimentation Ethics Committee of the University of Melbourne

Abbreviation: PEPCK, the cytosolic form of phosphoenolpyruvate carboxykinase.

3. RESULTS

PEPCK transcriptional activity in liver was determined up to 96 h after partial hepatectomy (Fig. 1). The lowest PEPCK transcriptional activity was 30% of the activity in starved controls 12 h after partial hepatectomy. The transcriptional activity increased to 65% of that in starved controls 18 h after partial hepatectomy, and then decreased again to 38% of that in starved controls 24 h after partial hepatectomy. Thereafter, transcriptional activity increased again to have a similar activity as the starved controls.

The PEPCK mRNA level (the proportion of PEPCK mRNA in total RNA) in liver was determined by Northern analysis (Fig. 2), 12 h after partial hepatectomy

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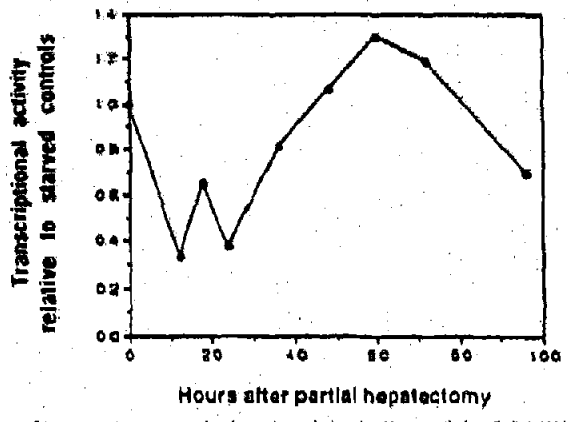

Fig. 1. Changes in transcriptional activity in liver of the PEPCK gene after partial hepatectomy. Nuclei were isolated from livers of 5–8 rats at indicated times after partial hepatectomy. Transcriptional activity relative to that in livers of starved rats was determined as described in section 2. Values given are the average of 2 experiments.

and was compared with fed and fasted unoperated and sham-operated controls. Well-defined, discrete bands for PEPCK identified by hybridisation with the cDNA probe, indicated the integrity of the prepared RNA. All operated animals were starved since it is difficult to ensure the same intake of food for different treatment groups. In this way, the effect of fasting can be distinguished from the effect of liver regeneration. The PEPCK mRNA level after partial hepatectomy was reduced to 37% of the level in fasted, unoperated con-

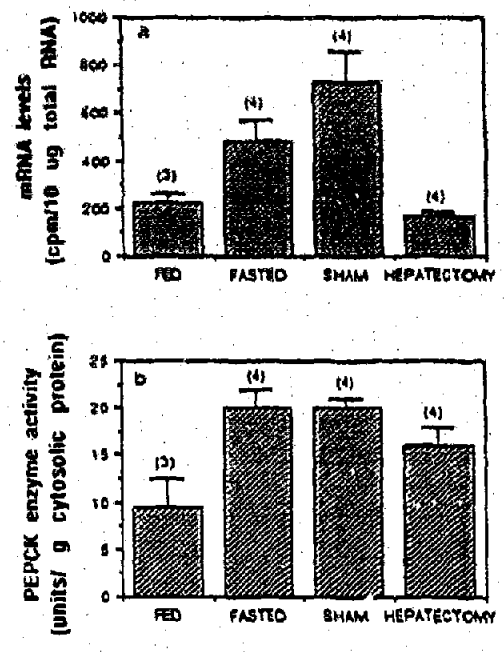

Fig. 3. (A) PEPCK mRNA in livers of fed, fasted unoperated, fasted sham-operated or fasted partially hepatectomised rats. Levels of mRNA were determined by Northern blot analysis as described in section 2. Values are means ± SE for the number of animals given in parentheses. (B) PEPCK enzyme activity in liver of fed, fasted unoperated, fasted sham operated or fasted partially hepatectomised rats. PEPCK activity was determined as described in section 2. Values are means ± SE for the number of animals given in parentheses.

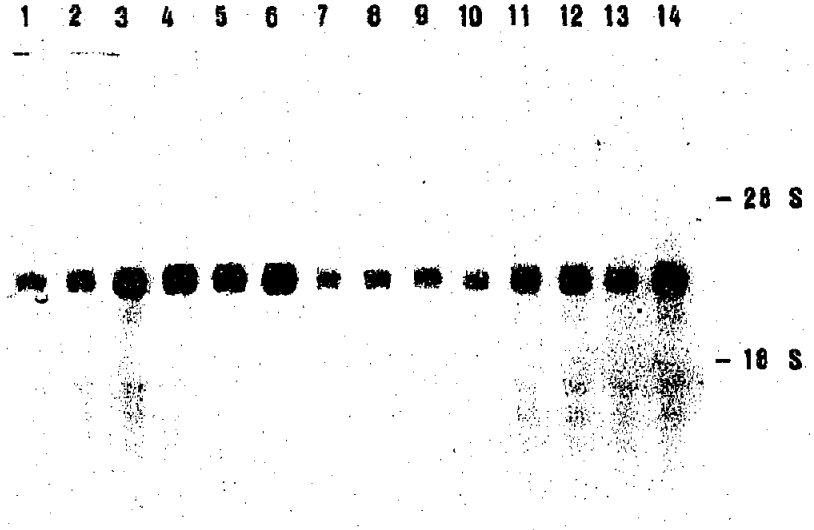

Fig. 2. Northern blot analysis of PEPCK mRNA in rat liver. Total RNA prepared from rat liver was separated by electrophoresis (10 μg/track), transferred to nitrocellulose and hybridised with ^{32}P-labelled PEPCK probe and quantified as described in section 2. The 28 S and 18 S ribosomal RNA bands were used as molecular size markers. Lanes 1–2 contain RNA from fed rats, lanes 3–6 from fasted rats 12 h after sham operation, lanes 7–10 from fasted rats 12 h after partial hepatectomy, and lanes 11–14 from fasted unoperated rats.

trols and 23% of the level in fasted, sham-operated controls (Fig. 3A). The reduction of PEPCK mRNA in regenerating liver compared to fasted controls was similar to that reported previously [9].

To confirm that the model of PEPCK activity in liver regeneration used in these experiments was behaving as others have reported [7,8], PEPCK enzyme activity was measured in the same rats used for the mRNA determination (Fig. 3A). The PEPCK enzyme activity per g of cytosolic protein was measured in liver 12 h after partial hepatectomy and compared with that in livers from fasted unoperated and sham-operated controls. PEPCK enzyme activity per g cytosolic protein was not significantly different between fasted unoperated, sham-operated and partially hepatectomised rats (Fig. 3B).

4. DISCUSSION

PEPCK gene expression is regulated at the transcriptional level after partial hepatectomy. PEPCK mRNA is induced by fasting prior to the partial hepatectomy (Fig. 3A). After partial hepatectomy, PEPCK transcriptional activity and mRNA are then de-induced. This is similar to the de-induction caused by insulin after PEPCK mRNA has been induced by glucagon [4]. After partial hepatectomy serum glucagon levels increase [14] and insulin has been reported to decrease slightly [14,15]. Since glucagon normally induces PEPCK mRNA [4], induction of gene expression would be expected to occur after partial hepatectomy. However the opposite was observed. Since insulin levels are not increased after partial hepatectomy [14,15], insulin probably did not cause the observed de-induction in PEPCK transcription and mRNA. All animals were fasted, and therefore the decrease in gene expression is unlikely to be due to a difference in serum insulin levels and glucose homeostasis caused by different feeding patterns between treatment groups. In view of the similarity between insulin and certain insulin-like growth factors whose mRNA levels increase after partial hepatectomy (for reviews see [16,17]), an insulin-independent de-induction of PEPCK gene expression is not surprising.

A similar decrease in transcriptional activity 24 h after partial hepatectomy (Fig. 1) has been observed previously for other genes [9]. Mitotic activity would be expected 24 h after partial hepatectomy (for review see [18]). Since RNA synthesis ceases during mitosis (for review see [19]), a decrease in transcriptional activity is not surprising [9].

Whilst PEPCK transcriptional activity and mRNA level are reduced compared to fasted controls, the enzyme activity per g cytosolic protein is maintained at the higher level of activity similar to that found in fasted rats. A similar result for PEPCK activity per g tissue has been reported [7,8].

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