Distribution of hepatic glutaminase activity and mRNA in perivenous and periportal rat hepatocytes

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Perivenous and periportal hepatocytes were isolated by the digitonin/collagenase perfusion technique. The specific activity of phosphate-activated glutaminase was 2.33-fold higher in periportal cells than in perivenous cells. Similarly, the relative abundance of glutaminase mRNA was 2.6-fold higher in samples from periportal cells. The distribution of glutaminase activity and mRNA was compared with those for glutamine synthetase (predominantly perivenous) and phosphoenolpyruvate carboxykinase (predominantly periportal). The results suggest that phosphate-activated glutaminase is predominantly expressed in the periportal zone of the liver acinus.

INTRODUCTION

Glutamine is an important substrate for hepatic gluconeogenesis and urea synthesis. Liver possesses a unique isoenzyme of phosphate-activated glutaminase (EC 3.5.1.2) [1–3]. Rat liver also has the capacity for glutamine synthesis (glutamine synthetase, EC 6.3.1.2) [1,4]. These two enzymes appear to be localized in different regions of the liver acinus. Glutamine synthetase activity, protein and mRNA have been shown to be confined to a discrete perivenous population of hepatocytes in adult rats [4,5]. In contrast, there is functional evidence for a predominantly periportal location for glutaminase [6–8]. The differential location of glutaminase and glutamine synthetase has resulted in the hypothesis of an intercellular glutamine cycle [6–8]. However, to date the exact intrahepatic location of glutaminase has not been established.

The purpose of the work described in this paper was to determine the distribution of glutaminase activity in isolated perivenous and periportal hepatocytes. The availability of a recently isolated cDNA for hepatic glutaminase also allowed investigation of glutaminase mRNA levels.

MATERIALS AND METHODS

Male Sprague–Dawley rats (175–300 g) obtained from Blue Spruce (Altamont, NY, U.S.A.) were maintained on a standard diet (Purina) and water *ad libitum*. Perivenous and periportal hepatocytes were isolated by the digitonin/collagenase perfusion technique of Quistorff [9] as modified by Chen & Katz [10]. Freshly isolated cell preparations were divided into two portions; one portion was homogenized in 5 vol. of ice-cold 0.33 M-sucrose/1 mM-EDTA/1 mM-dithiothreitol/5 mM-Hepes, pH 7.4, and used for enzyme assays; the other portion was used for RNA isolation by guanidinium thiocyanate extraction and ethanol precipitation [11]. Liver phosphate-activated glutaminase was assayed in the presence of 100 mM-glutamine, 2 mM-NH₄Cl and 50 mM-potassium phosphate [3]. The activities of glutamine synthetase [12] and phosphoenolpyruvate carboxykinase (PEPCK) [13] were assayed by standard procedures. Protein was

determined by the Bradford [14] method (Bio-Rad, Richmond, CA, U.S.A.), with bovine serum albumin as the standard.

Total RNA was analysed by Northern blotting [15]. Filters were probed with ³²P-labelled cDNA probes, and autoradiographs were quantified by densitometry. The probes used were hepatic glutaminase cDNA [16], the 1000 bp *Pst*I fragment from the PEPCK cDNA pcK10 [17], glutamine synthetase cDNA [18] and a 30-nucleotide fragment of cDNA to rat β -actin (Clontech Laboratories, Palo Alto, CA, U.S.A.). There were no significant differences in the relative abundance of β -actin mRNA in any of the samples. The abundance of other mRNAs was normalized to that of β -actin, to correct for differences in the amount of RNA loaded per lane. All other procedures and materials were as previously described [3].

RESULTS

The specific activities (Table 1) and relative abundance of mRNAs (Fig. 1, Table 2) for PEPCK and glutamine synthetase show that the digitonin/collagenase perfusion techniques allowed good separation of perivenous and periportal hepatocytes. Glut-

Table 1. Activities of glutaminase, PEPCK and glutamine synthetase in perivenous and periportal rat hepatocytes

Hepatocytes were isolated and enzyme activities measured as described in the Materials and methods section. Results are expressed as nmol of product formed/min per mg of protein, and are the means $\pm$ S.E.M. of five different cell preparations in each group. The ratio of activity in periportal to perivenous cells is given as PP/PV, and was determined from the mean activities.

Enzyme	Periportal	Perivenous	PP/PV
Glutaminase	70 $\pm$ 12	30 $\pm$ 4	2.33
PEPCK	12.8 $\pm$ 1.7	8.0 $\pm$ 2.0	1.60
Glutamine synthetase	2.6 $\pm$ 1.2	16.3 $\pm$ 4.5	0.16

Abbreviation used: PEPCK, phosphoenolpyruvate carboxykinase.

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Fig. 1. Relative abundance of mRNAs encoding hepatic glutaminase, PEPCK and glutamine synthetase (GS) in perivenous and periportal hepatocytes

RNA was isolated from perivenous and periportal hepatocytes and analysed by Northern blotting as described in the Materials and methods section. Each lane represents total RNA (10 μ g) from a separate cell preparation. PV indicates five preparations of perivenous cells and PP five preparations of periportal cells. The blot was sequentially probed with cDNAs to hepatic glutaminase, PEPCK, GS and β -actin (results not shown).

aminase activity and mRNA were almost exclusively found in the perivenous zone, whereas PEPCK activity and mRNA were predominantly periportal in location. There was some cross-contamination of fractions, as evidenced by one periportal sample with unusually high glutamine synthetase activity and mRNA (see Fig. 1). However, the periportal/perivenous ratios for PEPCK [10] and glutamine synthetase [19] activities were similar to those reported for cells isolated by this technique.

The specific activity of glutaminase was 2.33-fold higher in periportal cells (Table 1), and this was also reflected in the distribution of glutaminase mRNA (Fig. 1, Table 2).

Table 2. Relative abundance of mRNA encoding hepatic glutaminase, PEPCK and glutamine synthetase in perivenous and periportal hepatocytes

Northern blots (Fig. 1) were quantified by densitometry. Results are expressed as densitometry units representing cDNA hybridized per 10 μ g of total RNA, and are means $\pm$ S.E.M. for 5 preparations in each group. The results have been normalized for differences in total RNA as determined by hybridization to β -actin cDNA (see the Materials and methods section).

Enzyme	Periportal	Perivenous	PP/PV
Hepatic glutaminase	1321 $\pm$ 182	609 $\pm$ 195	2.17
PEPCK	2198 $\pm$ 232	1127 $\pm$ 248	1.95
Glutamine synthetase	185 $\pm$ 109	1079 $\pm$ 333	0.17

DISCUSSION

The concept of metabolic zonation in the liver has gained considerable acceptance in recent years (see [20] for review). The enzymes for gluconeogenesis and urea synthesis have been shown to be predominantly periportal, whereas those for glycolysis and glutamine synthesis are perivenous. Usually this distribution has been reported to be of a gradual nature, with most, if not all, cells possessing each activity. However, the distribution of glutamine synthetase (activity, protein and mRNA) has been found to be highly specific, located only in a layer, one to three cells thick, surrounding the central venule in adult rats [4,5,21–23].

The presence of high activities of glutamine synthetase and glutaminase within the liver gives rise to a potential futile (substrate) cycle. Häussinger and colleagues have proposed that such cycling does exist, but that it is intercellular in nature [6–8], i.e. periportal cells would possess higher glutaminase activity and utilize glutamine, with the ultimate products being urea and glucose. Perivenous cells, the only cells with glutamine synthetase activity, would serve to utilize NH_3 for glutamine synthesis. Häussinger *et al.* [6–8] have presented extensive functional evidence for a predominantly periportal location of hepatic glutaminase, but before the work reported here no direct measurements of the enzyme activity had been reported.

The results in the present paper confirm that glutaminase activity is predominantly located in periportal regions. However, such measurements are not definitive, since it is possible that the kidney-type glutaminase isoenzyme may be present and may contribute to the activity. The regulation of hepatic glutamine metabolism is believed to be a consequence of the properties of the unique hepatic isoenzyme of glutaminase. The availability of a cDNA to hepatic glutaminase (which does not hybridize to mRNA encoding the kidney isoenzyme [16]) enabled us to determine the distribution of the specific mRNA. The predominantly periportal distribution of hepatic glutaminase mRNA exactly matches the distribution of the enzyme activity, and indicates that the kidney-type enzyme is probably not present in adult liver. The results also indicate that the level and distribution of hepatic glutaminase activity are regulated at a pre-translation step. Similar findings have been reported for glutamine synthetase [5,21] and PEPCK [24].

The periportal location of hepatic glutaminase is in keeping with its role in the provision of substrate for gluconeogenesis and urea synthesis. It is also in agreement with the functional evidence of an intercellular glutamine cycle. The exact distribution of glutaminase within the liver cannot be determined by the isolated-cell technique, since there is obviously cross-contamination of fractions; however, this is the first demonstration of zonation of hepatic glutaminase activity and mRNA. More definitive dis-

tribution patterns will be established by using the cDNA and hybridization *in situ* in sections of liver.

This work was supported in part by grant DK-37301 from the National Institutes of Health and New York Hatch Project NY (C) 199448. E. M. S. was the recipient of a graduate fellowship from the U.S. Department of Agriculture. We thank Dr. R. W. Hanson and Dr. R. E. Miller for the gifts of cDNAs to PEPCK and glutamine synthetase respectively.

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Received 13 December 1989/5 February 1990; accepted 16 February 1990