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Cysteine metabolism in periportal and perivenous hepatocytes: Perivenous cells have greater capacity for glutathione production and taurine synthesis but not for cysteine catabolism

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Summary. Hepatocyte preparations highly enriched in cells from either the periportal or the perivenous zone of the liver acinus were prepared using a digitonin/collagenase perfusion method. Five enzymes of cysteine metabolism were assayed in both periportal and perivenous preparations. The ratios of periportal to perivenous activity were 0.76, 0.60, 0.81, 1.62, and 1.01 for cysteine dioxygenase, cysteinesulfinate decarboxylase, γ-glutamylcysteine synthetase, cystathionase, and asparate (cysteinesulfinate) aminotransferase, respectively. Only cysteinesulfinate decarboxylase activity was significantly different between periportal and perivenous cells. In incubations with 2 mmol/L [^{35}S]cysteine, total cysteine catabolism ([^{35}S]taurine plus [^{35}S]sulfate) between periportal and perivenous cells was not different, which is consistent with the observation of similar cysteine dioxygenase activity across the hepatic acinus. Consistent with the lower cysteinesulfinate decarboxylase activity in periportal cells, 16% of the total catabolism of [^{35}S]cysteine in periportal cells resulted in taurine synthesis compared to 28% in perivenous cells. A lower rate of [^{35}S]glutathione synthesis was observed in periportal cells compared to perivenous cells, but γ-glutamylcysteine synthetase activity was not significantly different between perivenous and periportal cells. Cysteinesulfnate decarboxylase can be added to the list of enzymes whose activities are markedly enriched in perivenous cells.

Keywords: Cystathionase – Cysteine dioxygenase – Cysteinesulfinate decarboxylase – γ-Glutamylcysteine synthetase – Periportal hepatocytes – Perivenous hepatocytes

Introduction

The concentration of a number of proteins in hepatocytes varies depending upon the location of the hepatocytes within the liver acinus, the microvascular unit of the liver. Generally, cells proximal to the afferent vessels (periportal zone) have been shown to contain higher amounts of the gluconeogenic and ureagenic enzymes such as glutaminase, phosphoenolpyruvate carboxykinase, lactate dehydrogenase, and alanine aminotransferase (Chen and Katz, 1985; Gebhardt, 1992; Quistorff, 1985). Cells from areas adjacent to the efferent vessels (perivenous zone) contain higher amounts of lipogenic enzymes, glutamine synthetase, enzymes for bile acid synthesis, and cytochromes P450 (Bars et al., 1992; Gebhardt, 1992; Gebhardt and Mecke, 1983; Ugele et al., 1991). Thus, both the concentration gradients of substrates in the sinusoidal plasma and the phenotypic differences in the hepatocytes of different zones contribute to differences in metabolism in periportal and perivenous hepatocytes in situ.

Little is known about the phenotypic differences in the capacity of hepatocytes in various zones to utilize and metabolize cysteine. In addition to being used for protein synthesis, cysteine is incorporated into the tripeptide glutathione, is converted to taurine, and is degraded to pyruvate plus inorganic sulfur as shown in Fig. 1. Saiki et al. (1992) reported the existence of a low K_m (~0.08 mmol/L) transport system for cysteine that is localized in the perivenous zone of the hepatic acinus. At concentrations of cysteine of 0.1 mmol/L or less, a gradient for uptake of cysteine was observed with low uptake by cells surrounding the portal vein and high uptake by cells surrounding the central vein. Although the higher cysteine uptake or cysteine availability in the perivenous zone would be expected to increase the rate of glutathione synthesis, Smith et al. (1979) reported that the glutathione concentration was lower in perivenous cells. Additionally, Kera et al.

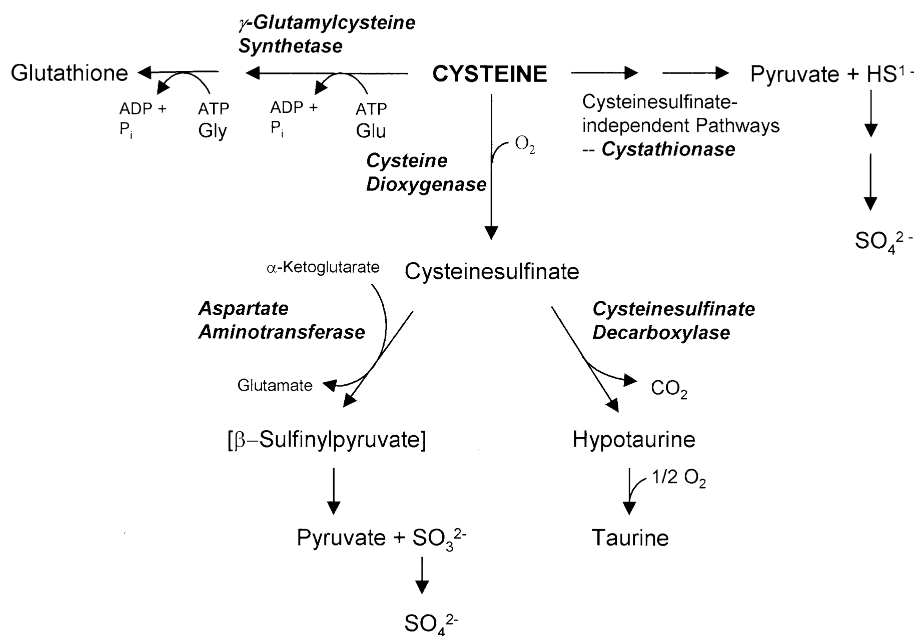

Fig. 1. Pathways of cysteine metabolism in mammalian tissues

(1988) reported that hepatocytes from the perivenous region have a lower capacity for glutathione synthesis, which they argued may contribute to the greater vulnerability of this region to xenobiotic damage. Subsequently, Penttila (1990) suggested that this apparent lower capacity for glutathione production may be due to a slightly lower γ-glutamylcysteine synthetase activity and a faster metabolism of cysteine to taurine by perivenous than by periportal hepatocytes. He hypothesized that the faster catabolism of cysteine to taurine may limit the availability of cysteine for glutathione synthesis by the perivenous hepatocytes.

The purpose of this study was to further evaluate the metabolism of cysteine in periportal and perivenous hepatocytes. To do this we have assayed the activities of a number of enzymes involved in cysteine metabolism and measured the rates of production of radiolabelled cysteine metabolites by hepatocytes incubated with [^{35}S]cysteine.

Materials and methods

Chemicals

L-Cysteine, taurine, glutathione, L-alanine, L-glutamine, L-glutamate, L-aspartate, α-ketoglutarate, ATP, NAD, NADH, the disodium salt of bathocuproine disulfonate, collagenase, and digitonin were purchased from Sigma Chemical (St. Louis, MO). Sodium sulfate was purchased from Mallinckrodt (St. Louis, MO). L-Cysteinesulfinate was purchased from Aldrich Chemical (Milwaukee, WI), and L-cystathionine was purchased from Calbiochem (San Diego, CA). L-[^{35}S]Cysteine was purchased as the hydrochloride from Amersham Corp. (Product # SJ141, Arlington Heights, IL) and was purified before use as previously described (Bagley and Stipanuk, 1994).

Animals and isolation of hepatocytes

Male Sprague-Dawley rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and were housed individually in stainless steel mesh cages. The room was maintained at 20°C and 60–70% humidity with light from 2000 to 0800 h. Rats were fed a non-purified diet (RHM 1000, Agway, Syracuse, NY) for at least two weeks before use for this study. Rats had ad libitum access to both diet and water. Rats were anesthetized with an intraperitoneal injection of 100 mg sodium pentobarbital/kg body weight. Hepatocytes from the periportal and perivenous regions were isolated by the digitonin/collagenase perfusion method of Quistorff (1985). Hepatocytes were selectively destroyed by a brief infusion of digitonin via the portal vein or the hepatic vein to disrupt periportal or perivenous hepatocytes, respectively. The digitonin infusion was followed by isolation of cells from the unaffected area using the method of Berry and Friend (1969) as modified by Krebs et al. (1974). Maintenance of cellular ATP at >2.0 μmol/g wet weight of cells throughout the experimental incubation period was used as a criterion for hepatocyte viability. The use and care of animals was approved by the Cornell University Institutional Animal Care and Use Committee.

Metabolism of cysteine by hepatocytes and enzyme activities

Freshly isolated hepatocytes (~150 mg wet weight of cells) were incubated with 0.2 mmol/L L-[^{35}S]cysteine in a volume of 2.5 mL. The production of [^{35}S]sulfate, [^{35}S]taurine (including [^{35}S]hypotaurine), and [^{35}S]glutathione (including disulfide forms) were measured as described previously (Bella and Stipanuk, 1995).

Aliquots of each freshly isolated hepatocyte preparation were used for analysis of total protein by the method of Smith et al. (1985) and of the initial taurine and glutathione concentrations using methods described previously (Bagley et al., 1995; Stipanuk et al., 1992).

Cellular ATP content was determined by the method of Lamprecht and Trautschold (1974); ATP analysis was done on cells at the end of the incubation with [^{35}S]cysteine to ensure that hepatocytes remained viable throughout the time during which metabolite production was measured.

For assay of enzyme activities, freshly isolated hepatocytes were homogenized and immediately assayed for various enzyme activities. Cysteine dioxygenase (EC 1.13.11.20), cysteinesulfinate decarboxylase (EC 4.1.1.29), and γ-glutamylcysteine synthetase (EC 6.3.2.2) activities in isolated hepatocytes were measured as described previously (Bella et al., 1999a). Aspartate (cysteinesulfinate): α-ketoglutarate aminotransferase (EC 2.6.1.1) activity in isolated hepatocytes was measured by the procedure described by Bergmeyer and Bernt (1974) except 20 mmol/L cysteinesulfinate was used instead of aspartate (Daniels and Stipanuk, 1982). Cystathionine-γ-lyase (cystathionase, EC 4.4.1.1) activity in isolated hepatocytes was measured by the procedure described by Stipanuk (1979). Glutaminase (EC 3.5.1.2, Watford et al., 1984) and glutamine synthetase (EC 6.3.1.2, Vorhaben et al., 1973) were assayed as markers for periportal or perivenous hepatocytes, respectively. All enzyme assays were performed under V_{max} conditions.

Statistics

Data were analyzed by the Student's t-test (Minitab 81.1, State College, PA). Differences were considered significant at P ≤ 0.05.

Results

As shown in Table 1, the freshly isolated periportal and perivenous hepatocytes had similar protein, ATP, taurine and glutathione contents. Hepatocytes were judged to be viable based on an ATP content of 2.0 μmol or more of ATP per gram of wet tissue. The activities of the periportal and perivenous marker enzymes are shown in Fig. 2. The activity of glutaminase was enriched in periportal hepatocytes, and the activity of glutamine synthetase was enriched in perivenous hepatocytes. The ratios of periportal to perivenous activities were 1.8 and 0.09 for glutaminase and glutamine synthetase, respectively.

The activities of the five enzymes involved in cysteine metabolism, which were measured in homogenates or extracts of the freshly isolated periportal or perivenous cells, are shown in Fig. 3, Cysteine dioxygenase, cysteinesulfinate decarboxylase, and γ-glutamylcysteine synthetase play key regulatory roles in determining the rates of cysteine catabolism, taurine synthesis, and glutathione production, respectively, in rat hepatocytes (Bella et al., 1999a and 1999b; Bagley and Stipanuk, 1994, 1995). Cysteinesulfinate decarboxylase activity was significantly greater (P = 0.01) in perivenous cells than in periportal cells. Cysteine dioxygenase (P = 0.59) and γ-glutamylcysteine synthetase (P = 0.33) activities were not significantly different. The gradient for cysteinesulfinate decarboxylase activity approached the reciprocal of that for glutaminase, with perivenous cells having 1.7-times as much cysteinesulfinate decarboxylase activity as periportal cells compared to periportal cells having 1.8 times as much glutaminase activity as perivenous cells.

Table 1. Protein, ATP, taurine and glutathione concentrations in hepatocyte preparations highly enriched in cells from the periportal or perivenous regions

	Periportal hepatocytes	Perivenous hepatocytes
Protein concentration, mg/g wet wt of hepatocytes	248 ± 11	266 ± 9
ATP concentration, μmol/g wet wt of hepatocytes	2.0 ± 0.1	2.1 ± 0.1
Initial taurine concentration, μmol/g wet wt of hepatocytes	6.1 ± 2.3	5.6 ± 2.4
Initial glutathione concentration, μmol/g wet wt of hepatocytes	2.07 ± 0.1	2.26 ± 0.1

Values are means ± SEM for hepatocytes preparations from 5 rats. Hepatocyte preparations were prepared to be enriched in periportal or perivenous cells as indicated

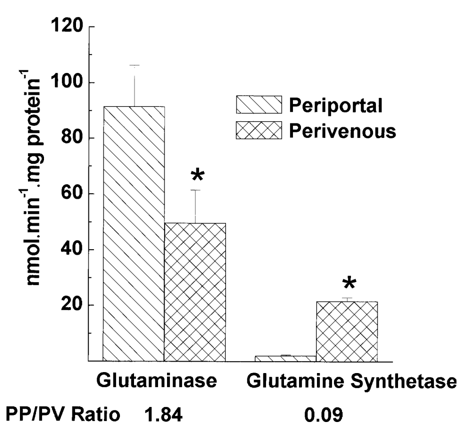

Fig. 2. Distribution of marker enzymes in hepatocyte preparations highly enriched in cells from the periportal or perivenous regions. Values are means ± SEM for hepatocyte preparations from 5 rats. An * indicates that the value was significantly different in perivenous vs. periportal hepatocytes (P ≤ 0.05). Periportal to perivenous ratios (PP/PV) are indicated for each set of values

Two other enzymes involved in cysteine metabolism, cystathionase, which can catalyze the β-cleavage of cyst(e)ine to yield pyruvate and sulfide, and aspartate aminotransferase, which can catalyze the transamination of cysteinesulfinate with α-ketoglutarate to a putative intermediate, β-sulfinylpyruvate, which yields pyruvate and sulfite (which is readily oxidized to sulfate) were also studied. The activities of

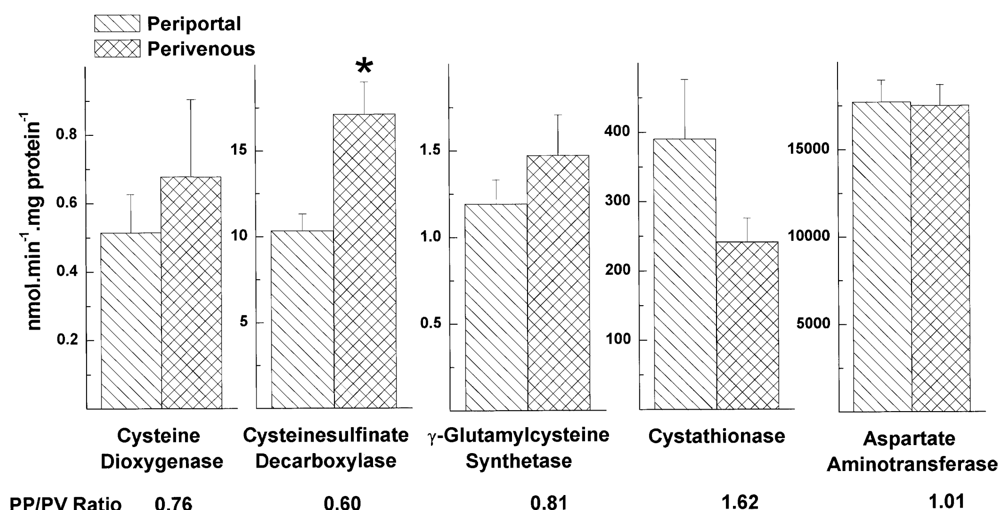

Fig. 3. Distribution of enzymes of cysteine metabolism in hepatocyte preparations highly enriched in cells from the periportal or perivenous regions. Values are means ± SEM for hepatocyte preparations from 5 rats. An * indicates that the value was significantly different in perivenous vs. periportal hepatocytes ($P \leq 0.05$). Periportal to perivenous ratios (*PP/PV*) are indicated for each set of values

Table 2. Comparison of cysteine metabolism in periportal versus perivenous hepatocytes

	[^{35}S]Taurine	[^{35}S]Sulfate	[^{35}S]Glutathione
	pmol·min^{-1}·mg·wet weight cells^{-1}		
Periportal hepatocytes	5.36 ± 0.8	25.43 ± 1.4	12.33 ± 1.3
Perivenous hepatocytes	8.81 ± 1.7	19.73 ± 2.2*	22.19 ± 2.1*

Values are means ± SEM for hepatocyte preparations from 5 rats. Hepatocyte preparations were prepared to be enriched in periportal or perivenous cells as indicated. An * indicates that the value was significantly different in perivenous vs. periportal hepatocytes ($P \leq 0.05$)

these two enzymes were not significantly different between periportal and perivenous cells ($P = 0.25$ and 0.92, respectively). Both of these enzymes contribute to sulfate production, but not to taurine production, from cysteine.

Rates of metabolism of exogenous [^{35}S]cysteine to [^{35}S]taurine, [^{35}S]sulfate, and [^{35}S]glutathione, which were measured in intact freshly isolated hepatocytes incubated with 0.2 mmol/L [^{35}S]cysteine, are shown in Table 2. The rate of [^{35}S]sulfate production was significantly less ($P = 0.05$) and the rate of [^{35}S]glutathione production was significantly greater ($P = 0.003$) in perivenous cells than in periportal hepatocytes. In contrast, the rate of production of [^{35}S]taurine was apparently greater in perivenous cells, though of borderline significance ($P = 0.11$) with the small sample size.

Discussion

Our studies with intact rats and with rat hepatocytes have indicated that cysteine dioxygenase activity plays the major role in determining the capacity for cysteine catabolism to taurine plus sulfate (Bagley and Stipanuk, 1994, 1995; Bella et al., 1999a, 1999b). The sums of [^{35}S]taurine and [^{35}S]sulfate production in assays with 0.2 mmol/L [^{35}S]cysteine were 30.8 and 28.5 pmol·min^{-1}·mg·wet weight cells^{-1} for periportal and perivenous hepatocytes, respectively. Thus, there were no differences in total cysteine catabolism between periportal and perivenous cells. The lack of any significant difference in either cysteine dioxygenase activity or total taurine plus sulfate production is consistent with a controlling role of cysteine dioxygenase in cysteine catabolism. Cystathionase can contribute to the cysteinesulfinate-independent catabolism of cysteine, but its contribution to sulfate production is normally minor unless the dietary supply of cysteine is below the requirement level (Drake et al., 1987; Bagley and Stipanuk, 1994).

Once cysteinesulfinate is formed via the oxidation of cysteine by cysteine dioxygenase, cysteinesulfinate is further metabolized either to pyruvate and inorganic sulfur (eventually sulfate) by aspartate aminotransferase or to hypotaurine (and hence to taurine) by cysteinesulfinate decarboxylase (Fig. 1). Although aspartate aminotransferase activity was essentially identical in periportal and perivenous hepatocytes, cysteinesulfinate decarboxylase activity was 1.67 times

as much in the perivenous hepatocytes as in the periportal cells. The higher cysteinesulfinate decarboxylase activity in perivenous cells is consistent with the greater proportion of cysteine catabolism that resulted in taurine vs. sulfate formation: 28% of the catabolism of [^{35}S]cysteine in perivenous cells resulted in taurine synthesis, whereas only 16% of the total catabolism of [^{35}S]cysteine in periportal cells led to taurine production. Our finding of a greater capacity for taurine synthesis in hepatocytes from the perivenous region is consistent with the observations of Penttila (1990). In our previous studies of cysteine metabolism in rat hepatocytes, we demonstrated that cysteinesulfinate decarboxylase activity plays a regulatory role in determining the partitioning of cysteinesulfinate between the decarboxylation and transamination pathways (Bagley and Stipanuk, 1994, 1995). This regulatory role of cysteinesulfinate decarboxylase is further supported in this study by the greater contribution of [^{35}S]taurine production to the total catabolism of cysteine ([^{35}S]sulfate plus [^{35}S]taurine production) in perivenous cells in which higher cysteinesulfinate decarboxylase activity was observed. The role of taurine in bile acid conjugation in the liver is well known. A greater conversion of cysteinesulfinate to taurine versus sulfate in the perivenous region correlates well with the higher levels of enzymes involved in bile synthesis in this region (Ugele et al., 1991).

Although we did not observe significantly greater γ-glutamylcysteine synthetase activity in perivenous hepatocytes, we did observe that perivenous hepatocytes produced [^{35}S]glutathione from [^{35}S]cysteine at a greater rate than did periportal hepatocytes. These higher rates of glutathione synthesis most likely reflect a slightly higher γ-glutamylcysteine synthetase activity in perivenous cells compared to periportal cells. Our previous studies in rat hepatocytes have shown a close association of γ-glutamylcysteine synthetase activity and capacity for glutathione synthesis (Ohta et al., 2000), Differences in [^{35}S]cysteine uptake seem an unlikely explanation for the higher rates of glutathione synthesis because the perivenous and periportal cells had similar rates of total cysteine catabolism (taurine plus sulfate production). In addition, differences in glutathione export cannot be the explanation because total [^{35}S]glutathione in the incubation mixture was measured as product. Our results clearly provide no support for the hypothesis of Kera et al. (1988) that hepatocytes from the perivenous region have a lower capacity for glutathione synthesis. Furthermore, our observations of similar cysteine dioxygenase activity and similar rates of cysteine catabolism in perivenous and periportal cells do not support the suggestion of Penttila (1990) that the greater rate of taurine synthesis in perivenous cells limits the availability of cysteine for glutathione synthesis by the perivenous hepatocytes or the report of Smith et al. (1979) that perivenous hepatocytes have a lower glutathione concentration.

Of the five enzymes of cysteine metabolism that we measured, cysteinesulfinate decarboxylase was the only one that exhibited a clear activity gradient across the hepatic acinus, with perivenous cells containing 1.67-times as much activity as periportal cells. The distribution of cysteinesulfinate decarboxylase across the hepatic acinus has not been reported previously, and this study demonstrates for the first time that cysteinesulfinate decarboxylase can be added to the list of enzymes that are markedly enriched in perivenous cells. Our finding that γ-glutamylcysteine synthetase activity was similar in perivenous and periportal hepatocytes is consistent with the report of Penttila (1990), but our results for metabolism of [^{35}S]cysteine to [^{35}S]glutathione suggest that the slightly higher (though non-significant) γ-glutamylcysteine synthetase activity in perivenous cells might be biologically significant.

The distribution of cysteine dioxygenase activity across the liver acinus has not been reported before, but conflicting reports for distribution of cysteine dioxygenase mRNA and protein have been published. Shimada et al. (1998), using an antisense cRNA probe for cysteine dioxygenase mRNA, found a stronger signal in the periportal region, whereas Parsons et al. (1998), using antibodies to an epitope of cysteine dioxygenase, reported a stronger signal in the perivenous region. Our results for cysteine dioxygenase activity and cysteine catabolism support a third possibility, that the distribution of cysteine dioxygenase is not different between periportal and perivenous cells. Aspartate aminotransferase activity was clearly similar between perivenous and periportal cells. More observations are required to determine whether cystathionase activity differs between periportal and perivenous cells. It is possible that cystathionase activity is enriched in periportal cells and that this difference was not significant in this study due to the high variance of cystathionase activity coupled with a small sample size or to insufficient enrichment of the periportal and perivenous cell preparations.

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