Role of cysteine and taurine in regulating glutathione synthesis by periportal and perivenous hepatocytes

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The uptake and metabolism of ^{35}S -labelled sulphur amino acids were compared in periportal (PP) and perivenous (PV) rat hepatocytes, isolated by digitonin/collagenase perfusion, to identify the factors underlying the previously observed [Kera, Penttilä & Lindros, *Biochem. J.* (1988) 254, 411–417] higher rate of GSH replenishment in PP cells. The buthionine sulfoximine-inhibitable synthesis of GSH was faster in PP than in PV hepatocytes with both cysteine (6.1 versus 5.0 $\mu\text{mol/h}$ per g of cells) and methionine (4.5 versus 3.3 $\mu\text{mol/h}$ per g) as well as with endogenous precursors and L-2-oxo-4-thiazolidinecarboxylate as substrates. However, the uptake of cysteine by PP cells was slower than by PV cells (8.6 versus 10.3 $\mu\text{mol/h}$ per g of cells), whereas methionine was taken up at similar rates. The activity of γ -glutamylcysteine synthetase (GCS) was slightly higher in digitonin lysates from the PP than from the PV zone. Production of sulphate, the major catabolite of [^{35}S]cysteine sulphur, as well as incorporation of the label into protein occurred at similar rates in PP and PV cells. Taurine, on the other hand, was produced from [^{35}S]cysteine much faster by PV than by PP cells (0.7 versus 0.1 $\mu\text{mol/h}$ per g of cells). Accordingly, the taurine content of PV hepatocytes tended to be higher and to increase faster during incubation with methionine. These results imply that metabolism of taurine is highly zoned within the acinus. They also suggest that both the slightly lower GCS activity and the fast metabolism of cysteine to taurine limit the capacity of PV hepatocytes to synthesize GSH.

INTRODUCTION

Results demonstrating that hepatocytes isolated from the periportal (PP) zone of the liver acinus accumulate GSH faster than do perivenous (PV) hepatocytes were recently reported from this laboratory [1]. Since GSH serves as a cofactor in cellular protection against reactive electrophiles and peroxidative agents [2], limited replenishment capacity of GSH, resulting, for instance, from limited availability of substrates or inhibition of synthesis, potentiates the cellular injury triggered by toxic chemicals [3,4]. Within the liver acinus, activation of numerous xenobiotics to reactive metabolites predominates in the PV zone, owing to higher expression of most cytochrome *P*-450 isoenzymes as compared with the PP zone [5–7]. Distribution of various GSH-related and other enzyme activities involved in detoxication [5,6,8] may also affect the zonal differences in hepatocyte vulnerability. The lower capacity of PV hepatocytes to replenish GSH during chemical attack may also limit the detoxication process and thus further augment the toxicity of, e.g., electrophilic metabolites of the cytochrome *P*-450 system.

The major factors regulating GSH biosynthesis (reviewed in [9]) are the availability of cysteine [3,10,11] and the activity of the first enzyme of the synthesis sequence, γ -glutamylcysteine synthetase (GCS) [12–14]. Intracellular cysteine, the content of which is relatively low (0.1–0.2 $\mu\text{mol/g}$ of liver [10,15,16]), can be derived from protein degradation, via transport across the plasma membrane or in hepatocytes, via the cystathionine pathway from methionine and serine [11,17,18]. The activity of the cystathionine pathway is not governed by cysteine content [18,19], and hence the intracellular content of cysteine is largely regulated, besides by uptake, by its catabolism to sulphate and taurine [15,18].

Biosynthesis of GSH has been shown to depend on the uptake rate of cystine [14,20,21]. However, the amino acid transport system x_c^- mediating cystine uptake is absent from freshly isolated hepatocytes [20,22], and consequently they cannot utilize cystine for GSH synthesis [3,16]. On the other hand, cysteine and

methionine, taken up by hepatocytes via transport systems ASC [23] and L [24] respectively, are rapidly incorporated into GSH [3,11,17].

The higher capacity of PP hepatocytes to replenish their GSH was evident with both endogenous and added sulphur amino acids as substrates [1]. Since neither the activity of GCS [1] nor the degradation rate of cellular proteins [25] was found to differ significantly in the two groups of cells, the difference in GSH accumulation was attributed to some dissimilarity in the transport and/or metabolism of GSH-related amino acids [1]. In the present work the uptake of sulphur amino acids and subsequent metabolism of [^{35}S]cysteine by PP and PV cells was studied. In addition, the acinar distribution of GCS was re-investigated by assaying the activity in digitonin lysates obtained from the PP and PV zones.

MATERIALS AND METHODS

Materials

Batches of digitonin producing a clear 7 mM solution [26] were purchased from BDH Chemicals, Poole, Dorset, U.K., or Merck, Darmstadt, Germany, and collagenase was from Boehringer, Mannheim, Germany (type H), or from Sigma Chemical Co., St. Louis, MO, U.S.A. (type IV). L-Buthionine *SR*-sulphoximine (BSO) was obtained from Chemical Dynamics Co., South Plainfield, NJ, U.S.A., and propargylglycine (PPG) and L-2-oxo-4-thiazolidinecarboxylic acid (LOTC) were from Sigma. Cysteine and methionine labelled with ^{35}S (sp. radioactivity 1450 Ci/mmol) and [^3H]inulin (330–620 $\mu\text{Ci/mg}$) were obtained from Amersham International, Amersham, Bucks., U.K. [^3H]Inulin was dissolved in 4% (v/v) ethanol to 2.5 mCi/ml and stored above liquid N_2 . Portions of labelled sulphur amino acids were stored likewise and diluted with non-radioactive solutions to yield daily 100 mM stocks with a specific radioactivity of 2–5 mCi/mmol. Eagle's minimum essential medium (EMEM)

Abbreviations used: BSO, buthionine sulfoximine; EMEM, Eagle's Minimum Essential Medium; GCS, γ -glutamylcysteine synthetase; LOTC, L-2-oxo-4-thiazolidinecarboxylic acid; OPA, *o*-phthalaldehyde; PPG, propargylglycine; PP, periportal; PV, perivenous.

with Earle's salts and non-essential amino acids, but without sulphur amino acids and Phenol Red, was specially produced by NordVacc AB, Skärholmen, Sweden. Glutamine and fatty-acid-free BSA (25 mg/ml; Boehringer) were added before use. 1-Bromododecane and dodecane were products of Aldrich KG, Steinheim, Germany, and liquid-scintillation chemicals and ATP Monitoring Kit were from LKB-Wallac, Turku, Finland.

Isolation of PP and PV hepatocytes

Male rats of the Alko mixed strain weighing 180–340 g had free access to standard R3 diet (Ewos AB, Södertälje, Sweden) and tap water. Hepatocytes originating primarily from the PP or the PV region were isolated under sodium pentobarbital anaesthesia (60 mg/kg, intraperitoneally) by using digitonin/collagenase perfusion [26] with slight modifications [1,27]. Digitonin was infused via the portal vein for 11–32 s, or via hepatic veins for 22–39 s, in order to disrupt the hepatocytes of the PP or PV zone respectively. The cells of the opposite zone were subsequently isolated, suspended in EMEM, washed [1,27] and adjusted to a final concentration of about 20 mg/ml. They were continuously kept under CO₂/air (1:19).

Incubation of hepatocytes

Portions (about 4 ml) of hepatocytes were preincubated for 15 min in 25 ml Erlenmeyer flasks in a shaker at 37 °C, followed by addition of inhibitors or substrates as 100–500-fold-concentrated stock solutions immediately after drawing the zero-time sample. Samples (0.2 ml) for metabolite determinations were deproteinized with 0.2 ml of 0.8 M-HClO₄/8 mM-EDTA. The uptake of amino acids was assayed by layering 0.2 ml of cell suspension on to 0.4 ml of 1-bromododecane/dodecane mixture (55.07:1, w/w) in a 1.5 ml tube and centrifuging the hepatocytes through the organic layer in a Beckman Microfuge B for 10 s. A 25 µl sample was taken from the medium for determination of radioactivity, the remaining medium plus the organic phase were removed by aspiration, and the walls of the tube were dried. The tip of the tube was cut off, and the cells were solubilized with 0.5 ml of OptiSolv solubilizer in a glass scintillation vial. Contamination of the cell pellet by extracellular radioactivity was assessed by mixing 240 µl of cell suspension with 10 µl of [³H]inulin (80 µCi/ml in EMEM) and treating a 0.2 ml sample from this mixture as described above. Cellular ³⁵S radioactivity was corrected for the percentage of [³H]inulin adherent to cells. Contamination of the cells was 0.4–1.6 % of total ³H radioactivity present in the 0.2 ml sample.

In some experiments the cells were suspended and incubated at 40 mg/ml in Krebs–Henseleit bicarbonate buffer supplemented with physiological amino acids except sulphur amino acids [1]. Intracellular samples were obtained by centrifuging cells from 0.5 ml of suspension to HClO₄ through the organic mixture as described previously [27].

Analytical methods

Alanine aminotransferase was assayed by using an UV-test kit (Boehringer), and glutamine synthetase was determined radioisotopically [28]. The activity of GCS was assayed with L-glutamate and L-α-amino-n-butyric acid [12] as formation of P_i that is inhibited by 5 mM-BSO. P_i was determined as described by Taussky & Shorr [29]. GSH was assayed in protein-free supernatants fluorimetrically [30] with 1 mg (final concn.) of o-phthalaldehyde (OPA)/ml.

Samples used for determination of ³⁵S radioactivity in sulphate and taurine were neutralized with 2.0 M-KOH/0.24 M-Mops (4+1 by vol.). For [³⁵S]sulphate assay a sample was further diluted (1:10) with eluent, 50 µl was injected into a Dionex ion chromatograph equipped with an AG5A guard column and an

ASSA analytical column, and the sulphate fraction was collected. [³⁵S]Taurine was separated after pre-column derivative formation for 90 s (12 µl of sample and OPA) by h.p.l.c. Method I, consisting of mobile phase (pH 6.0), a C₁₈ column and an electrochemical detector as described in [31]. The analytical column was preceded by a µBondapak C₁₈ pre-column (Waters Associates). After isocratic elution for 20 min (1.0 ml/min) [31], the elution was changed as a step gradient to buffer/methanol (44:56; flow 1.2 ml/min) for 20 min, followed finally by equilibration for 15 min with the first buffer. Retention time for taurine was about 19 min. In both h.p.l.c. methods the pre-column derivative formation was carried out automatically with a Waters Intelligent Sample Processor (WISP).

The amount of ³⁵S label incorporated into protein was measured after resuspending and washing the protein pellet twice with 0.4 M-HClO₄ and solubilizing it in 0.5 ml of OptiSolv. Radioactivities were measured in 10 ml of OptiPhase HiSafe II scintillant and were corrected for quenching by the sample channels-ratio method and, for sulphate and taurine, for background eluted from the chromatography systems. Molar amounts of ³⁵S-labelled amino acids and metabolites were calculated based on the specific radioactivity of added substrate.

For amino acid determination the intracellular extracts were neutralized with NaOH, norvaline was added as internal standard and pre-column derivatives (5 µl of sample and OPA for 60 s) of amino acids were determined by h.p.l.c. with fluorescence detection as described in [32] (referred to as Method II).

Statistics

The significances of the differences between PP and PV hepatocytes were calculated by using Student's *t* test for unpaired data, unless otherwise noted.

RESULTS

Good selectivity in the isolation of PP and PV hepatocytes was indicated by the activity of a periportal marker enzyme, alanine aminotransferase, for which the PP:PV ratio was 1.8 [*n* = 19 + 19 (numbers of cell preparations or experiments with PP and PV hepatocytes respectively), *P* < 0.001], and by an exclusively perivenous marker, glutamine synthetase, with an observed PP:PV ratio of 0.006 (*n* = 13 + 13, *P* < 0.001). Cellular ATP content depended neither on the acinar origin of, nor on the additions made to, the hepatocyte preparations, but did increase slightly from a mean of 2.6 to 2.8 µmol/g of cells during the incubation. A histological demonstration of the efficient zonal selectivity of digitonin treatment has recently been presented [33].

Glutathione accumulation and synthesis

Cellular GSH accumulated faster in PP than in PV hepatocytes with both endogenous and added substrates (Table 1), as shown previously [1]. Of the substrates added, cysteine (0.5 mM) was a more efficient precursor for GSH than was methionine (0.5 mM), whereas the intracellular cysteine-delivery agent LOTC [34] (1 mM) promoted GSH accumulation even less than did methionine. Doubling the concentration of cysteine to 1.0 mM accelerated the initial synthesis of GSH (Table 1), whereas increasing methionine concentration to 2.5 mM did not further stimulate GSH production in either cell type (*n* = 4 + 4; results not shown).

In the presence of BSO, a specific inhibitor of GCS [35], cellular GSH declined at similar rates in PP and PV hepatocytes. The decrease was slower when GSH synthesis only from endogenous methionine was blocked by the cystathionase inhibitor PPG [17] (Table 1). Based on GSH changes in the absence and

Table 1. Rates of net change and total synthesis of GSH by PP and PV hepatocytes in the presence of various substrates and inhibitors

Freshly isolated cells were incubated at 20 mg/ml in EMEM, or in some experiments at 40 mg/ml in amino acid-supplemented Krebs–Henseleit bicarbonate buffer, devoid of sulphur amino acids. Inhibitors (BSO, PPG) and/or substrates were added at zero time. The rate of net change in GSH was calculated from the change in GSH content during 20–60 min of incubation, so that the same time interval was used for both types of cells with a particular substrate. The 20 min time was applied for cysteine, which was rapidly oxidized [3,16] in the medium with a half-time of less than 10 min. The rate of GSH synthesis was calculated for each experiment by subtracting the rate of GSH change in the presence of BSO from the rate observed in its absence. Addition of cysteine or methionine with BSO, or methionine with PPG, had absolutely no effect on the depletion of GSH, confirming that the enzymes were completely inhibited. The GSH contents of PP and PV cells at zero time were 2.9 ± 0.4 ($n = 18$) and 3.1 ± 0.6 $\mu\text{mol/g}$ of cells ($n = 18$) respectively (not significantly different). Means $\pm$ s.d. are given, with the numbers of experiments in parentheses: * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$.

Substrate or inhibitor	Rate of change or synthesis of glutathione in cells ($\mu\text{mol/h}$ per g of cells)	
	PP	PV
Change of GSH content with		
Endogenous cysteine and methionine	0.2 ± 0.2 (13)	-0.2 ± 0.2 (13)***
BSO (5 mM)	-1.3 ± 0.1 (12)	-1.4 ± 0.3 (12)
PPG (0.5 mM)†	-0.5 ± 0.2 (4)	-0.6 ± 0.3 (4)
Cysteine (0.5 mM)	4.8 ± 0.9 (11)	3.6 ± 0.7 (11)**
Cysteine (1.0 mM)	7.0 ± 0.6 (2)	5.7 ± 1.7 (2)
Methionine (0.5 mM)	3.2 ± 0.7 (7)	1.9 ± 0.7 (7)**
LOTIC (1 mM)	1.7 ± 0.3 (5)	0.9 ± 0.2 (5)***
GSH synthesis with		
Endogenous cysteine and methionine	1.5 ± 0.2 (12)	1.2 ± 0.3 (12)‡
Endogenous methionine§	0.8 ± 0.1 (4)	0.4 ± 0.0 (4)**
Cysteine (0.5 mM)	6.1 ± 1.0 (10)	5.0 ± 0.8 (10)*
Cysteine (1.0 mM)	8.3 ± 0.6 (2)	7.2 ± 2.1 (2)
Methionine (0.5 mM)	4.5 ± 0.6 (6)	3.3 ± 0.6 (6)**

† Endogenous methionine not available for GSH production.

‡ $P = 0.06$.

§ Synthesis from methionine converted into cysteine via cystathionine pathway (equivalent to GSH accumulation that is inhibitable by 0.5 mM-PPG).

Table 2. Uptake of [^{35}S]cysteine and [^{35}S]methionine by PP and PV hepatocytes

Cells were incubated for 20 min in EMEM as described in the legend to Table 1. Cells were separated from medium and the intracellular radioactivity was counted as described in the Materials and methods section. Means $\pm$ s.d. are given, with the numbers of experiments in parentheses: * $P < 0.02$ for difference between periportal and perivenous hepatocytes; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ for the effect of BSO or PPG in combined PP and PV data (paired t test, $n = 5-6$).

Additions	Rate of uptake into cells ($\mu\text{mol/h}$ per g of cells)	
	PP	PV
Cysteine (0.5 mM)	8.6 ± 1.3 (11)	10.3 ± 1.5 (11)*
+ BSO (5 mM)	4.1 ± 1.3 (3)	6.0 ± 0.9 (3)†††
Cysteine (1.0 mM)	11.3 ± 2.2 (2)	18.2 ± 1.0 (2)
Methionine (0.5 mM)	4.5 ± 0.7 (5)	4.1 ± 0.8 (5)
+ BSO (5 mM)	3.1 ± 0.3 (3)	3.4 ± 0.4 (3)††
+ PPG (0.5 mM)	6.1 ± 1.0 (2)	4.7 ± 0.6 (3)†

presence of BSO, values representing the rates of GSH synthesis were calculated (Table 1). As suggested by the net accumulation rates of GSH, the rates of true GSH synthesis were higher in PP than in PV cells. This PP dominance was evident with both endogenous and added substrates.

Uptake of [^{35}S]cysteine and [^{35}S]methionine

A possibility that the lower rate of GSH synthesis in PV cells results from limited uptake of added substrates was investigated by comparing the accumulation of [^{35}S]cysteine and [^{35}S]methionine in PP and PV hepatocytes. Contrary to GSH synthesis, PV cells were found to take up cysteine at a higher rate than PP cells (Table 2). The uptake was further augmented by increasing the concentration of cysteine from 0.5 to 1.0 mM. Methionine, on the other hand, was taken up at a similar rate in the two cell populations, but more slowly than cysteine. BSO inhibited the uptake of the sulphur amino acids, whereas PPG accelerated the uptake of methionine (Table 2).

Incorporation of [^{35}S]cysteine into sulphate, taurine and protein

As there seemed to be a relative excess of cysteine taken up by PV cells with respect to its utilization for GSH synthesis, the metabolic fate of cysteine sulphur was investigated. [^{35}S]Cysteine (0.5 mM) was metabolized to [^{35}S]sulphate at approximately the same rate in PP and PV cells during 30 min incubation (Table 3). On the other hand, in PV cells the label appeared in taurine several-fold faster than in PP hepatocytes. Sulphate, however, clearly predominates over taurine as a principal catabolite of cysteine sulphur in these isolated cell preparations. No difference could be observed in incorporation of [^{35}S]cysteine into proteins in PP and PV cells (Table 3).

Intracellular contents of taurine, serine and glutamine

In general, the taurine content in PV cell suspensions tended to be higher than in PP suspensions (Table 4). Incubation of the cells with methionine elevated the taurine content in both PP and PV cells. Cysteine, on the other hand, did not bring about any apparent change in taurine content.

The intracellular content of serine decreased during 120 min incubation in sulphur-amino-acid-free medium to about half of the initial value (Fig. 1a). Addition of methionine further accentuated the decrease, which was at least partly due to utilization of serine for the synthesis of cystathionine. The extent of methionine-induced serine depletion was similar in PP and PV cells, but the intracellular serine content tended to be higher in PV cell preparations.

Table 3. Conversion of [^{35}S]cysteine into sulphate, taurine and protein by PP and PV hepatocytes

Samples of cell suspension were precipitated with an equal volume of 0.8 M-HClO₄, and radioactivity in each sulphur pool was determined as described in the Materials and methods section. Formation of radiolabelled sulphate and taurine was dependent on both time and cysteine concentration (0.5 or 1.0 mM). The rates, given as means $\pm$ s.d. with the numbers of experiments in parentheses, are based on total incorporation of radioactivity during 30 min incubation: * $P < 0.001$.

Metabolic sulphur pool	Rate of ^{35}S incorporation in cells ($\mu\text{mol/h}$ per g of cells)	
	PP	PV
Sulphate	5.6 ± 0.7 (6)	4.7 ± 1.0 (6)
Taurine	0.1 ± 0.1 (6)	0.7 ± 0.2 (5)*
Protein	2.5 ± 0.4 (3)	2.5 ± 0.3 (4)

Cellular glutamine content decreased slightly in PP cells during incubation, whereas in PV cells it approximately doubled, thus exaggerating the PP–PV difference present already at the start of incubation (Fig. 1b).

Activity of GCS

The activity of GCS was slightly, but not significantly, higher in PP- than in PV-cell preparations (Table 5). Furthermore, when its acinar distribution was probed by analysing effluents

obtained by digitonin pulse perfusion, a slight but statistically significant dominance of the activity in portal (PP) over venous (PV) eluates was found (Table 5). In some experiments where the relative duration of digitonin infusion was very short, i.e. less

Table 4. Taurine content in PP and PV hepatocytes

Two h.p.l.c. methods were used for taurine determination. Method I: hepatocytes were incubated at 20 mg/ml in EMEM, and taurine was determined in the supernatant after direct precipitation of the cell suspension with HClO_4 . The taurine content of the cells did not change upon incubation without or with 0.5 mM-L-cysteine, and therefore the mean of up to four samples drawn from those incubations was taken as the taurine content of a cell preparation. Method II: hepatocytes were incubated with or without methionine for 120 min in Krebs–Henseleit bicarbonate buffer (three PP–PV pairs), and taurine was determined in intracellular extracts as described in the Materials and methods section. Incubation in the absence of methionine did not alter the intracellular taurine content. The PP and PV group means of taurine content obtained by methods I ($n = 4+4$) and II ($n = 7+7$) were within $\pm 15\%$ of the combined group means ($n = 11+11$) that are presented. Means $\pm$ S.D. and numbers of samples from separate cell preparations are shown: * $P = 0.10$ for difference between periportal and perivenous hepatocytes; † $P < 0.01$ for the effect of methionine in combined PP and PV data (paired t test, $n = 6$).

Taurine ($\mu\text{mol/g}$ of cells)	Cells	
	PP	PV
Content	1.1 ± 0.5 (11)	1.9 ± 1.4 (11)*
Increase during incubation with 0.5 mM-methionine	1.0 ± 0.7 (3)	1.7 ± 0.4 (3)†

Table 5. Activity of GCS in PP and PV hepatocytes and cell lysates obtained from liver after a short digitonin infusion

The activity was determined in 105000 g supernatants of sonicated cells and in cell lysates obtained from the PP or PV zone of liver by first infusing 7 mM-digitonin at 10 ml/min and then collecting the effluent for 1 min while perfusing in the opposite direction with buffer [8]. The relative duration of digitonin infusion, calculated as $100 \times (\text{duration of digitonin infusion})/(\text{body weight})$, was 5.6–6.2 and 8.3–9.8 for portal and hepatovenous infusions respectively. For comparison, in isolation of PV and PP cells the relative duration was 6.9 ± 2.0 ($n = 16$) for portal and 12.4 ± 1.9 ($n = 15$) for hepatovenous infusions respectively. Activity of alanine aminotransferase is included to demonstrate the distinct acinar origin of periportal and perivenous cells or lysates. Means $\pm$ S.D. are shown for six pairs of cell preparations and eight pairs of cell lysates: * $P < 0.05$, ** $P < 0.001$.

Enzyme activity	Cells or cell lysates	
	PP	PV
Cells		
GCS (nmol of P_i/h per mg of cells)	79 ± 15	65 ± 16 †
Alanine aminotransferase (nmol/min per mg of cells)	98 ± 14	51 ± 12 **
Cell lysates		
GCS (μmol of P_i/h per mg of protein)	0.55 ± 0.08	0.48 ± 0.04 *
Alanine aminotransferase ($\mu\text{mol}/\text{min}$ per mg of protein)	1.08 ± 0.18	0.27 ± 0.13 **

† $P = 0.15$.

Fig. 1. Cellular contents of serine and glutamine in PP and PV hepatocytes during incubation

PP and PV hepatocytes were incubated for 120 min in Krebs–Henseleit bicarbonate buffer supplemented with 0.5 mM-methionine (+) or buffer (–) at zero time. Intracellular extracts of cells were obtained and assayed for serine (a) and glutamine (b) by h.p.l.c. Method I as described in the Materials and methods section. Changes in the content of amino acids were time-dependent during the 120 min incubation. Means, S.D. and numbers of samples from separate cell preparations are shown. The presence of L-methionine during incubation had no effect on glutamine content. Therefore the glutamine content at 120 min was calculated as a mean value from incubations without and with L-methionine.

than 0.055 and 0.08 for portal and venous infusions respectively (see legend to Table 5), the activity of GCS was slightly lower in portal than in venous effluents, whereas the activity of alanine aminotransferase was still higher in portal effluents (results not shown).

DISCUSSION

The present results confirm that the faster accumulation of GSH by PP than by PV hepatocytes, observed both without [1] and with preliminary chemical depletion of GSH [27], results directly from the higher biosynthesis rate of the tripeptide in PP cells. The slower GSH synthesis in PV cells, in turn, appears to be related both to their larger capacity to catabolize intracellular cysteine to taurine and to their slightly lower activity of GCS, rather than to a lower rate of sulphur amino acid uptake, as compared with PP hepatocytes. This conclusion is supported by the fact that GSH synthesis also from endogenous precursors was faster in PP than in PV cells, resulting in opposite changes of the cellular GSH content in the absence of added substrates. Methionine derived from protein degradation and metabolized through the cystathionine pathway contributed by about 30–50% to endogenous GSH synthesis, as previously estimated [11]. Re-investigation of the acinar distribution of GCS activity suggests that the 15–20% higher activity both in portal lysates and in PP cells as compared with the corresponding PV samples represents a true, although slight, dominance of the activity in hepatocytes from the PP acinar region. The notion that less GCS activity was detected in portal effluents when very short infusions of digitonin were given suggests that there is a non-linear activity distribution within the acinus, the activity being maximal in hepatocytes adjacent to those surrounding the portal entrance of the sinusoid.

Transport of cyst(e)ine has been suggested to be a limiting factor in GSH biosynthesis [20]. Accordingly, rates of cysteine uptake and GSH synthesis depended on the concentration of cysteine (0.5 or 1.0 mM). Therefore it was an unexpected finding that PV cells, although synthesizing GSH at a lower rate, accumulated cysteine (0.5 mM) 20% faster than did PP cells. This may indicate a larger abundance of cysteine transport sites, or reflect the faster conversion of cysteine into taurine (see below), in hepatocytes originating from the PV zone. So far, the acinar distribution of only the amino acid transport systems A, N and G⁻, and of the analogous Na⁺-independent activities, has been directly assessed in isolated PP and PV hepatocytes [36]. The strong dominance of glutamate transport by system G⁻ in PV cells and its association with glutamine synthetase activity [36] conform with the observed net accumulation of glutamine in isolated PV cells. These results directly confirm the perivenous dominance of glutamate uptake observed in perfused liver [37,38] and underlie the strict acinar zonation of nitrogen metabolism.

Transport system ASC is *trans*-stimulated, e.g. by glutamine and serine in cultured human fibroblasts. A possibility that the faster accumulation of cysteine by PV cells is due to their higher intracellular content of glutamine (and possibly serine) is, however, less likely, since in hepatocytes the transport of glutamine is largely mediated by system N (see [20] for references).

The suppression by BSO of the uptake of cysteine, observed previously in Chinese-hamster ovary cells [21], and also of methionine, may represent a regulatory response triggered by elevation of intracellular cysteine that presumably results from inhibition of GSH synthesis. Accordingly, the stimulation of methionine uptake by PPG could indicate an inverse type of response, possibly via *trans*-stimulation of system L [24], to lowered intracellular cysteine. Whether cysteine exerts these regulatory actions in hepatocytes or whether BSO and PPG act directly on the transport site is not known.

A single bolus of [³⁵S]cysteine was recently shown to be taken up and rapidly incorporated into GSH in the proximal acinar zone in the direction of perfusion (antegrade or retrograde) [39]. The efficiency of cysteine uptake ([39]; the present work) and the predominant exposure of the PP zone to plasma cysteine suggest that the greater capacity of PP cells for GSH synthesis serves in converting cysteine into its non-toxic storage form GSH [10], even during high dietary supply [15].

Methionine, although transported into PP and PV cells at similar rates, promoted GSH synthesis more in PP hepatocytes. Methionine taken up further accentuated the decrease in intracellular serine that took place when endogenous methionine alone was utilized for cysteine production. It can be inferred from the extent of serine depletion that the cystathionine pathway functions at similar rates in PP and PV cells. Consequently, the PP–PV differences in GSH production from methionine can obviously be ascribed to regulation of GSH synthesis at the level of cysteine utilization.

LOTIC is an efficient cysteine delivery agent capable of promoting GSH repletion *in vivo* [40–42]. In isolated hepatocytes, however, it was even less potent than methionine in supporting GSH accumulation. As LOTIC is efficiently converted into S-carboxycysteine by 5-oxo-L-prolinase [34], it is presumed that LOTIC is transported into hepatocytes more slowly than methionine or cysteine.

Metabolism of cysteine is thought to be initiated by cysteine dioxygenase-catalysed oxygenation to cysteinesulphinat. This is followed either by decarboxylation catalysed by cysteinesulphinat decarboxylase to yield taurine via hypotaurine intermediate, or by transamination and subsequent decomposition to yield finally pyruvate and inorganic sulphate (reviewed in [18]). Recent results support the existence also of a cysteinesulphinat-independent pathway [43–45], where reduced inorganic sulphur may be formed as an intermediate [18]. Regardless of the pathways involved, however, the ultimate catabolic products of cysteine sulphur observed in mammalian tissue are taurine and sulphate. The observed metabolism of [³⁵S]cysteine sulphur principally to sulphate is in accordance with observations made in intact male rats [46,47] and in hepatocytes from rats and cats [43,44]. On the contrary, other studies with rats [15,48] or rat hepatocytes [49], cats [48] and mice [45] suggest that a substantial portion of cysteine is metabolized to taurine.

The observation that the ³⁵S label of cysteine is incorporated into taurine much faster in PV than in PP hepatocytes indicates that the capacity for taurine production is strongly zoned in the hepatic acinus of the male rat. Accordingly, the basal level of taurine tended to be higher, and to be elevated by methionine during incubation to a greater extent, in PV than in PP hepatocytes. Moreover, the result implies that PV cells contain higher activity of cysteinesulphinat decarboxylase, thought to be rate-limiting for taurine biosynthesis [45,48], or of cysteine dioxygenase, which has a *K_m* value for cysteine (0.4 mM; [50]) similar to that of GCS [12]. Furthermore, the predominant utilization of cysteine for synthesis of taurine in PV hepatocytes obviously limits the availability of cysteine for GSH synthesis as compared with PP cells.

Although taurine is not further metabolized in mammals [18], it is used for conjugation with bile acids, which takes place mostly in the PP zone [6,51]. However, it has not been considered whether a single sub-population of hepatocytes is capable of synthesizing and conjugating taurine. The finding that PP cells synthesize taurine relatively slowly suggests that they may utilize taurine coming from diet or from enterohepatic circulation (as bile salts), or formed in the PV zone or in other tissues having relatively high activity of cysteinesulphinat decarboxylase, such as kidney and brain [52]. Taurine synthesized in the PV zone

could be transported to PP hepatocytes via the systemic circulation or with secreted bile via the bile canaliculus. This kind of inter-organ and intra-organ cycles would be analogous to those described for metabolism of nitrogen [37,38] and glutathione [53].

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