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Glutamine metabolism and signaling in the liver

Dieter Häussinger and Freimut Schliess

Heinrich-Heine-University Düsseldorf, Clinic for Gastroenterology, Hepatology and Infectiology, Moorenstrasse 5, 40225 Düsseldorf, Germany

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1. ABSTRACT

Glutamine is the most abundant amino acid in the human body and can be synthesized by almost all tissues by the glutamine synthetase (GS)-catalyed amidation of glutamate. Hepatocytes have access to extracellular glutamine by the concentrative uptake via members of the sodium-dependent neutral amino acid transport systems N and A. Hepatic glutamine metabolism in connection with urea synthesis is importantly involved in systemic ammonia detoxication and pH regulation due to the unique regulatory properties of the liver-type glutaminase, the acinar compartimentation of urea and glutamine synthesis, and a cycling of glutamine between periportal and perivenous hepatocytes. Upregulation of GS expression in hepatocellular carcinoma is related to growth advantage and an enhanced metastatic potential. Glutamine is a potent activator of signal transduction. Recent progress concerns the understanding of glutamine-induced hepatocyte swelling and the downstream activation of integrins, Src, and MAP-kinases in the regulation of autophagic proteolysis, canalicular bile acid excretion, glycogen and fatty acid synthesis, insulin signaling, and protection from apoptosis. Most recently the first primary GS defect leading to inherited glutamine deficiency with fatal outcome was described in human. This review summarizes recent progress in the understanding of glutamine metabolism and signal transduction, which provides further rationale for the use of glutamine as a therapeutic tool.

2. INTRODUCTION

Glutamine is the most abundant free amino acid in human plasma, skeletal muscle and cerebrospinal fluid. Glutamine can be synthesized by most cells and tissues by the glutamine synthetase (GS)-catalyzed ATP-dependent reaction of glutamate with ammonia. Glutamine plays a central role in nitrogen metabolism and represents a storage and transport form of glutamate and ammonia. Astroglial glutamine synthesis plays a role in glutamine-glutamate cycling between neurons and astrocytes in the brain (1) and is important for reduction of the cerebral ammonia load in hyperammonemic states (2). In the liver, parenchymal glutamine synthesis is restricted to perivenous hepatocytes which scavenge ammonia escaping periportal urea synthesis (3). Intercellular glutamine cycling (glutaminase-catalyzed deamidation by the periportal hepatocytes and GS-catalyzed resynthesis by the perivenous hepatocytes) is important for urea cycle regulation in the liver, which plays an important role for ammonia detoxication and systemic pH regulation. Glutamine represents a major donor of the amino moiety in the the synthesis of amino acids, purines, pyrimidines, AMP, carbamoylphosphate, amino sugars, and other metabolites. Further, glutamine is a precurser for gluconeogenesis (4) and glutathione synthesis (5).

Sodium-dependent glutamine transport is performed by the systems ATB^0, $B^{0,+}$, y^+L, and by members of the sodium-coupled neutral amino acid

transporters (SNAT) of the SLC38 gene family, which correspond to the earlier described systems A (SNAT1, SNAT2, SNAT4) and N (SNAT3 and SNAT6) (6-7). SNAT-mediated glutamine transport displays pH sensitivity, which in the case of system N-type transporters arises from coupled H^+ antiport. Depending on the sum of driving forces SNATs may mediate cellular glutamine uptake or export, respectively (6-7). Cellular glutamine transport underlies regulation by nutritions, hormones, and the cellular hydration state (8).

Glutamine availability critically determines cell hydration. Intracellular accumulation of glutamine leads to cell swelling, whereas glutamine starvation contributes to cell shrinkage (8-9). Accordingly, glutamine among other amino acids plays a role as an osmolyte in cell volume recovery under hyperosmotic conditions (10).

Glutamine not only acts as a metabolic precursor and substrate for protein synthesis but additionally activates and modulates signal transduction pathways involved in regulation of proliferation, apoptosis, gene expression, and metabolism. For instance, glutamine induces intestinal cell proliferation by stimulating an extracellular signal-regulated kinase (Erk)- and c-Jun-N-terminal kinase (JNK)-dependent DNA binding activity of activator protein AP-1 (11). Interestingly, the presence of glutamine was essential for epidermal growth factor (EGF)-stimulated Erk signaling and proliferation in intestinal epithelial cells (11), providing another example for the cooperation of growth factors and amino acids in signal transduction (12). Some of the proliferative and metabolic glutamine signals depend on cellular registration of glutamine-induced cell swelling, which leads to activation of cell volume-sensitive signal transduction pathways (13-14). Antiapoptotic signaling of glutamine may also involve the suppression of oxidative stress by support of glutathione synthesis (5), the stimulation of heat shock protein expression (15), the inhibition of the apoptosis signal-regulating kinase ASK1 by binding to glutaminyl-transfer RNA synthetase (16) and upregulation of Bcl-2/downregulation of CD95 and CD95 ligand expression (17). Recently, the interference of glutamine with the apoptotic volume decrease was shown to antagonize CD95-mediated apoptosis (18-19).

In catabolic states due to critical illness glutamine levels in plasma and skeletal muscle are strongly reduced, which is associated with a worse prognosis (4;20). For instance in severe infection glutamine release by the skeletal muscle exceeds endogenous glutamine synthesis, leading to depletion of the intracellular glutamine pool. However, plasma glutamine does not increase due to elevated glutamine uptake and metabolim by the liver, reflecting the fact that the skeletal muscle serves as the major glutamine supplier whereas the liver represents a major consumer of glutamine in sepsis and infection (6;20). Also the activated immune system utilizes large amounts of glutamine and glutamine availability can become limiting for immune cell function (20). Depletion of intracellular glutamine contributes to tissue dehydration which in turn triggers protein catabolic states and insulin resistance in sepsis and various other diseases (21-22).

Only recently, a primary defect of GS was described mamely in two independent patients from Turkish origin. Congenital systemic glutamine deficiency, which has not been described before, was the leading biochemical finding resulting in multisystemic disease and neonatal multiple organ failure. As the most striking finding, severe brain malformation with abnormal gyration and marked white matter lesions were detected (23). Also secondary deficiencies of GS have been described; the underlying mechanisms are still unclear (24-26). In the endotoxemic rat liver and the ammonia-exposed rat brain GS is tyrosine nitrated, which relates to the inhibition of GS activity (27-28).

In the following we present a state of the art overview about hepatic glutamine metabolism and signal transduction. Where useful also work on non hepatic cells and tissues will be considered.

3. HEPATIC GLUTAMINE TRANSPORT AND METABOLISM

In the early work by Krebs in 1935, the liver was identified to play a particular role in glutamine metabolism, thereby contrasting other organs like brain or kidney in properties of glutaminase and GS. Balance studies across the whole liver gave either conflicting results or demonstrated no net glutamine turnover at all, favoring an early view in which the liver did not play a major role in glutamine metabolism, although the activities of glutamine metabolizing enzymes were found to be high. Later, a fundamental conceptional change in the field of hepatic glutamine metabolism was derived from the understanding of the unique regulatory properties of hepatic glutaminase, the discovery of hepatocyte heterogeneities in glutamine metabolism with metabolic interactions between subacinar hepatocyte populations, and the role of intercellular glutamine cycling in the liver acinus for the maintenance of systemic ammonia and bicarbonate homeostasis (3;29).

3.1. Glutamine transport

In the liver glutamine transporters of the system N (SNAT3, SNAT5) and system A (SNAT2) subfamilies are expressed (7). System N plays a major role in hepatic glutamine transport and is largely attributed to plasma membrane expression of SNAT3 (older synonyms: SN1, mNAT, g17), which in rodents is most strongly expressed by the liver (30-32). SNAT 3 is expressed throughout the liver acinus but there is a gradient from lower expression levels in periportal to higher expression levels in perivenous hepatocytes (32-33). SNAT3 displays a glutamine-driven Na^+/H^+ exchanger activity due to the countertransport of H^+ associated with transport of 2 Na^+ and glutamine (Figure 1) (30-31). Thus, Na^+-dependent glutamine import via SNAT3 leads to intracellular alkalinization, whereas SNAT3-mediated export of Na^+ and glutamine leads to intracellular acidification. SNAT3-mediated glutamine transport is electrogenic by coupling transport of one glutamine and two Na^+ to the efflux of one H^+ (31). The collective driving forces including membrane electrical potential and the transmembrane concentration gradients of Na^+, glutamine, and H^+ determine the direction

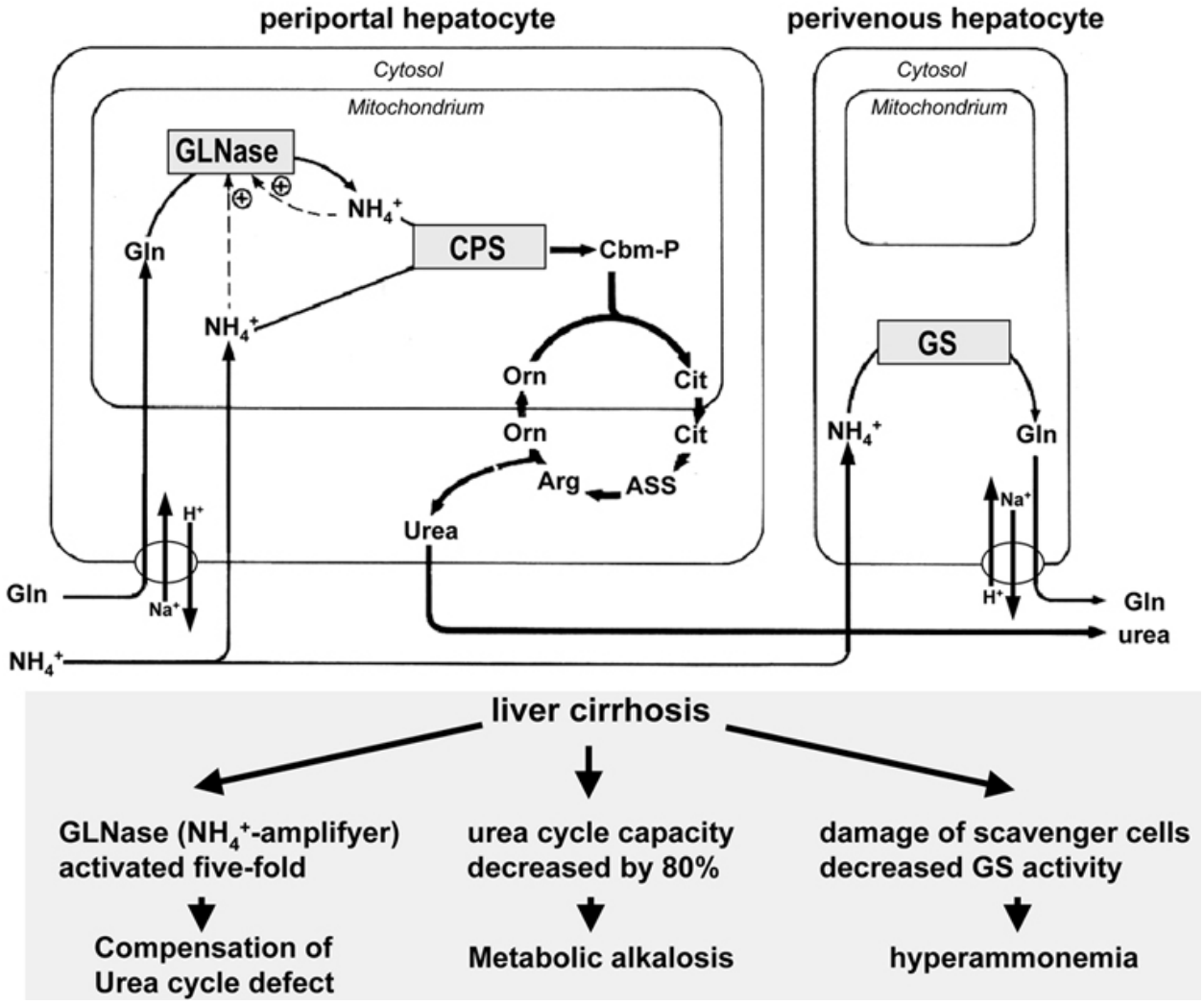

Figure 1. Hepatic ammonia and glutamine metabolism: structural and functional organization of intercellular glutamine cycling and ureogenesis and pathogenetic implications in liver cirrhosis. Periportal hepatocytes express glutaminase (GLNase) and urea cycle enzymes including the carbamoylphosphate synthethase (CPS), but no glutamine synthetase (GS). GS expression is confined to a small rim of perivenous hepatocytes. The later do virtually not express urea cycle enzymes. Periportal GLNase is activated by ammonia and acts as a pH- and hormone-sensitive ammonia amplifier within the mitochondria, thereby determining flux through the urea cycle (low affinity system for ammonia detoxication). Ammonia that escapes periportal urea synthesis is converted by the perivenous hepatocytes (scavenger cells) into Gln as catalyzed by the GS (high affinity system for ammonia detoxication). At normal extracellular pH GLNase flux equals GS flux, so that there is no net Gln turnover by the liver, but portal ammonia is converted efficiently into urea despite the low ammonia affinity of the CPS. Glutamine transport across the plasma membrane is performed by the SN1-type transporter SNAT3. The exposure of the periportal hepatocytes to high concentrations of glutamine and the outwardly directed H^+-gradient due to bicarbonate consumption by the CPS-catalyzed reaction drive the uptake of Gln via SNAT3 via its Na^+/H^+ exchange activity. SNAT3 expression is increased in perivenous hepatocytes (not shown in the scheme). Gln release by perivenous hepatocytes is supported by the diminished exposure of these cells to extracellular glutamine, high cytoplasmic glutamine levels and less acidic cytoplasm. In liver cirrhosis the urea cycle capacity is decreased by up to 80%. Due to a reduced bicarbonate disposal this leads to metabolic alkalosis, which is in part counteracted by increased glutaminase activity. Defective scavenger cells are accountable for hyperamonemia, which plays a key role in the pathogenesis of hepatic encephalopathy.

of glutamine transport by SNAT3 (6). A model was proposed for the role of SNAT3 within the hepatic intercellular glutamine cycle (Figure 1) (6-7;29). Urea synthesis is confined to the periportal hepatocytes. In these cells consumption of bicarbonate for the synthesis of carbamoylphosphate produces a more acidic intracelluar environment, which together with high glutamine concentrations in the periportal blood drives glutamine uptake. In the perivenous hepatocytes glutamine efflux is supported by the lower plasma glutamine concentration, the

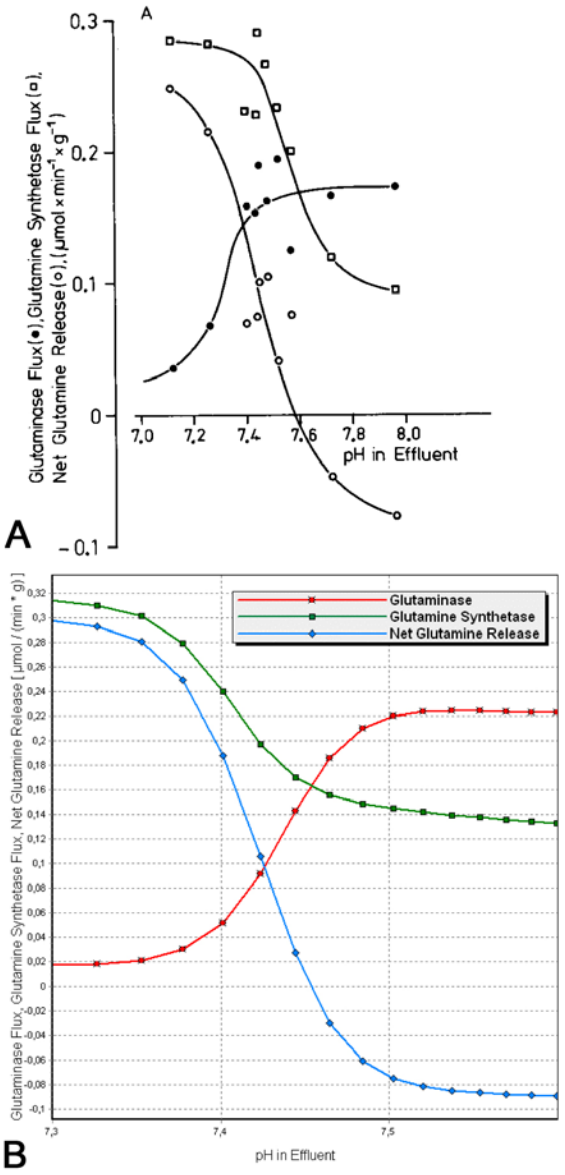

Figure 2. pH-sensitivity of fluxes through glutaminase and glutamine synthetase. Acidosis requires a systemic retention of bicarbonate. The liver responds to acidosis with a decrease of urea synthesis achieved by a reduced NH_4^+ production by the glutaminase-catalyzed reaction. Simultaneously, NH_4^+ passing by the acinus is scavenged by the perovenous hepatocytes: flux through glutamine synthetase increases. (A) Data obtained from liver perfusion experiments (107) (B) Modeling (112).

less acidic cytoplasm, and intracellular glutamine accumulation by the GS-catalyzed synthesis. Also SNAT2 and SNAT5 contribute to periportal glutamine uptake (7;34-35).

The concentrative glutamine transport across the mitochondrial membrane is pH-regulated (36-37). Nonaqueous fractionation analysis of livers perfused with a physiological glutamine concentration (0.6 mmol/L) revealed an apparent three- to fourfold accumulation of glutamine inside the mitochondria when compared to the cytosol (38). This gradient and, consequently, the intramitochondrial glutamine concentration rose about threefold, when the extracellular pH was increased from 7.3 to 7.7, whereas the cytosolic glutamine concentration did not change significantly (39). It was concluded that pH control of flux through hepatic glutaminase is mediated by variations of the mitochondrial glutamine concentration.

For a long time it has been accepted that glutamine transport across biological membranes in the liver is not controlling its metabolism. This view has changed completely, when it became clear that the glutamine transport systems in the plasma and mitochondrial membrane build up steady-state glutamine concentration gradients in the respective subcellular compartments (39-41), which determine the flux through glutamine metabolizing enzymes. With a physiological extracellular glutamine concentration of 0.6 mM and a physiological extracellular pH 7.4 *in vivo* and in the isolated perfused rat liver, the cytosolic and mitochondrial glutamine concentrations are about 6 and 20 mM (38). Raising the extracellular pH from 7.3 to 7.7, the mitochondrial glutamine concentration increases from 15 to 50 mM (39). Such pH-dependent fluctuations of the mitochondrial glutamine concentration, despite constancy of the extracellular concentration, critically determine flux through mitochondrial glutaminase, which displays a K_m (glutamine) of 22-28 mM. For this reason flux through glutaminase is increased four- to fivefold upon raising the extracellular pH from 7.3 to 7.7. (Figure 2). Thus, the control of glutamine metabolism by transport is exerted by the setting of the subcellular glutamine concentrations, rather than by the absolute velocity of initial glutamine import into the cells.

Endotoxin treatment of rats strongly increases the hepatic glutamine transport activity of system N due to the appearance of increased numbers of transporter molecules in the hepatocyte plasma membrane (42). Likewise starvation of rats increases hepatic glutamine uptake and it was found that starvation and endotoxin synergize in stimulation of hepatic glutamine transport (43). Tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, glucocorticoids and other compounds mediate the increase of hepatic glutamine transport under endotoxemic conditions. Glucocorticoids in part mediate the increase of hepatic glutamine transport achieved by *in vivo* administration of TNF-alpha to rats (44). Although largely without effect *per se*, TNF-alphaand IL-6 increased glutamine transport in isolated human hepatocytes in presence of dexamethasone (45). It was shown that dexamethaone increased the hepatocyte expression of IL-6 receptors (46). Also burn injury produces a negative nitrogen balance and increases hepatic glutamine consumption. In hepatocytes of IL-6 knockout mice burn injury failed to increase hepatic glutamine uptake, leading to the suggestion that IL-6 plays a key role for stimulation of hepatic glutamine transport in response to burn injury (47).

Glutamine uptake by hepatoma cells exceeds rates observed in normal hepatocytes more than 10-fold due to the expression of high affinity glutamine transporters of the ATB^0/ASCT2 family (48-50). ATB^0 mRNA is undetectable in normal human liver but is found in human hepatoma cell lines and hepatoblastoma biopsies (50). ATB^0 mRNA was also found in a human liver epithelial cell line and cirrhotic liver (50). System N isoforms (SNAT3, SNAT5) were detected only in well differentiated hepatomas (50). Glutamine in hepatoma cells represents a major energy source and is essential for progression through the cell cycle (51-52). It was suggested that ATB^0-type transporters guarantee sufficient glutamine supply under conditions of low extracellular glutamine availability and limited GS activity (50). In line with this, knockdown of ATB^0 expression induces apoptosis in human hepatoma cells, suggesting ATB^0 to be a potential target for selective treatment of human hepatocellular carcinoma (53).

3.2. Liver-type glutaminase

The phosphate-dependent liver glutaminase (EC 3.5.1.2) is a mitochondrial enzyme (54) which is predominantly expressed at the periportal zone of the liver acinus (55-56). The glutaminase is activated by phosphate (K_a = 5 mM), the K_m für glutamine is 28 mM (57) and the pH optimum is between 7.8 and 8.2. Due to the concentrative properties of glutamine transporting systems in the plasma and mitochondrial membrane, the enzyme is exposed even at the physiologically low extracellular glutamine concentrations (about 0.6 mM) to substrate concentrations close to its K_m. The liver glutaminase has been cloned (58) and is expressed only in neonatal and adult but not in fetal liver. Liver glutaminase is activated by ammonia (59-60), with NH_3 being the activating species (61). Most importantly, already physiological ammonia concentrations (0.2-0.3 mM) stimulate glutaminase activity (62). Mitochondrial swelling activates glutaminase as does an increase in pH (54;63). Different from the kidney enzyme, liver glutaminase is not subject to inhibition by glutamate and underlies regulation at the transcriptional level (55;64). Ammonia formed by the glutaminase-catalyzed reaction again stimulates glutaminase activity, leading to an autoamplification of mitochondrial ammonia production, which in view of the low $K_{m\ (ammonia)}$ of the the carbamoylphosphate synthetase importantly determines flux through the urea cycle (62;65). In addition, the glutaminase-catalyzed reaction provides glutamate for synthesis of N-acetylglutamate, an activator of both, glutaminase itself and carbamoylphosphate synthetase I (66-67).

3.3. Glutamine synthetase

Glutamine synthetase (GS, E.C.6.1.3.2) is encoded by one of the oldest existing and functioning genes (68). Liver GS is a cytosolic enzyme (69) and the reaction mechanism has been studied in detail (70). GS catalyzes in the presence of a divalent cation (Mg^{2+}, Mn^{2+}, or Co^{2+}) the ATP-dependent conversion of glutamate and ammonia to glutamine (3). L-glutamine is the only substrate of the gamma-glutamyl transfer reaction of GS but not D-glutamine (71). The enzyme is activated by alpha-ketoglutarate and is inhibited by methionine sulfoximine (MSO), glycine, and carbamoylphosphate (70). Mammalian GS consists of eight subunits with a subunit molecular size of 44 kDa. GS expression is upregulated in many cell types and tissues by glucocorticoids due to the presence of a glucocorticoid responsive element (GRE) in the first intron of the gene (72).

In the adult mammalian liver GS expresion is restricted to a small population (about 7%) of hepatocytes which form only a small rim of one to three hepatocytes around the central veins (73) (Figure 3). This is unique for mammalian liver and no zonation is found in avian and amphibian liver (74). The early fetal rat uniformly expresses GS and carbamoylphosphate synthetase mRNA and the heterogeneous and complementary distribution of these enzymes is fully developed two weeks after birth (75). However, the first indications for a heterogenous distribution of GS, which is related to the vascular architecture, are detectable at the mRNA level after 18 embryonic days and 2 days later at the protein level (76). In GS-positive hepatocytes about 1% of total cellular protein consists of GS.

The strict confinement of GS expression to the a small rim of hepatocytes surrounding the terminal hepatic venules is remarkably stable and modified only under extreme experimental conditions. Glucocorticoids can upregulate GS expression, however, the acinar compartment containing the enzyme does not enlarge. Following destruction of the GS containing hepatocytes by CCl_4 or a temporary expression of transgenic TGF-beta1 the scavenger cell compartment reappears within several days (77-78). Whereas in livers of mice starved for 1-2 nights a reduction of GS-positive hepatocytes was observed, multiple cycles of starvation and refeeding led to a remarkable increase of the number of GS-positive hepatocytes around the central veins (78). GS expression in originally GS-negative periportal hepatocytes was induced by their co-cultivation with the liver epithelial cell line RL-ET-14 (79) or transplantation into interscapular fat bodies (80). The unability of glucocorticoids to induce GS expression in GS-negative hepatocytes is explained by the action of a silencer element downstream from the glucocorticoid-responsive element (GRE), named GS silencer element of the rat (GSSEr), which binds a yet unknown nuclear protein (GSSE-BP) (81). The GSSEr also supresses GS expression driven by the action of the main 5´-enhancer of the GS gene (82).

Recently, the mechanism underlying the zonation of GS expression has been elucidated (82). Using hepatocytes isolated from both, the periportal and the perivenous zone of the liver, it was demonstrated that the GSSEr is active only in GS-negative hepatocytes. Accordingly, gel retardation assays using DNA probes representing the silencer element unravelled a significantly reduced GSSE-BP binding if nuclear extracts from cell preparations enriched with perivenous hepatocytes were subjected to the assay. Finally, by *in situ* co-immunostaining of GS and a luciferase reporter controlled by the 5´-upstream region and the first intron of the GS gene, it was visualized that the reporter is indeed expressed exclusively in GS-positive hepatocytes (82).

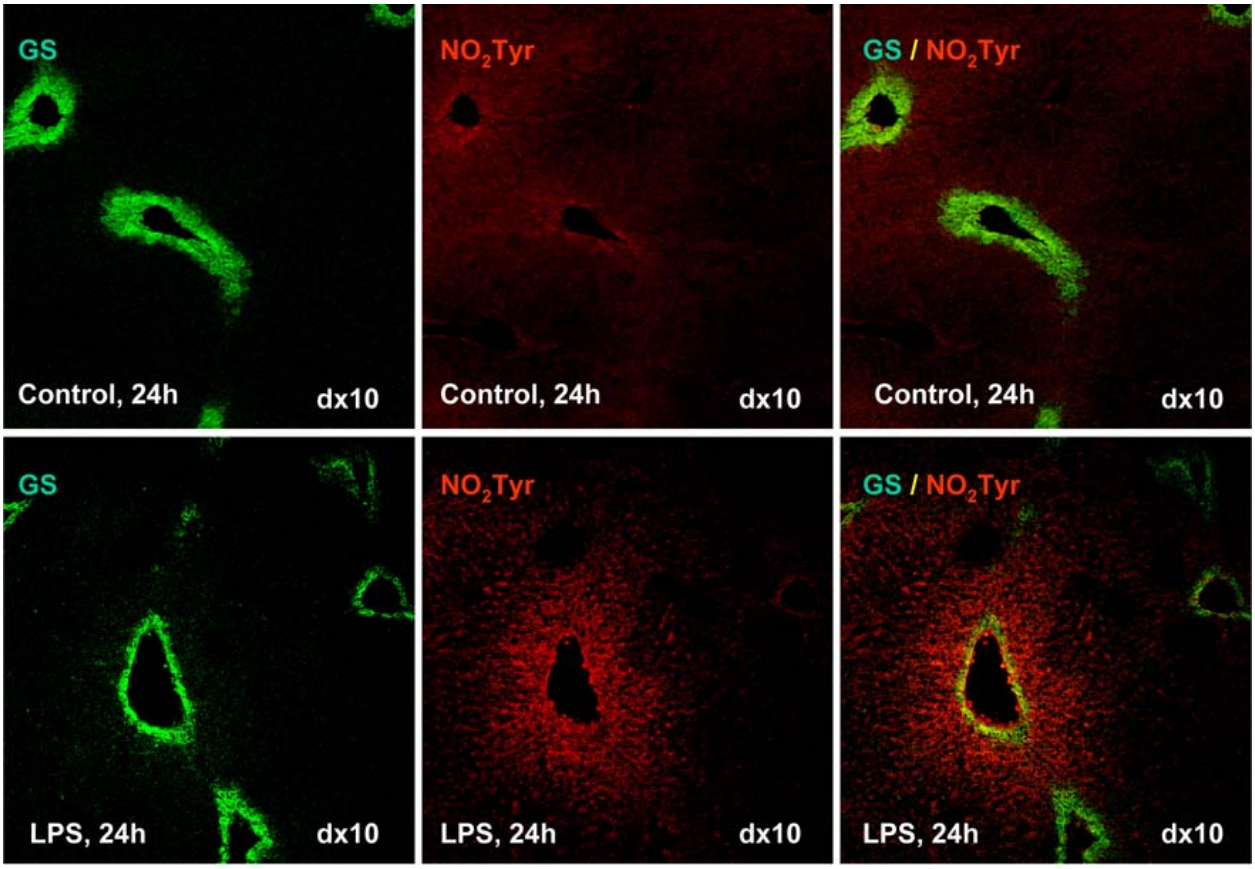

Figure 3. Immunohistochemical representation of hepatic glutamine synthetase expression and protein tyrosine nitration in lipopolysaccharide-treated rats. Liver samples from rats were taken 24h after intraperitoneal administration of lipopolysaccharide (LPS, 4 mg/kg body wt) or saline (Control), respectively. Liver sections were probed with antibodies raised against 3´-nitrotyrosine (NO_2Tyr), and glutamine synthetase (GS), respectively. Immunoreactivities were visualized by using Cy3-coupled anti-rabbit and FITC-coupled anti-mouse secondary antibodies (final dilution 1:200) with a confocal laser scanning microscope. LPS-induced protein tyrosine nitration is accentuated in the perivenous area of the liver acinus and is found in GS-positive hepatocytes.

In the injured liver also hepatic stellate cells (HSCs) may express GS (83). HSCs represent 3-8 % of all liver cells and liver injury stimulates their transformation from a quiescent to an activated myo-fibroblast-like phenotype, which is accompanied by increased alpha-smooth muscle actin (alpha-SMA) expression, proliferation and contractility. HSC transformation on the one hand plays a role in tissue repair but on the other promotes the development of liver fibrosis in case of persistent liver injury (84). HSC transformation is observed also during culture of isolated HSCs on plastic dishes, where the occurence of alpha-SMA is paralleled by an increase of GS expression and activity (83). Interestingly, despite absence of GS protein and activity, high levels of GS mRNA are found already in quiescent HSCs (83), a situation similar to that found in developing rat liver (75). GS expression in cultured HSCs may be regulated at the transcriptional and the posttranscriptional level (83), but the underlying mechanisms are currently not known. GS expression by transformed HSCs suggests that these cells could play a role in ammonia detoxication in the diseased liver and thereby in part compensate for the markedly reduced parenchymal GS activity seen in human liver cirrhosis (85) and CCl_4-intoxicated rats (86-87). In addition, the increased GS expression may reflect an increased glutamine demand of the proliferating cells and contribute to the relative resistance of cultured activated HSCs to apoptotic stimuli. GS mRNA, protein, and activity was also found in rat liver macrophages (Kupffer cells) but not in rat sinus endothelial cells, blood monocytes and RAW 264.7 mouse macrophages (88).

Recent work established a potentially important role of GS in hepatocellular carcinogenesis. GS expression is upregulated in a subset of hepatocellular carcinomas (HCC) (89-91) which is related to activation of beta-catenin signaling (92). In different mouse models with de-regulated beta-catenin signalling, hepatic expression of GS, ornithine amidotransferase (OAT) and the glutamate transporter GLT-1 is upregulated, which is a specific outcome of beta-catenin signaling and not a general response to hepatocellular proliferation (92). Activation of beta-catenin signaling also affected the zonation of GS expression. ΔN131beta-catenin mice exhibit a hyperplastic liver and

most of the hepatocytes in the liver lobule of ΔN131beta-catenin mice were GS-positive (92). In addition, expression of GLT-1 was identified to be strictly confined to the GS-positive perivenous hepatocytes but diffuse in the liver of the ΔN131beta-catenin mouse model (92). Further *in vivo* examination showed that the GS promoter is responsive to beta-catenin-dependent signaling in the liver (92). Analysis of human HCC samples revealed a significant correlation between beta-catenin activation and induction of the GS gene (92) and a relation between beta-catenin activation and GS expression levels in HCCs has also been established in phenobarbital plus N-nitrosdiethylamine-treated mice (93). Further, the induction of GS in fetal mouse hepatocytes by the RL-ET-14 cell line requires beta-catenin signaling. Here, induction of GS and the activation of transgenic reporter constructs under the control of regulatory regions of the GS gene was repressed by a knockdown of beta-catenin expression (94). Likewise, inhibition of GSK-3beta by lithium chloride, which activated beta-catenin, resulted in stimulation of GS expression. (94). Loss of the GS silencer element binding protein during tumorigenesis may be another mechanism contributing to GS expression by HCCs (82).

A study including patients with a single advanced HCC nodule revealed that high GS expression by the tumor was associated with a higher risk for disease recurrence (95). High GS expression by HCCs may make the tumor cells more independent from the availability of extracellular glutamine and thereby provide growth advantage and increase the metastatic potential. The role of extracellular glutamine and GS activity for tumor cell growth and viability was examined in leukemia cells in an exemplary fashion. Inhibition of GS by MSO in human MOLT-4 cells does not affect cell growth and viability, unless the extracellular glutamine concentration drops below a certain threshold (96), suggesting that GS becomes essential in maintaining cell growth in situations where extracellular glutamine is limited. Likewise, GS inhibition in rat fibrosarcoma cells is without effect on cell viability (97). However, depletion of extracellular glutamine and asparagine by asparaginase treatment induces apoptosis. Interestingly, in a rat fibrosarcoma cell line resistant to asparaginase, GS expression and activity are upregulated and GS inhibition by MSO in presence of asparaginase induces apoptosis (97). For this reason GS was suggested to represent a potential target for suppression of asparaginase-resistant phenotypes in the treatment of acute lymphoblastic leukemia (97). One observation in the two families affected by congenital GS deficiency due to mutations in the GS encoding gene further illustrates the importance of GS function (23). In both patients and in one terminated fetal sibling of one of the patients, fibroblast cultures failed to grow despite a large excess of glutamine in the culture medium, suggesting that the mutated GS somehow might interfer with the utilization of extracellular glutamine.

3.4. Acinar organization of hepatic glutamine metabolism

3.4.1. GS: a perivenous ammonia scavenger

In the intact liver lobule, the two major ammonia-detoxicating systems, urea and glutamine synthesis, are anatomically connected in series (Figure 1). Accordingly, the portal blood will first get into contact with hepatocytes capable of urea synthesis, before glutamine-synthesizing (scavenger) cells just at the end of the acinar bed are reached. In functional terms, this organization represents the sequence of a periportal low affinity, but high-capacity system (ureogenesis) and a perivenous high-aftinity system for ammonia detoxication (glutamine synthesis) (65). *In vivo* and *in vitro* a considerable fraction of the ammonia delivered via the portal vein from the intestine reaches the perivenous end of the liver acinus. Here, perivenous GS acts as a high-affinity scavenger for the ammonia, which escaped periportal detoxication by urea synthesis (3;29;65). This also holds for ammonia produced by amino acid breakdown in the much larger periportal compartment. The pivotal role of the perivenous GS-expressing hepatocytes for scavenging ammonia is supported by the observation that hepatic uptake of alpha-ketoglutarate, glutamate, and malate is restricted to this hepatocyte population (98-101). In addition, expression of the ammonia transport protein RhBG (a non-erythrocyte rhesus glycoprotein) is strictly confined to the GS-expressing hepatocytes (102), suggesting its particitation on high affinity ammonia detoxication.

The important scavenger role of perivenous glutamine synthesis for the maintenance of physiologically low ammonia concentrations in the hepatic vein becomes evident after inhibition of GS by MSO (65) or after destruction of perivenous hepatocytes by CCl_4 treatment (86). In the latter case, hyperammonemia ensues due to an almost complete scavenger cell failure to synthesize glutamine, although periportal urea synthesis is not affected. Similarly, in livers of endotoxin-treated rats a scavanger cell defect accounts for the reduced capacity for ammonia detoxication (27). This was related to reduced GS expression levels and a decline of specific activity of the residual GS due to tyrosine nitration of the enzyme (27). As shown in Figure 3, 3′-nitrotyrosine immunoreactivity in livers of LPS-treated rats is accentuated in the perivenous area of the liver acinus and in part colocalizes with GS (27).

3.4.2. Liver-type glutaminase: a pH- and hormone-sensitive ammonia amplifyer

Whereas GS is perivenous, glutaminase is expressed in periportal hepatocytes (65) and has a joint mitochondrial localization together with carbamoylphosphate synthetase (Figure 1). In view of the potent activation of liver glutaminase by its product ammonia in the physiological concentration range (62), glutaminase function is seen to amplify ammonia inside the mitochondria (65). Because urea synthesis is normally controlled by flux through carbamoylphosphate synthetase (103), which largely depends on the actual ammonia concentration inside the mitochondria, the extent of ammonia amplification importantly determines flux through the urea cycle in view of the physiologically low ammonia concentration. The glutamine-derived ammonia may even be a preferred substrate for urea synthesis, because incorporation of the glutamine nitrogen into urea in contrast to the incorporation of infused ammonia is almost insensitive to carbonic anhydrase inhibition (104).

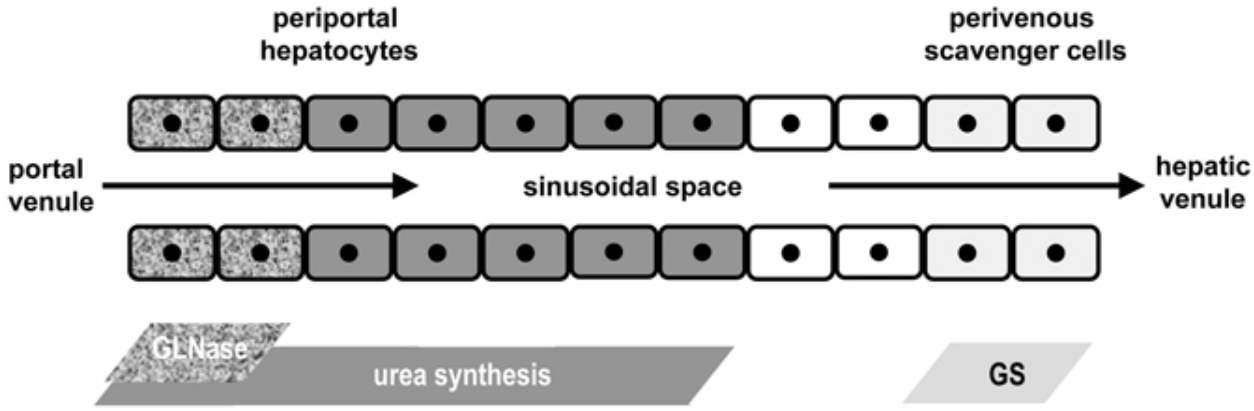

Figure 4. Hepatic ammonia detoxication: structural and functional organization. *In situ* hybridization experiments on the distribution of glutaminase (GLNase) mRNA (56) suggest that GLNase expression is restricted to a small subpopulation of periportal urea synthesizing hepatocytes at the very inflow of the acinus. Thus, GLNase may fulfill a role as ammonia amplifyer also for the more downstream urea synthesis in the midzone compartment.

In view of the fact that glutaminase is restricted to a subpopulation of urea cycle enzyme-containing periportal hepatocytes (56), it seems likely that glutaminase-mediated ammonia amplification may also increase ammonia delivery to more downstream hepatocytes containing urea cycle enzymes. If this is true, the small periportal cell population containing both glutaminase and urea cycle enzymes would function as an ammonia amplifying compartment, which determines the ammonia supply for ureogenic hepatocytes being devoid of glutaminase (Figure 4).

As anticipated from a control of urea cycle flux by glutaminase activity, factors known to affect the urea cycle flux are associated with parallel activity changes of glutaminase. Apart from the portal ammonia concentration this includes the effects of glucagon, alpha-adrenergic agonists, vasopressin, acidosis/alkalosis, and feeding of a high protein diet (3). Interestingly, liver-type glutaminase is activated by hepatocyte swelling, as it occurs due to concentrative uptake of amino acids (105). Thus, an increased portal amino acid load will increase hepatocyte volume and the accompanying activation of glutaminase is expected to favor amino acid-nitrogen disposal via urea synthesis. Inhibition of flux through liver glutaminase in acidosis (106-107) inhibits urea cycle flux and thereby hepatic bicarbonate consumption associated with ureogenesis.

3.4.3. The intercellular glutamine cycle: role in ammonia detoxication and systemic pH regulation by the liver

In the intact liver acinus, periportal glutaminase and perivenous GS are simultaneously active, resulting in a periportal breakdown and perivenous re-synthesis of glutamine (Figure 1) (62;65). This energy-consuming cycling of glutamine was termed the intercellular glutamine cycle (65). Glutamine cycling is under complex metabolic, hormonal, and pH control (65;106-107). In the special case of a well-balanced acid-base situation, intercellular glutamine cycling allows to maintain a high urea flux, despite the low affinity of carbamoyl phosphate synthetase for ammonia and the presence of physiologically low ammonia concentrations: glutamine consumed during ammonia amplification in periportal hepatocytes is re-synthesized in the perivenous compartment from the ammonia that escaped upstream urea synthesis. Intercellular glutamine cycling, however, also provides an effective means for adjusting ammonia flux into urea or glutamine according the needs of the acid-base situation (Figure 2). In acidosis, flux through glutaminase decreases and flux through GS increases, resulting in a net production of glutamine at the expense of urea (107). Thus, intercellular glutamine cycling provides an effective means by which the liver can switch from net glutamine consumption to net glutamine output (106-108).

Urea synthesis is a major pathway performing irreversible bicarbonate disposal. By this means, the liver is considered as an important organ that is involved in the regulation of the systemic acid-base homeostasis (107;109-111). The structural and functional organization of ammonia and glutamine metabolizing pathways in the liver lobule is one prerequisite for such a role of the liver in acid-base homeostasis (Figure 1) (3;107). Due to this organization, periportal urea cycle flux can be adjusted to the needs of systemic acid-base balance without threat of hyperammonemia. The reason is that glutamine synthesis in perivenous scavenger cells acts as a "back-up system" for ammonia detoxication, guaranteeing non-toxic ammonia levels in effluent hepatic venous blood even when urea cycle flux is repressed in order to diminish hepatic bicarbonate consumption in acidosis. In general, pH control of urea cycle flux occurs at the level of substrate provision for carbamoylphosphate synthetase, but not within the urea cycle itself (3). The remarkable pH sensitivities of glutamine transport and glutaminase play an important role among the various factors that adjust bicarbonate-consuming urea cycle flux to the needs of pH homeostasis. As outlined above, mitochondrial glutaminase acts as a pH-regulated ammonia amplifier: lowering the extracellular pH from 7.4 to 7.3 already inhibits the enzyme by 70%. Inhibition of flux through glutaminase in acidosis diminshes urea synthesis and therefore bicarbonate

consumption in the liver. The liver becomes a net producer of glutamine due to diminished glutamine consumption in periportal cells and an increased glutamine synthesis in perivenous scavenger cells (106-108). Recently, a mathematical model has been developed and applied to simulate glutaminase flux, net glutamine release and urea synthesis of perfused rat liver under varying conditions of the perfusate (Figure 2) (112). In acidosis, the glutamine is hydrolyzed by renal glutaminase and surplus ammonium ions are excreted into urine. Thus, an interorgan team effort between liver and the kidney maintains both bicarbonate and ammonia homeostasis.

3.4.4. Pathobiological aspects

As explained more extensively already in (3;113), metabolic alkalosis is frequently found in patients with liver cirrhosis even in the absence of alkalosis precipitating factors including vomiting, diuretic or antacid therapy. This can be simply explained by an impaired bicarbonate disposal by urea synthesis in the diseased liver (Figure 1). Alkalosis due to impaired bicarbonate disposal via urea synthesis in turn represents one signal for activation of liver glutaminase. Indeed, flux through glutaminase was shown to be increased about five-fold in human liver cirrhosis (114-117). This compensatory response via increased mitochondrial ammonia amplifying augments urea synthesis leading to the maintainance of near-normal rates of urea synthesis in the compensated cirrhotic patient. Thus, the cirrhotic patient approaches a new, albeit more alkaline steady state, which allows him to maintain a life-compatible urea cycle flux despite a marked reduction of the capacity for urea synthesis. This compensation, however, requires the presence of metabolic alkalosis in order to keep the ammonia amplifying system glutaminase active. When acidosis develops e.g. during infection, sepsis, or cardiac insufficiency, this compensatory mechanism is shut off and severe hyperammonemia develops.

Human liver cirrhosis is characterized by a defect of perivenous scavenger cells (Figure 1): the capacity to synthesize glutamine is decreased by about 80% in cirrhosis (114;116). This scavenger cell defect may be related to portosystemic shunting (118) and is a major factor for the development of hyperammonemia in liver cirrhosis. Hyperammonemia can also be induced by drugs and toxins, which primarily impair the function of perivenous hepatocytes, whereas the periportal urea synthesizing hepatocytes need not to be affected (86). In addition, reversal of sinusoidal blood flow, as it is sometimes observed in cirrhosis, can produce hyperammonemia, because under these conditions the perivenous scavenger cells (high-affinity ammonia detoxication) are switched before the periportal urea synthesis (low-affinity detoxication) (65). A scavenger cell defect in cirrhosis may also be responsible for hyperammonemia induced by diuretics (119). In healthy individuals with intact perivenous scavenger cells a 5-10 % inhibition of urea synthesis e.g. by mefruside, xipamide, and thiazides in near-therapeutic concentrations will not impair hepatic ammonia detoxication. However, in cirrhosis, the scavenger cells are defective and even a slight inhibition of urea synthesis will trigger systemic hyperammonemia.

Mammalian GS is highly susceptible to oxidative and nitrative modification which is associated with inactivation and proteolytic degradation of the enzyme (120-121). The age-related increase in oxidized protein in rat liver and brain is accompanied by a loss of GS activity (121-123). Ammonia in cultured rat astrocytes increases the generation of reactive oxygen and nitrogen species leading to increased tyrosine nitration of protein including GS (28). Astroglial GS tyrosine nitration (Figure 3) is related to inactivation of the enzyme (28). *In vitro* studies with purified sheep GS showed that already 20μM peroxynitrite were sufficient to potently nitrate the enzyme (27), whereas 100-200μM peroxynitrite were required to nitrate GAPDH to a similar extent (124). This indicates an exceptionally high susceptibility of the GS to tyrosine nitration by peroxynitrite. Nitration of sheep GS by peroxynitrite was associated with a remarkable loss of activity (27). Epicatechin, a flavonoid which interferes with protein tyrosine nitration by intercepting the tyrosyl radical (125) completely abolished tyrosine nitration and partly prevented inactivation of the GS by peroxynitrite (27). Inactivation of the hepatic GS by tyrosine nitration was recently suggested to play a role in the pathogenesis of hyperammonemia in cirrhotic patients with sepsis and infection (27).

4. HEPATIC GLUTAMINE SIGNALING

It is meanwhile well established that amino acids not only are important precursers in metabolic pathways but in addition are important players in the regulation of cellular signal transduction and gene expression (8;12;14;126-129).

4.1. Glutamine signaling via mTOR

Amino acids, especially leucine, potently activate signaling via the mammalian target of rapamycin (mTOR) to the mTOR effectors p70 ribosomal S6 protein kinase (p70S6-kinase) and the eukaryotic initiation factor 4E (eIF4E) binding protein 4E-BP1 in may cell types and tissues including isolated hepatocytes (130-132) and perfused rat liver (133). Further, the presence of amino acids is essential to receive the entire response of mTOR-dependent signaling to insulin (12;14;126-127). Accordingly, deprivation of extracellular amino acids leads to dephosphorylation of p70S6-kinase and 4E-BP1, respectively (134). Interestingly, amino acid starvation of H4IIE rat hepatoma cells was ineffective to block insulin-induced phosphorylation of p70S6 kinase and 4E-BP1 and this was assigned to the intracellular provision of amino acids due to stimulation of autophagic proteolysis (135). Sparing amino acids by inhibition of cellular protein synthesis likewise maintained insulin signaling in the absence of added extracellular amino acids (136), suggesting a particular importance of intracellular amino acid availability for regulation of mTOR. mTOR is critically involved in glutamine signaling in Jurkat (137) and leukemic (138) cells and is a major mediator of the cardiomyocyte transcriptional response to glutamine (139).

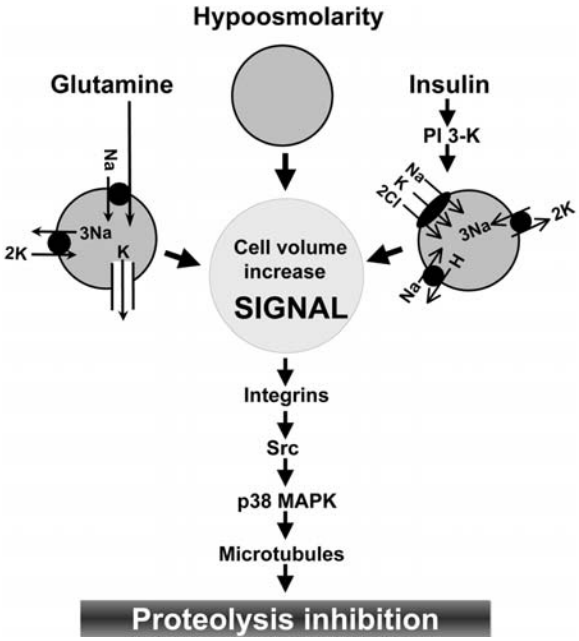

Figure 5. Glutamine-induced proteolysis inhibition critically depends on integrin-dependent cell volume sensing and signaling. Concentrative glutamine uptake leads to hepatocyte swelling, which is only in part counterregulated by a volume-regulatory K^+ release. Insulin induces hepatocyte swelling by stimulating a PI 3-kinase-dependent net K^+ uptake. Ambient hypoosmolarity triggers cell swelling due to osmotic water influx, which is in part counterregulated by a volume-regulatory loss of anorganic ions and organic osmolytes (not shown). Hepatocyte swelling induced by either glutamine, insulin or hypoosmolarity is registrated by the integrin system. In the case of hypoosmolarity and insulin it was shown that the beta1 integrin subunit changes into the active conformation in a swelling-dependent manner. Integrin activation leads to a Src-dependent p38 MAPK activation which upstream (or at the side of) microtubule-dependent signals participates on inhibition of autophagic proteolysis. For references see text.

mTOR was suggested to play a central role for the coordinated regulation of protein synthesis and autophagic proteolysis (140-141). However, a general role of mTOR in regulation of proteolysis was recently questioned due to the insensitivity of autophagic proteolysis inhibition by hypoosmolarity, insulin, and amino acids in isolated hepatocytes or perfused rat liver to rapamycin (133;142-143), a highly specific inhibitor of mTOR signaling (144). This topic is discussed more detailed in (145). Glutamine activates p70S6-kinase in isolated hepatocytes, which depends on a calyculin A-sensitive protein phosphatase and is inhibited by activation of the AMP-activated protein kinase (131). mTOR-dependent glutamine signaling is not involved in stimulation of glycogen synthesis and acetyl-CoA carboxylase activity by the amino acid (131-132;146) but may play a role in regulation of protein synthesis.

4.2. Cell hydration and glutamine signaling

Uptake of extracellular glutamine potently induces hepatocyte swelling (8;14). It has been meanwhile well established that cell volume alterations induced by either anisoosmotic environments or under the influence of hormones, oxidative stress and substrates represent an independent signal contributing to the regulation of cell function and gene expression. Changes in cell hydration within a narrow, physiological range markedly affect carbohydrate and protein metabolism as well as hepatic bile flow. Somewhat generalizing one can state that moderate swelling stimulates anabolic pathways and proliferation and protects the cells from different kinds of stress, whereas moderate shrinkage triggers catabolism and insulin resistance, and sensitizes cells to apoptotic challenges. Isoosmotic cell swelling due to retention of inorganic ions and amino acids is obligatory during the cell cycle whereas cell shrinkage as a result of osmolyte release is an early hallmark of apoptosis (147-150). Hepatic cell hydration critically determines protein and carbohydrate metabolism, bile salt transport, proliferation, and the susceptibility to oxidants, heat, and CD95 (151). In particular, a close correlation between autophagic proteolysis and the hepatocellular hydration state was observed, i.e. stimulation/inhibition of proteolysis under different conditions corresponds well to the respective degree of cell swelling or shrinkage (152).

Among the substrates inducing cell swelling glutamine is especially potent due to the high concentrative capacity of the system N (SNAT 3) in liver and skeletal muscle, which in essence combines the activities of glutamine/Na^+ symport with a Na^+/H^+ exchanger activity. Thus, the availability of extracellular glutamine and the activity of the Na^+-coupled system N transport are major determinants of cell hydration (8;14;29). Multiple glutamine effects are mediated by glutamine-induced cell swelling, which include stimulation of glycogen synthesis (153), activation of fatty acid synthesis (154), inhibition of autophagic proteolysis (21;155-156), alkalinization of endocytotic vesicles (157-159), stimulation of canalicular bile salt excretion (160-161), reduction of oxidized glutathione excretion into bile (147) and prevention of apoptosis (18-19).

Cell volume changes are registered by osmosensing structures, which activate signal transduction contributing to the regulation of cellular metabolism and gene expression. Progress during the last ten years in the understanding of cell volume sensing and signaling has been made not only in response to hypoosmolarity but more importantly as an integrated part of overall signal transduction triggered by insulin and glutamine, respectively (13-14;22). Currently, cell volume-dependent glutamine signaling towards inhibition of autophagic proteolysis, stimulation of canalicular bile acid excretion, glycogen and fatty acid synthesis and inhibition of apoptosis are fairly well understood.

4.2.1. Proteolysis inhibition

Hypoosmotic swelling, glutamine, and insulin in perfused rat liver potently inhibit autophagic proteolysis (Figure 5) (155;162-165). It was shown that proteolysis inhibition by glutamine and insulin largely depends on the degree of cell swelling induced by the respective agents

(152). For instance in livers from starved rats cumulative glutamine accumulation is enhanced compared to that found in livers from fed rats, leading to increased cell swelling and accordingly enhanced inhibition of autophagic proteolysis by glutamine (13;152;155). Proteolysis inhibition by insulin in perfused rat liver is abolished if insulin-induced swelling is blocked by loop diuretica, glucagon, starvation, hyperosmolarity, or PI 3-kinase inhibitors (163;165;166). Colchicine interferes with swelling dependent proteolysis inhibition by hypoosmolarity, insulin and glutamine without affecting the cell volume increase, indicating that microtubules are involved in coupling swelling to proteolysis inhibition (167).

More recent investigations unravelled the role of integrins and p38 MAP-kinases in swelling-dependent inhibition of hepatic proteolysis (Figure 5). Already 250 nM of the p38 inhibitor SB203580 potently abolishes proteolysis inhibition but not cell swelling induced by hypoosmolarity, insulin, and glutamine in perfused rat liver, indicating that p38 inhibition uncouples the cell volume increase from proteolysis inhibition (142). In the case of insulin it was shown that antagonizing cell swelling by bumetanide, PI 3-kinase inhibitors and hyperosmolarity largely inhibited p38 activation by insulin (166). Although the immediate effector of p38 signaling in this context is currently unknown, swelling dependent p38 activation was localized upstream or at the side of the microtubular system due to the insensitivity of hypoosmotic p38 activation to colchicine (168).

As the volume sensitive signaling system most upstream in osmosensing/signalling towards hepatic proteolysis inhibition recently the integrins were identified (Figure 5) (133;169). Integrins are highly conserved transmembrane adhesion receptors which link extracellular matrix components to intracellular signaling modules (170-172). Integrins are essentially involved in the sensing of mechanical perturbances and transducing them into intracellular signaling (mechanotransduction). In addition, integrins are known components in growth factor signaling: the binding of integrins to extracellular matrix ligands may activate growth factor receptors and/or increase the efficacy of growth factor-induced signaling (173-175). The perturbation of integrin/matrix interactions with the hexapeptide GRGDSP potently inhibits the antiproteolytic response to hypoosmolarity, insulin and glutamine, respectively (133;169). Likewise, inhibition of the tyrosine kinase Src with PP2 prevents proteolysis inhibition by hypoosmolarity, insulin and glutamine (133;169). In the case of hypoosmolarity and insulin it was shown that both, GRGDSP and PP2 prevent p38 activation and that GRGDSP abolishes the activating Src phosphorylation, suggesting Src to act here as an effector of integrins localized upstream of p38 (133;169). GRGDSP and PP2 do not reduce cell swelling by hypoosmolarity, insulin and glutamine and thus like SB203580 (142) uncouple swelling from proteolysis inhibition (133;169). Interestingly, swelling-independent proteolysis inhibition by phenylalanin is insensitive to colchicine, SB203580, GRGDSP, and PP2, respectively (142;167;169), underlining the specific interference of these inhibitors with swelling-dependent signaling. Overall the findings suggest that sensing of cell swelling by integrins essentially contributes to insulin and glutamine signaling, thereby defining a novel way of integrin involvement in growth factor and amino acid signaling.

4.2.2. Canalicular bile acid excretion

Bile formation is an osmotic process brought about by the vectorial transport of solutes from the sinusoidal space to the canalicular lumen (151;176-178). A major site of control is the excretion of cholephilic compounds across the canalicular membrane of the hepatocyte by transport ATPases including the conjugate export pump multidrug resistance-associated protein (MRP)2 for transport of glutathione and glucuronide conjugates and the bile salt export pump (BSEP) for excretion of conjugated bile acids. Canalicular secretion by these transporters is strongly regulated by the hepatocellular hydration state (160-161). Roughly, an increase of cell water by 10% almost doubles the transport capacity for taurocholate into bile within minutes. This occurs in a microtubule-dependent way (179) and is explained by the rapid insertion of MRP2 and BSEP transporter molecules, which are stored underneath the canalicular membrane inside the cell, into the canalicular membrane (180-183). Conversely, cell shrinkage triggers cholestasis by a rapid retrieval of these transporters from the canalicular membrane.

Similar to hypoosmotic exposure, glutamine, at physiologically relevant concentrations, stimulates biliary excretion due to glutamine-induced cell swelling (160-161). Endotoxin exerts its cholestatic effect in liver on a short-term time scale by transporter retrieval from the canalicular membrane (181) and on a long-term time scale by downregulation of BSEP and MRP2 expression (184). Under these conditions, the canalicular excretion of leukotriene C4 metabolites, which are MRP2 substrates, is compromised, and this phenomenon may be of relevance in septic shock. Interestingly, hepatocyte swelling by glutamine (and likewise hypoosmolarity) significantly stimulates excretion of leukotriene C4 metabolites into bile under these conditions (185).

The signaling events that trigger swelling-dependent insertion of BSEP and MRP2 by hypoosmolarity into the canalicular membrane were studied in detail and are most likely identical to those triggering the choleretic effect of glutamine. Inhibition of integrins, Src, p38, and Erk signaling by GRGDSP, PP2, SB203590 and PD098059, respectively in perfused rat liver unraveled that an integrin-dependent Src activation essentially mediates the activation of p38 and Erk-type MAP-kinases (186-188) and it was suggested that p38- and Erk-dependent signals converge upstream of the microtubule-dependent signaling step(s) (168). The therapeutic potential of glutamine in cholestatic liver disease has yet to be evaluated, but it is conceivable that its beneficial effects in septic states are due to multiple sites of action, including an augmentation of canalicular secretion.

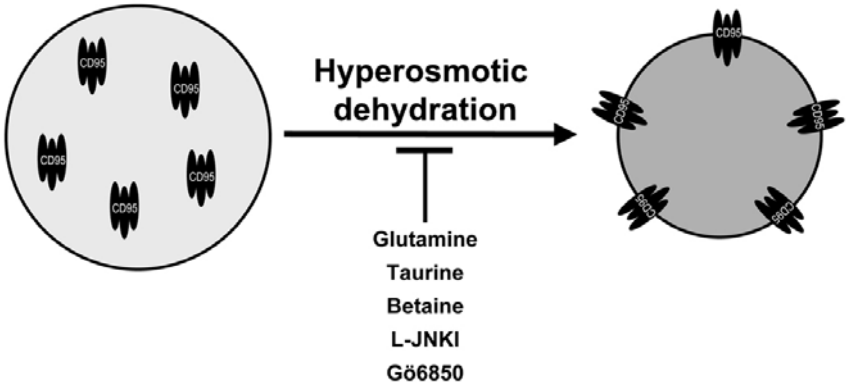

Figure 6. Glutamine antagonizes the hyperosmotic trafficking of CD95 from intracellular compartments to the plasma membrane. Hyperosmotic sensitization of rat hepatocytes to CD95L-induced apoptosis is related to the translocation of CD95 from intracellular pools to the plasma membrane (19). Hyperosmotic CD95 membrane trafficking is potently antagonized by glutamine but also by betaine and taurine, suggesting that glutamine acts as a compatibe organic osmolyte supporting the cellular adaptation to the dehydrating condition. Also the c-Jun-N-terminal kinase (JNK) inhibitory peptide L-JNKI and the PKC inhibitor Gö6850 effectively antagonize hyperosmotic CD95 trafficking to the plasmamembrane. To what extent glutamine, taurine and betaine affect the hyperosmotic activation of JNKs and PKCs is currently not known. For further discussion see text.

4.2.3. Glycogen and fatty acid synthesis

Isolated hepatocytes were used to examine the stimulation of liver glycogen and fatty acid synthesis due to hypoosmotic or glutamine-induced swelling (132;146;153). The swelling-dependent activation of hepatic glycogen synthase by glutamine has been attributed to the stimulation of glycogen-synthase-phosphatase resulting from both, an increase of the intracellular concentration of glutamate and aspartate and a volume-regulatory decrease in intracellular chloride (153). A similar mechanism has been suggested for the swelling-dependent activation of the acetyl-CoA synthetase (154). In addition, both, hypoosmolarity and glutamine activate PI 3-kinase in isolated hepatocytes, which was found to mediate the activation of glycogen synthase and acetyl-CoA synthease by hypoosmolarity and glutamine, respectively (146) The PI 3-kinase inhibitor wortmannin does not affect swelling by glutamine and hypoosmolarity, respectively (146), corroborating the view that hepatocyte swelling mediates PI 3-kinase activation by glutamine. The mechanisms of cell volume sensing and signaling upstream of PI 3-kinase in isolated hepatocytes are currently unknown. Because cell matrix interactions in isolated hepatocytes are largely absent, the integrin system is most likely not involved. The importance of integrins for the entire metabolic response to hepatocyte swelling is supported by the resistance of isolated hepatocytes to hypoosmolarity and insulin with regard to proteolysis inhibition (130;189) and Erk activation (146).

4.2.4. Insulin resistance and apoptosis

The dehydration of insulin target tissues represents an important factor contributing to insulin resistance. This may explain why endocrine and metabolic disturbances in severely diabetic patients are in part reversible already after adaequate rehydration therapy. In addition, rehydration improves the patient´s sensitivity to low doses of therapeutically applicated insulin (190-191). Vice versa an artificial systemic hyperosmolarity induces insulin resistance in healthy subjects (192). A hyperosmotic insulin resistance was also observed in *in vitro* studies with isolated tissues and cell culture models. Both, hyperosmotic NaCl and urea impair insulin-induced glucose uptake by epididymal fat pads (193). Likewise, insulin-stimulated glucose uptake by rat hemidiaphragms (194) and isolated rat adipocytes (195) is suppressed by hyperosmolarity. At least some insulin-responsive signaling components which are sensitive to hyperosmolarity are localized around mTOR. The hyperosmotic inhibition of insulin-induced glucose uptake, glycogen synthesis and lipogenesis in 3T3L1 adipocytes is related to a hyperosmotic inactivation of PKB (196) and a hyperosmotic inhibition of insulin-induced PKB activation (197). Hyperosmolarity also interferes with the mTOR-dependent induction of MAP-kinase phosphatase MKP-1 expression by insulin in H4IIE rat hepatoma cells (198). Similar to hyperosmolarity, amino acid starvation leads to cell shrinkage and cell shrinkage was suggested to mediate certain responses to amino acid starvation including stimulation of the tonicity-sensitive element binding protein (TonEBP) (199) and stimulation of osmolyte transport (200). In view of the importance of glutamine for maintaining cell hydration one is tempted to speculate that cellular dehydration by glutamine deficiency plays a major role in mediating insulin resistance by amino acid starvation.

Cell shrinkage at the beginning of apoptosis, (apoptotic volume decrease, AVD) is an early prerequisite for the apoptotic machinery. The contribution of AVD to apoptotic signal transduction is not yet completely understood; hyperosmotic shrinkage does not necessarily trigger apoptosis. For instance in cultured rat hepatocytes, moderate hyperosmolarity (405 mosmol/l) activated the CD95 system (19;201-202). Hyperosmolarity within one minute increased production of reactive oxygen species and trafficking of the CD95 from inside the hepatocyte to the plasma membrane (Figure 6) (19). Although ineffective to induce apoptosis by itself, hyperosmolarity sensitized the hepatocytes towards CD95 ligand-induced apoptosis (19),

indicating a synergistic interplay between signals triggered by hyperosmotic shrinkage and CD95 ligand, respectively. Glutamine, similar to compatible organic osmolytes taurine and betaine, prevents hyperosmotic CD95 trafficking to the plasma membrane (19), perhaps by facilitation of cell volume recovery. Almost complementary to these findings, glutamine deprivation of leukemia-derived CEM and HL-60 cells leads to cell shrinkage, which in turn triggers CD95-mediated and ligand-independent apoptosis (18). Compatible organic osmolytes prevented apoptosis by glutamine deprivation in these cells (18).

5. PERSPECTIVE

As detailed in this article hepatic glutamine metabolism is importantly involved in systemic pH regulation and ammonia detoxification. Glutamine in the liver not only acts as a substrate in biosynthetic pathways, but also represents a trigger of signals involved in the regulation of cell growth and proliferation. Importantly, the availability of glutamine by either uptake or synthesis may critically determine HCC growth and metastatic potential probably by acting as both, substrate in energy metabolism and nucleotide synthesis and by activating proliferative and anti-apoptotic signal transduction, in part by the induction of cell swelling. Progressive analysis of inherited defects of GS and other glutamine metabolizing enzymes as well as glutamine transporting systems as well as appropriate genetic animal models will provide further insights into the specific actions of glutamine and give further rationale for the application of glutamine as a therapeutic tool.

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Abbreviations: AP-1: activator protein 1; ASK: apoptosis signal-regulating kinase; caspase: cystein-aspartate perotease; BSEP: bile salt export pump; CoA: coenzyme A; eIF-4E: eukaryotic initiation factor 4E-BP; EGF: epidermal growth factor; Erk: extracellular signal-regulated kinase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; GRE: glucocorticoid responsive element; GS: glutamine synthetase; GSSE-BP: GS silencer element of the rat binding protein; GSSEr: GS silencer element of the rat; GSK: glycogen synthaase kinase; HCC: hepatocellular carcinoma; HSC: hepatic stellate cells; IL: interleukin; JNK: c-Jun-N-terminal kinase; MAP: mitogen-activated protein; MKP: MAP-kinase phosphatase; MRP: multidrug resistance-associated protein; MSO: methionine sulfoximine; mTOR: mammalian target of rapamycin; OAT: ornithine amidotransferase; p70S6-kinase: p70 ribosomal S6 protein kinase; PI 3-kinase: phosphoinositide 3-kinase; PKB: protein kinase B; PKC: protein kinase C; SNAT: sodium-coupled neutral amino acid transporters; SMA: smooth muscle actin; TGF: tissue growth factor; TNF: tumor necrosis factor; TonEBP: tonicity-sensitive element binding protein

Key Words: Acidosis, Amino acids, Ammonia, Apoptosis, Bile acids, CD95, Cell cycle, Cell volume, Cirrhosis, Glutaminase, Glutamine synthetase, Epidermal growth factor, Inflammation, Insulin, Integrins, Interleukin-6, MAP-kinases, Mammalian target of rapamycin, Microtubules, Mitochondria, Modeling, Proliferation, Proteolysis, Sepsis, tumor, Tyrosine nitration, Urea, Review

Send correspondence to: Professor Dr. Dieter Häussinger, Universitätsklinikum der Heinrich-Heine-Universität Düsseldorf, Klinik für Gastroenterologie, Hepatologie und Infektiologie, Moorenstrasse 5, D-40225 Düsseldorf, Germany, Tel.: 49-211-81-17569, Fax: 49-211-81-18838, E-mail: haeussin@uni-duesseldorf.de

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