Rat liver endothelial cell glutamine transporter and glutaminase expression contrast with parenchymal cells

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Lohmann, Rüdiger, Wiley W. Souba, and Barrie P. Bode. Rat liver endothelial cell glutamine transporter and glutaminase expression contrast with parenchymal cells. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G743–G750, 1999.—Despite the central role of the liver in glutamine homeostasis in health and disease, little is known about the mechanism by which this amino acid is transported into sinusoidal endothelial cells, the second most abundant hepatic cell type. To address this issue, the transport of L-glutamine was functionally characterized in hepatic endothelial cells isolated from male rats. On the basis of functional analyses, including kinetics, cation substitution, and amino acid inhibition, it was determined that a Na+-dependent carrier distinct from system N in parenchymal cells, with properties of system ASC or B0, mediated the majority of glutamine transport in hepatic endothelial cells. These results were supported by Northern blot analyses that showed expression of the ATB0 transporter gene in endothelial but not parenchymal cells. Concurrently, it was determined that, whereas both cell types express glutamine synthetase, hepatic endothelial cells express the kidney-type glutaminase isozyme in contrast to the liver-type isozyme in parenchymal cells. This represents the first report of ATB0 and kidney-type glutaminase isozyme expression in the liver, observations that have implications for roles of specific cell types in hepatic glutamine homeostasis in health and disease.

The pleiotropic role of circulating glutamine as an important metabolic fuel for dividing cells and as the major nontoxic shuttle of ammonia between tissues is underscored by its rapid cellular turnover rates (13) and presence at 0.6 mM in the plasma. It is well established that the liver plays a unique role in glutamine metabolism and that its ability to rapidly switch from net production to consumption of this amino acid is afforded by the differential position-dependent expression of specific glutamine-metabolizing enzymes in parenchymal cells (PCs) along the liver acinus (20). Portal and arterial glutamine is taken up by the large parenchymal cells (PCs) surrounding the proximal end of each sinusoid. Translocated glutamine is subsequently hydrolyzed to ammonia and glutamate by the mitochondrial glutaminase isozyme, and the ammonia generated is detoxified by the urea cycle (44). In contrast, ammonia that escapes detoxification by the urea cycle is “scavenged” and utilized to generate glutamine via the enzyme glutamine synthetase (GS) by a small population (representing 5–7% of the total PCs) of “periportal hepatocytes” surrounding the venous outflow. Thus the relative flux through each opposing pathway of this intercellular glutamine cycle (21) determines the net glutamine balance across the liver and may be controlled by the transport of glutamine across the plasma membrane (22, 32).

Although PCs constitute 70% of the total number of cells and nearly 90% of liver mass, sinusoidal nonparenchymal cells (NPCs) such as lipocytes and endothelial and Kupffer cells comprise ~30% of hepatic cellularity and 27% of its total plasma membrane content (3). Among the NPC types, sinusoidal endothelial cells that form the “sieve plates” are the most abundant, representing 2.5% of the lobular parenchyma (3) and from 38% (18) to 75% (31) of the total number of NPCs, based on protease-digested rat liver cell suspensions. Glutamine transport has been well characterized in isolated PCs from both rat (29) and human (5) livers. In both species, it is mediated by a unique Na+-dependent transporter that plays a central role in support of hepatic nitrogen metabolism (7, 22, 32) and has been termed system N for its narrow substrate specificity of only glutamine, histidine, and asparagine, amino acids with nitrogen-containing side chains. To date, transport processes in hepatic endothelial cells have focused exclusively on the receptor-mediated uptake of ligands such as lipoproteins, plasma proteins, and matrix components (8, 15), but, to our knowledge, no studies have yet examined amino acid transport processes in this second most abundant liver cell type. Given the important role of glutamine in hepatic physiology and metabolism, the studies presented here were undertaken to compare the uptake of this amino acid in liver endothelial cells (LECs) with the well-characterized PCs. The results show that the expression of isozymes for the transport and metabolism of glutamine is cell specific, reflecting the differential metabolic role of this amino acid in each cell type.

Materials and Methods

Radiolabeled L-[3H]glutamine and [α-32P]dCTP were obtained from DuPont NEN (Boston, MA). The digestive enzymes pronase and collagenase were from Boehringer Mannheim (Indianapolis, IN), and chemicals, perfusion media [suspension culture minimal essential medium (S-MEM)], and unlabeled amino acids were from Sigma (St. Louis, MO). Tissue culture medium and all additives were from Gibco BRL Life Technologies (Gaithersburg, MD), and supplies and chemicals for scintillation spectrophotometry were from Packard Instrument (Meriden, CT).

LEC isolation. Male Sprague-Dawley rats (500–700 g) were obtained from Charles River Laboratories (Wilmington,
GLUTAMINE TRANSPORT IN RAT HEPATIC ENDOTHELIAL CELLS

MA). Animals were housed in the Massachusetts General Hospital animal facility under controlled conditions of 12:12-h light-dark cycles and ad libitum access to chow and water. All experimental procedures were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee/Subcommittee on Research Animal Care, according to the guidelines in the Guide for the Care and Use of Laboratory Animals. To minimize nutritional influences on experimental results, all animals were subjected to an overnight fast before surgery. LECs were isolated by a modified pronase-collagenase digestion procedure established by others (18, 26, 30, 31), followed by density gradient centrifugation (18) and a final centrifugal elutriation step (18, 26, 30, 31). Briefly, rats were anesthetized intraperitoneally (75 mg/kg ketamine, 5 mg/kg xylazine, and 1 mg/kg acepromazine; Henry Schein, Port Washington, NY), laparatomy was performed, and the portal vein and inferior vena cava were cannulated with 16- and 14-gauge Teflon angiocatheters, respectively. The liver was cleared of blood by antegrade perfusion (20 ml/min) with 100 ml of warm (37°C) Ca2+-free S-MEM. Thereafter, the flow rate was reduced to 10 ml/min and the liver was first digested with pronase (2 mg/ml) in 100 ml of a Ca2+-containing balanced salt solution [BSS; containing (in mM) 140 NaCl, 10 HEPES (pH 7.4), 5 KCl, 5 glucose, 25 NaHCO3, and 0.5 CaCl2] followed by collagenase digestion (0.35 mg/ml) in 100 ml of Ca2+-containing S-MEM. The digested liver was removed, and cells were released by gentle agitation into 100 ml of BSS containing 30 mg pronase in a siliconized Erlenmeyer flask and then placed on a heat-calibrated (37°C) stir plate with a magnetic stir bar for an additional 30 min. In a subset of experiments, the pronase steps were eliminated and only collagenase was used for liver digestion; isolation of PC was performed in these preparations as described previously (17) for use in comparison studies. Afterward, 10 ml of fetal bovine serum were added to the mixture to help neutralize proteolytic activity, and the suspension was passed over eight-ply sterile gauze in a metal cell strainer into two 50-ml centrifuge tubes.

The suspension was subjected to two centrifugations at 50 g for 2 min to remove any remaining PC, and the supernatant containing the NPC was subjected to a final centrifugation at 500 g for 5 min. The NPC pellet was resuspended in 25 ml of S-MEM containing 10 µg/ml of DNase I, passed over a 40-µm cell strainer, and carefully layered on top of a discontinuous gradient of arabinogalactan density medium (Cellsep, Larex, St. Paul, MN) (18). After centrifugation at 2,500 g for 5 min. The NPC pellet was resuspended in 25 ml of S-MEM. Thereafter, the flow rate was reduced to 10 ml/min and the liver was first digested with pronase (2 mg/ml) in 100 ml of a Ca2+-containing balanced salt solution [BSS; containing (in mM) 140 NaCl, 10 HEPES (pH 7.4), 5 KCl, 5 glucose, 25 NaHCO3, and 0.5 CaCl2] followed by collagenase digestion (0.35 mg/ml) in 100 ml of Ca2+-containing S-MEM. The digested liver was removed, and cells were released by gentle agitation into 100 ml of BSS containing 30 mg pronase in a siliconized Erlenmeyer flask and then placed on a heat-calibrated (37°C) stir plate with a magnetic stir bar for an additional 30 min. In a subset of experiments, the pronase steps were eliminated and only collagenase was used for liver digestion; isolation of PC was performed in these preparations as described previously (17) for use in comparison studies. Afterward, 10 ml of fetal bovine serum were added to the mixture to help neutralize proteolytic activity, and the suspension was passed over eight-ply sterile gauze in a metal cell strainer into two 50-ml centrifuge tubes.

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Amino acid transport assay. Measurement of initial rate of glutamine uptake was carried out via the duster-tray method (19), as reported previously (5). The uptake of [L-3,4-3H(N)]glutamine was measured in the presence of specific amounts of unlabeled L-glutamine as indicated. For kinetic studies, the amount of unlabeled glutamine in the transport buffer varied from 10 µM to 8 mM. After an initial two rinses with Na+-free Krebs-Ringer phosphate buffer (KRP), all transport measurements were carried out at 37°C in either Na+-free KRP or Na+-containing KRP and were terminated after 1 min. Intracellular radiolabeled glutamine was extracted with 0.2 ml iodoacetic acid buffer (0.2% SDS + 0.2 N NaOH; after 1 h, 0.1 ml of the lysate was neutralized with 10 µl of 2 N HCl and subjected to scintillation spectrophotometry (TopCount, Packard Instruments). The remaining lysate was utilized for determination of cellular protein by the bicinchoninic acid method (Pierce Chemical, Rockford, IL). Rates of glutamine transport were calculated from the counts per minute (cpm) per sample and the specific activity of the uptake mix (in cpm/nmol) and normalized to cellular protein content in a 0.1 ml sample. Transport values obtained in the presence of extracellular Na+ (diffusion and Na+-dependent uptake) were subtracted from those in the presence of Na+ (total uptake) to yield Na+-dependent rates (reported in units of mmol/mg protein·min-1). All transport values depicted are the average ± SD of four separate determinations. Kinetic analysis of transport data was performed by linear and nonlinear regression analysis in Cricket Graph (Computer Associates, Islandia, NY).

Statistical analysis. Differences in specific measured parameters between experimental conditions were evaluated for statistical significance by paired t-test (Microsoft Excel) or ANOVA for multiple comparisons (Statview, Abacus Concepts, Berkeley, CA). Relative differences were considered significant at P < 0.050.
RNA isolation and Northern blot procedure. Total cellular RNA was isolated from isolated LECs, PCs, or frozen tissues (liver or kidney) by the one-step acid-phenol guanidinium procedure (11) using Trisolve reagent (Biotecx, Houston, TX), followed by an additional acid-phenol, phenol-chloroform-isoamyl alcohol, chloroform extraction and ethanol precipitation in the presence of sodium acetate. Equal amounts of total RNA (20 µg), as determined both spectrophotometrically and through ethidium bromide staining, were fractionated by electrophoresis through denaturing 1% agarose gels containing 0.2 M formaldehyde, transferred to nylon membranes by capillary action, and ultraviolet cross-linked to the membrane. The cDNAs utilized in this study to generate radiolabeled probes were full-length rat ATB0 (rATB0; SalI/NotI, 2.7-kb fragment) in pSPORT1 and kindly provided by Dr. Vadivel Ganapathy (27). Rat liver glutaminase (GAl; EcoRI, 0.6- and 1.4-kb fragments) in pBluescript II SK was kindly provided by Dr. Malcolm Watford (39), rat kidney type glutaminase (GAK; ClaI/AccI, 1.3-kb fragment) in pBluescript (2) was kindly provided by Dr. Norman Curthoys (38), and rat GS (BamHI, 0.8-kb fragment), originally derived from pGSRK-1 (9) in pBluescript II KS, was kindly provided by Dr. John F. Mill. The cDNA inserts containing primarily coding sequence were excised from the plasmid with appropriate restriction enzymes, separated on agarose gels, excised, eluted, and used as templates to generate [α-32P]dCTP-labeled probes using a random primer labeling kit (Megaprime, Amersham, Arlington Heights, IL) according to the manufacturer’s protocol. Hybridization with radiolabeled probe was performed overnight at 65°C in 5× sodium chloride-sodium phosphate-EDTA (SSPE) with 7.5× Denhardt’s reagent, 0.5% SDS, and 0.1 mg/ml sheared herring sperm DNA, after the membrane was preblocked for 2 h under the same conditions. Blots were washed at 55°C three times each for 10 min in decreasing concentrations of SSPE and increasing concentrations of SDS until 0.1× SSPE and 1.0% SDS was reached. Autoradiographic detection of the hybridization was achieved by exposure of Fuji Medical X-ray film at −80°C. The hybridized probe was stripped off the membrane by boiling in 0.1% SDS, and the blots were reutilized for the Northern analyses of other genes.

RESULTS

Purity of endothelial cells. On attachment, cells eluted from the rotator at 25 ml/min displayed the characteristic “spider web” cytoplasmic fenestrations characteristic of LECs, as well as cytoplasmic (red) fluorescence in preparations from the animals injected with Di-I-AcLDL before liver digestion (Fig. 1). In contrast, isolated PCs failed to display this fluorescence as did uninjected animals (not shown). Furthermore, the cells in the 25-ml/min elutriated fraction failed to display the perinuclear peroxidase-dependent diaminobenzidine staining characteristic of Kupffer cells (4, 45). This staining was evident, however, in (presumably) Kupffer cells in the 40-ml/min eluted fraction plated on uncoated tissue culture dishes. Collectively, the data suggest that the isolated NPC fraction utilized in these studies is highly enriched in LECs. The cell purity and transporter characteristics (listed below) remained unaffected whether a combination of pronase and collagenase or collagenase alone was utilized.

Glutamine transport in LECs. Glutamine transport in isolated LECs was determined in the presence of choline, Li+, or Na+ as the major cation for 1 min. The...
1-min assay time was determined to be appropriate for initial rate transport measurements, based on preliminary time courses as previously described (5). As shown in Fig. 2, similar to PCs the majority of glutamine uptake in LECs is Na\(^{+}\)-dependent (62%), whereas Na\(^{+}\)-independent and nonsaturable uptake accounted for 24% and 14% of total transport values, respectively. When the glutamine concentration was reduced to 10 \(µ\)M, the Na\(^{+}\)-dependent component increased to 78% of total transport values (0.090 ± 0.016 nmol·mg protein\(^{-1}\)·min\(^{-1}\), respectively), whereas at 600 \(µ\)M glutamine (physiological levels) the Na\(^{+}\)-dependent contribution (2.26 ± 0.9 nmol·mg protein\(^{-1}\)·min\(^{-1}\)) remained at 62% of the total uptake (3.62 ± 0.68 nmol·mg protein\(^{-1}\)·min\(^{-1}\)) values. Figure 2 also shows that the LECs were intolerant to Li\(^{+}\)-for-Na\(^{+}\) substitution to drive the concentrative uptake of 50 \(µ\)M L-glutamine, in contrast to PCs, where such tolerance is a well-established system N functional characteristic (5, 7, 29). These data suggest that the Na\(^{+}\)-dependent carrier expressed in LECs is distinct from the system N glutamine transporter in PCs.

The Na\(^{+}\)-dependent component in LECs was kinetically characterized (Fig. 3) and found to possess a much higher affinity for glutamine [Michaelis-Menten constant (\(K_m\)) = 127 ± 31 \(µ\)M] than system N (\(K_m\) = ~1 mM) (5, 7, 29). The maximal velocity of this LEC glutamine carrier (2.4 ± 0.5 nmol·mg protein\(^{-1}\)·min\(^{-1}\)) is in the same range as we have observed for PC system N in past studies (17). Further functional characterization of the high-affinity Na\(^{+}\)-dependent LEC glutamine transporter was carried out with amino acid inhibition analysis (Fig. 4). In contrast to system N in PC, histidine was less effective than alanine and serine in inhibiting LEC Na\(^{+}\)-dependent glutamine uptake. The average glutamine transport values relative to control rates in four separate LEC preparations were 57 ± 18, 22 ± 17, and 32 ± 29% in the presence of 5 mM unlabeled histidine, alanine, and serine, respectively (P < 0.010 vs. control for all). In contrast, 5 mM histidine blocked 98% of glutamine uptake, whereas alanine and serine were ineffective inhibitors in PC, characteristic of system N. In both cell types, glutamine uptake was insensitive to the system A-specific substrate \(\alpha\)-(methylamino)isobutyric acid (MeAIB). Arginine also inhibited glutamine uptake significantly but to a lesser extent in both cell types [to 62 ± 31 and 70 ± 16% of control values for LECs and PCs, respectively (P < 0.050)]. The branch chain amino acid analog 2-amino-2-norbornane-carboxylic acid exerted a small degree of inhibition on LEC glutamine uptake [to 79 ± 20% of control values (P < 0.050)] but failed to attenuate uptake in PCs. In summary, when coupled with the higher affinity and Li\(^{+}\)-intolerance of the LEC carrier, such alanine- and serine-inhibitable and MeAIB-insensitive glutamine transport is indicative of system ASC (5, 12, 43) or system B0 activity (16, 27, 28).

LEC gene expression. Recently, cDNAs were isolated that encode for system ASC (43) and system B0 (27, 28) glutamine transporters with characteristics similar to those reported here for the LECs. The system ASC gene was isolated from a mouse cDNA library and designated as ASCT2. The system B0 genes were isolated from human (27, 28) and rabbit (28) cDNA libraries, as well as from a rat cDNA library (V. Ganapathy, unpublished observations), and designated as ATB0 for amino acid transporter B0. Both the ASCT2 and ATB0 genes encode for glutamine transporters with nearly identical
substrate specificities and kinetic characteristics. The deduced amino acid sequences between mouse ASCT2 (553 amino acids) and human ATB0 (541 amino acids) are highly similar (85%), with a 79% amino acid identity. Both are members of the rapidly emerging glutamate transporter superfamily (24), which includes ASCT1, a system ASC isoform that, in contrast to ASCT2 and ATB0, does not transport glutamine or asparagine (1, 37). Because our studies focused on rat liver cells, we utilized the rat ATB0 cDNA (rATB0) to test for its expression in the LECs by Northern blot analysis (Fig. 5). A single band of ~2.8 kb was observed in both LEC and kidney but was not evident in RNA from whole liver or isolated PCs. The apparent single 2.8-kb ATB0 mRNA species and the lack of detectable expression in liver (probably attributable to the “swamping out” of LEC-derived by PC-derived RNA) and evident expression in the kidney are consistent with results reported previously for this glutamine transporter (27, 28). Nonetheless, the expression of ATB0 in the LEC is consistent with the functional glutamine transport characteristics presented above; unfortunately, the gene responsible for system N activity has not yet been isolated.

Figure 5 also illustrates that, whereas both liver cell types express GS (apparent 3.1- and 1.7-kb bands), LECs express GAx (apparent 6.1- and 3.2-kb bands) and PCs express GAL (single band of ~2.6 kb). These observed mRNA sizes are consistent with those reported previously for each of these genes (9, 38, 39). Similar to ATB0, the GAx isozyme possesses a much higher affinity for glutamine (Km = ~2–5 mM) compared with GAL (Km = 8–40 mM) and different regulatory properties as well (44). These results indicate that both cell types possess the ability to produce glutamine, but LECs express markedly different genes for its transport and hydrolysis compared with those in PCs.

DISCUSSION

This study represents the first report on differences between liver cell types with respect to glutamine transport and the first report on the expression of ATB0 and GAx in normal liver, a finding previously masked by the preponderance of hepatic PC-derived RNA. Prior studies showed that LECs utilize glutamine as a respiratory substrate more readily than PCs (41). We show here that the concurrent expression of ATB0 and GAx in LECs probably contributes to this phenotype. The
ammonia generated from the action of GA\textsubscript{L} is readily channeled into the urea cycle via carbamoyl phosphate synthetase-1, whereas the ammonia generated from GA\textsubscript{K} often escapes as free ammonia (44). Thus each glutaminase isozyme serves a different role in the metabolism of individual liver cell types. With respect to glutamine uptake in endothelial cells, this process has also been attributed to system \textsuperscript{ASC-like} transporters in porcine pulmonary artery (25), rat lung microvascular (35), and human umbilical vein (10) endothelial cells. However, despite their common mesodermal origin, there are profound morphological and biochemical differences between LECs and vascular endothelium, including the pronounced fenestrae, which account for the \textquoteleft;sieve-plate\textquoteright morphology of the hepatic sinusoids (26). As demonstrated here, the system \textsuperscript{ASC-like} glutamine uptake in LECs is attributable to the expression of the \textit{ATB\textsubscript{0}} gene (Fig. 5), which raises the possibility that this gene product may also mediate vascular endothelial cell glutamine transport previously described as system ASC by us (25, 35) and others (10).

System N-mediated glutamine across the plasma membrane of hepatocytes has been shown to represent a rate-limiting step in glutaminase-dependent glutamine metabolism, especially when intracellular utilization rates are accelerated (22, 23, 32). Similarly, it has been shown that system ASC-mediated glutamine transport plays an important role in metabolic regulation. For example, transport rates account for the 90% of glutamine turnover rates in cultured fibroblasts (14) and also govern growth rates in a human hepatoma cell line (6). Because Na\textsuperscript{+}-dependent transporters such as systems N and ASC/B\textsubscript{0} utilize the energy present in the Na\textsuperscript{+} electrochemical gradient to drive the concentrative uptake of substrates against their transmembrane gradient, their operation maintains cytoplasmic amino acid levels far above equilibrium. One reason that the Na\textsuperscript{+}-dependent components of glutamine uptake play a quantitatively important role in governing cellular metabolic rates is that their activity is much higher than the Na\textsuperscript{+}-independent components that allow net glutamine efflux (see Fig. 2). It is also the hepatic Na\textsuperscript{+}-dependent components of glutamine uptake that increase during catabolic states, whereas there is little alteration in the Na\textsuperscript{+}-independent components that allow dissociation of transmembrane amino acid gradients (33). In a well-designed study, Low and colleagues (32) quantitatively evaluated the contribution of system N to hepatocyte glutamine metabolism. The contributions of Na\textsuperscript{+}-dependent and -independent transporters, mitochondrial transporter, glutaminase, proteolysis, and minor metabolic pathways were assessed for their role in observed glutamine utilization rates. Because GS is present in only 5% of the total liver PCs, this pathway was not considered in that study, although the capacity of this small population of cells to synthesize glutamine is enormous (Fig. 5 and Ref. 21). The authors concluded that, in the presence of physiological extracellular levels of histidine, system N possesses a significant flux control coefficient (0.51) and regulates glutaminase rates by adjusting cytoplasmic glutamine levels through electrochemical-dependent changes in its activity. System N activity was also found to be less than that of the mitochondrial transporter that supplies glutaminase, the major glutamine metabolizing enzyme in liver; in this sense, it can be considered a rate-limiting step in the regulation of glutamine catabolism. Together, it is apparent that cellular glutamine is governed by relative rates of synthesis and utilization, but Na\textsuperscript{+}-dependent transporters contribute significantly to flux through pathways such as glutaminase.

The upregulation of hepatic transport activities during catabolic states may be especially crucial, when intracellular amino acid utilization rates are accelerated. Previous studies from our laboratory have demonstrated a marked increase in system N activity in PCs isolated from animals treated with bacterial lipopolysaccharide (LPS) (17), a response that probably contributes to the increase in net hepatic glutamine uptake observed during endotoxemia (2). Spolarics and Wu (40, 42) have studied LPS-induced effects on LEC pathways of reactive oxygen species detoxification, which are activated by this inflammatory agent and largely dependent on the pentose phosphate pathway and maintenance of intracellular glutathione levels. Because glutamine plays an essential role in glutathione metabolism, its uptake in LECs might be expected to be accelerated in response to LPS, similar to system N in PCs. Enhancement of \textit{ATB\textsubscript{0}} expression and activity in LECs during catabolic states may be necessary, given its much broader substrate specificity than system N, where glutamine must compete for uptake with more circulating amino acids. As shown in Fig. 5, however, LECs not only possess the ability to transport and metabolize glutamine but also have an appreciable capacity to produce it via GS. On the basis of the markedly different enzymes for glutamine transport and hydrolysis in LECs, the response of the LECs may deviate from that of the metabolically compartmentalized PCs during catabolic states. Nonetheless, the studies presented here should serve as the basis for future investigations into the contribution of LECs to global hepatic glutamine economy.

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REFERENCES


