Tumor-influenced amino acid transport activities in zonal-enriched hepatocyte populations

ALEXANDRA M. EASSON, TIMOTHY M. PAWLIK, CRAIG P. FISCHER, JENNIFER L. CONROY, DENNIS SGROI, WILEY W. SOUBA, AND BARRIE P. BODE
1Surgical Oncology Research Laboratories, Department of Surgery, and 2Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Received 22 December 1999; accepted in final form 22 June 2000

Cancer is known to influence the nitrogen economy of the host, with a net efflux of amino acids such as glutamine from host tissues to tumors, where they are rapidly utilized (37). The methylcholanthrene (MCA)-induced fibrosarcoma is a transplantable tumor model in rats and has been used extensively by our group (10, 13, 15, 19, 41, 51) and others (7, 40, 47, 48) to study metabolic alterations in the host with cancer. It is a rapidly growing tumor that rarely metastasizes but is locally aggressive. Previous work with this model has shown that the liver switches from an organ of net glutamine balance to one of net release during tumor growth (10, 51). Surprisingly, concentrative (Na+ dependent) plasma membrane glutamine transport via system N is coordinately stimulated in the livers of tumor-bearing rats (TBR) by a mechanism that may partially involve the autocrine production of tumor necrosis factor-α by hepatocytes (21, 29). Liver arginine transport is also stimulated by tumor growth via an increased maximal velocity at the plasma membrane level (16) by a mechanism that may also involve tumor necrosis factor-α (29). These effects are tumor dependent, inasmuch as resection results in normalization of transport rates for both amino acids, albeit with different temporal kinetics (15).

The functional heterogeneity in glutamine metabolism along the liver acinus has been well established, with glutaminase (GAL) and urea cycle enzymes in the periportal (PP)- and perivenous (PV)-enriched rat hepatocyte populations. Na+ -dependent glutamate transport rates were eightfold higher in PV than in PP preparations but were relatively unaffected during tumor growth. System N-mediated glutamine uptake was 75% higher in PV than in PP preparations and was stimulated up to twofold in both regions by tumor burdens of 9 ± 4% of carcass weight compared with hepatocytes from pair-fed control animals. Excessive tumor burdens (26 ± 7%) resulted in hypophagia, loss of PV-enriched system N activities, and reduced transporter stimulation. Conversely, saturable arginine uptake was enhanced fourfold in PP preparations and was induced twofold only after excessive tumor burden. These data suggest that hepatic amino acid transporters are differentially influenced by cancer in a spatial and temporal manner, and they represent the first report of reciprocal zonal enrichment of system N and saturable arginine uptake in the mammalian liver.

Address for reprint requests and other correspondence: B. P. Bode, Dept. of Biology, St. Louis University, 3507 Laclede Ave., St. Louis, MO 63103-2010 (E-mail: bodebp@slu.edu).

http://www.ajpgi.org 0193-1857/00 $5.00 Copyright © 2000 the American Physiological Society G1209
den and food intake on these transport activities at the cellular level. We also chose to measure glutamine and arginine transport rates in hepatocytes from different positions in the liver acinus, inasmuch as this more refined examination may also offer initial clues into the disposition of each during tumor growth. These studies provide the first report of zone-dependent transport activities in the tumor-influenced liver, and the results indicate that glutamine and arginine transport rates are reciprocally enhanced along the acinus and are differentially enhanced by tumor growth.

MATERIALS AND METHODS

Animal tumor model. Adult male Fischer 344 rats (125-150 g; Charles River Laboratories, Wilmington, MA) were used in all studies, which received approval from the Massachusetts General Hospital Animal Care and Use Committee, according to the Guide for the Care and Use of Laboratory Animals. The animals were housed in individual wire cages under standard conditions (12:12-h light/dark cycle and ad libitum access to standard chow and water). After 1 wk of acclimatization, rats were randomly chosen to undergo bilateral subcutaneous flank implantation of 3-mm³ MCA-induced fibrosarcomas (TBR), as described previously (13), or a sham implantation procedure (control) under anesthesia. Control rats were pair fed to matched TBR chow consumption to control for tumor-induced anorexia. At specific times after tumor implantation, hepatocytes were isolated by the technique described below.

Tumor-induced anorexia. Food intake was measured daily, and tumor mass (in cm³) was determined as described by Morrison (38) every 48 h to day 27. To distinguish between the effects of duration and degree of tumor burden on the characteristic anorexia that develops in these animals, a separate group of 24 rats were implanted with one, two, or four MCA tumors on the same day (6 rats/group), and food intake was monitored daily thereafter.

Isolation of hepatocytes. To minimize the acute influence of prandial status on experimental results, all animals were subjected to an overnight fast before surgery on the following morning. Hepatocytes were isolated from the livers of animals by a modification (20) of the two-step collagenase perfusion technique of Seglen (49). For cell preparations enriched in hepatocytes from the PV or portal vein (PV) acinar zones, the livers were first perfused antegrade or retrograde, respectively, with a 0.5% (wt/vol) digitonin solution, as originally described (32, 42). Briefly, after laparotomy the portal vein and inferior vena cava were cannulated and sutured and food intake was monitored daily thereafter.

Livers were perfused exactly as outlined above but were removed before the collagenase perfusion step. Slices were obtained from the left median lobe and placed in 4% neutral-buffered formalin. Formalin-fixed samples were embedded in paraffin, mounted on glass slides, stained with hematoxylin and eosin, and photographed.

RNA isolation and Northern blot procedure. A portion of the final hepatocyte suspension (10⁷ cells) was removed from each preparation, centrifuged, and resuspended in Tri-Solv reagent (Biotex, Houston, TX). Total cellular RNA was isolated by the one-step acid-phenol guanidinium procedure followed by an additional acid-phenol, phenol-chloroform-isooamyl alcohol, chloroform extraction and ethanol precipitation in the presence of sodium acetate. Equal amounts of total RNA (20 μg), as determined spectrophotometrically and by 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 for Northern blot assays were rat phosphoehyruvate carboxykinase (PEPCK; Pst I, 2.67-kb fragment in pBR322, pPCK-10; American Type Culture Collection, Manassas, VA), rat liver Gα (Eco R I, 0.6- and 1.4-kb fragments in pBluescript SK II; kindly provided by Dr. Malcolm Watford), rat GS (1.6-kb Xho I fragment in pBluescript SK M13 (–); Stratagene, La Jolla, CA). 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 probes labeled with deoxy-[α-32P]CTP (NEB, Boston, MA) with 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 blocked 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 to 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 blot analyses of other genes. Quantitation of the hybridized mRNA bands was performed by laser densitometry (Molecular Dynamics, Sunnyvale, CA). The densitometric values for PP (PEPCK and Gα) and PV (GS) mRNA “markers” were normalized to those for β-actin in the same sample.
Arginine transporter expression. Expression of catio
tic amino acid transporter genes was examined by Northern 
blot analysis using probes specific for CAT-2, CAT-2A, and CAT-1
(35). The CAT-1 cDNA [EcoRI XhoI, 6.5-kb fragment in
pBluescript KS(−)] was kindly provided by Dr. Maria 
Hatzoglou (2). CAT-2 and CAT-2A were examined with syn-
thetical 58-base oligodeoxynucleotides complementary to the
unique alternatively spliced mRNA region of each of the
following transporter isoforms (30): CAT-2 (accession no.
M62838) 1196–1253 and CAT-2A (accession no. L03290)
1100–1157. Northern blot analysis with the CAT-1 cDNA
was carried out as described above; the CAT-2/2A oligode-
oxynucleotides were end labeled with T4 polynucleotide ki-
nase (Promega, Madison, WI) and [γ-32P]ATP (NEN) and
hybridized with UltraHyb (Ambion, Austin, TX) according to
the manufacturer’s instructions. All subsequent steps were
carried out as described above.

Amino acid transport. The transport of radiolabeled amino
acids (all from Amersham) by hepatocyte monolayers was
ْ **RESULTS**

Tumor burden and food intake. TBR and matched
control animals had similar carcass (tumor-excised)
weights (198 ± 15 and 192 ± 18 g for controls and TBR,
respectively) in the feeding study, suggesting that pair
feeding was effective. Daily food intake decreased by
20–25% (on average by 4–5 g/day) only after the tumor
burden reached 15–20% of carcass weight, consistent
with earlier observations (10). Inasmuch as some tu-
mors took longer to grow than others, the possibility
was considered that the duration, rather than the
magnitude, of tumor burden elicited hypophagia. How-
ever, when tested directly with multiple tumor implanta-
tions per animal, it was determined that the size of the
tumor burden influenced food intake more than the
duration of the tumor burden. Decline in average daily
food intake was first observable when tumor burden
exceeded 15–20%, which occurred in the tetra-im-
planted TBR at day 17 postimplantation, and at day 24
in the mono- and di-implanted TBR. On the basis of
these results, the experimental groups were divided
into small (<15%) and large (>15%) TBR to distin-
guish between tumor-specific and nutritional effects.
Mean percent tumor burden and tumor weight were
9 ± 4% and 17 ± 8 g in the small and 26 ± 7% and 48 ±
12 g in the large TBR, respectively.

Zonal-enriched hepatocyte populations. The selective
destruction of PP or PV hepatocytes via ante- or retro-
grade digitonin perfusion, respectively, before collagen-
ase digestion is a well-established technique (32, 44)
for the isolation of cells enriched in populations from
“zones 1 and 2” or “zones 2 and 3” (24), as defined in
Rappaport’s classic functional model of the liver acinus
(46). Figure 1 shows histologically the typical 20–30%
PP or pericentral destruction patterns obtained after
perfusion with 0.5% digitonin. The zonal enrichment of
the cell preparations was confirmed using well-estab-
lished specific mRNA markers (Fig. 2A). GS was
enriched 13-fold in PV hepatocyte preparations com-
pared with PP preparations in the large and small TB
groups. This marked enrichment is a function of the
restricted expression of GS mRNA in
5–7% of the hepatocytes surrounding the terminal
hepatic venules, which are more completely destroyed
with digitonin. Conversely, GA1 mRNA was enriched
2.1 (large TB) to 3.5-fold (small TB) in PP compared
with PV preparations, confirming the enrichment of
each population (Fig. 2B). Consistent with previous
observations (54), PEPCK mRNA was slightly less
enriched in PP preparations than GA1 (2.6-fold in
small TB), but in the large TB group the PP enrich-
ment disappeared. The reason for this observation is
unclear but may be associated with progressively in-
creased gluconeogenesis rates in the TBR (6, 39, 47).
Nonetheless, the enrichments of PP and PV hepatocyte
populations in this study were confirmed and consist-
tent with previous reports. Finally, on the basis of the
blots in Fig. 2A and other Northern analyses per-
formed on unenriched hepatocyte preparations (not
shown), there were no consistent tumor-dependent
changes in GA1 or GS mRNA abundance, suggesting
that altered flux through these pathways does not
involve changes in cognate mRNA levels (10).

Tumor burden and hepatic amino acid transport. Consistent
with previous studies (5, 27), PV cells dis-
played accelerated Na+-dependent glutamate trans-
port rates compared with PP hepatocytes [59 ± 5 and
9 ± 2 pmol·mg protein−1·min−1 in PV and PP, respec-
induced by tumor growth in a position-dependent manner along the acinus, the system A-specific substrate α-(methylamino)-isobutyric acid was included at a concentration of 5 mM in the glutamine transport assay. As shown in Fig. 3, there was no evidence of a significant system A contribution to hepatic glutamine uptake in either region over the entire range of tumor burdens. This indicated that tumor-dependent glutamine transport stimulation was attributable to enhanced system N activity and is consistent with data reported previously in studies with plasma membrane vesicles from whole liver (29, 41).

More detailed analysis of the hepatic response to tumor growth revealed some surprising and interesting results. Tumor burdens of 9 ± 4% elicited a 2.2-fold increase in system N activity in unenriched (no digitonin) hepatocyte preparations (P < 0.010) compared with those from pair-fed controls (Table 1). A separate study with tumors ranging from 0.004 to 9% of carcass weight indicated that tumor-dependent effects on glutamine uptake required a ≥4% burden. In contrast, large tumor burdens (26 ± 7%) collectively resulted in only a 26% induction of glutamine uptake, an effect that did not achieve statistical significance. These data indicated that cancer-dependent stimulation of system N activity occurs relatively early and wanes with excessive tumor burden. This effect appeared to be attributable in part to an 18% decrease in large TBR glutamine uptake (not significant) coupled with a 1.5-fold increase in large TBR control system N rates compared with small TBR controls (P < 0.010). Enhanced control system N activity may in turn result from reduced caloric intake during pair feeding (20).

When these studies were extended to zonal-enriched hepatocyte populations, another surprising observation was made. System N activities were enhanced by an average of 75% in PV- vs. PP-enriched hepatocyte preparations from control and TBR early in tumor growth (P < 0.010; Table 1). Likewise, Na⁺-dependent glutamine transport rates were increased 60–70% in PP and PV tumor-influenced hepatocytes compared with controls (P < 0.050; Table 1), indicating that system N is equally induced by tumor burden in both regions. During excessive tumor burden (26 ± 7%), enrichment of system N activity in PV preparations was no longer evident in TBR (Table 1) because of a significant drop in PV transport rates (P < 0.050), an effect that partially contributes to decreased tumor-dependent activation in these animals. Control PV-to-PP system N activity ratios also decreased, but not as profoundly. Although all the values did not achieve statistical significance, this loss of PV system N enrichment was collectively attributable to increased PP activities and decreased PV activities in both groups of animals (Table 1). Thus prolonged tumor burden and its associated nutritional effects shift the emphasis on concentrative glutamine uptake from PV (zones 2 and 3) to PP (zones 1 and 2) regions of the liver.

Saturable arginine transport in the liver is mediated largely by a low-affinity Na⁺-independent transporter (55) that has been shown to be encoded by CAT-2A (11).
Indeed, the activity was considerably lower than that for glutamine in control and TBR unenriched hepatocyte preparations (Table 1). In these hepatocyte preparations, arginine uptake was enhanced by 33% (not significant) and 67% \( (P \leq 0.050) \) in the small and large TBR groups, respectively. This observation was consistent with the graded induction of arginine uptake in liver plasma membrane vesicles with progressive tumor growth reported earlier (16). Contrary to system N activity, saturable arginine transport rates were enhanced fourfold in PP- vs. PV-enriched hepatocytes \( (P \leq 0.010) \) from small TBR groups and pair-fed controls. It is unclear why arginine transport rates in unenriched hepatocyte preparations failed to display intermediate values between those of PV and PP preparations, similar to that seen for glutamine (Table 1). This PP-enriched activity vanished in large TBR pair-fed controls, suggesting that the 20–25% decrease in caloric intake in these animals may elicit a metabolic/hormonal-dependent inhibition of augmented PP arginine uptake. Similarly, heightened PP arginine transport rates were attenuated by 33% in large TBR hepatocytes relative to small TBR, whereas the activities in PV preparations were enhanced by 33% compared with similar preparations from small TBR (Table 1). On the basis of the data, it appears that the presence of a tumor counteracts the nutritional effects in large TBR and preserves a portion of the PP-enriched arginine activity.
To our knowledge, this represents the first report of enhanced arginine uptake in zone 1 of the liver, so we sought to determine whether CAT-2A mRNA was correspondingly enhanced in this region. The Northern blot analysis shown in Fig. 4 suggests, however, that there is no obvious PP-enriched distribution of the CAT-2A mRNA (6.7 kb) in the control or tumor-influenced liver acinus, nor is there any apparent tumor influence on CAT-2A mRNA levels. Therefore, heightened arginine uptake in the PP preparations does not correspond to changes in CAT-2A mRNA, as originally hypothesized. Alternatively, we considered the possibility that another CAT isoform may underlie the fourfold-enhanced activity in PP preparations, but Northern blot analysis with CAT-1 and CAT-2 probes did not yield any detectable hybridizations (data not shown).

DISCUSSION

The MCA fibrosarcoma model of cancer has been used extensively to study host-tumor metabolic relationships. It is now well established that tumor growth induces muscle proteolysis and amino acid efflux (9) as well as accelerated hepatic metabolism, such as protein synthesis and gluconeogenesis (47, 52). In support of heightened liver amino acid metabolism during tumor growth, the activities of several amino acid transport activities are coordinately stimulated as measured in plasma membrane vesicles isolated from whole liver homogenates (12, 15, 16, 29, 41). Initially, it was proposed that these activities increase in proportion to the tumor burden (12, 16). More recent reports in isolated hepatocytes, however, yielded preliminary indications that this may not be the case for glutamine (13, 19), although the problem was not specifically addressed in those reports. Many studies with this tumor model have been performed when the burdens exceeded 20% of carcass weight (12, 18), causing some to question its physiological relevance. The studies presented here were therefore undertaken to address these problems and comprehensively examine the effects of progressive tumor burden on specific amino acid transport activities in hepatocytes from different acinar zones. One of the reasons we chose to utilize hepatocytes instead of plasma membrane vesicles was the fact that they represent a more comprehensive assessment of tumor-influenced physiology. Factors that collectively contribute to measured transport rates, such as transmembrane electrochemical potentials and intracellular amino acid levels, are retained in hepatocytes but are lost during subcellular fractionation. Inasmuch as plasma membrane vesicles offer unequivocal assessments of changes only at the plasma membrane level, the two systems are complementary. Lastly, we chose to focus on the hepatic transport of glutamine and arginine, inasmuch as these two amino acids have been proposed for use in immunomodulatory nutritional therapies for cancer patients (1), and each plays an integral role in liver nitrogen metabolism.

Enriched system N activity (Table 1) in PV hepatocytes (zones 2 and 3) was an unexpected finding, because no precedent for this observation exists. An earlier study on amino acid transport activities in PP and PV hepatocytes reported no difference in \( \text{Na}^+ \)-dependent histidine (another system N substrate) transport rates between both zones (5). However, several differences in methodologies between our studies might provide reasons for this discrepancy. For example, in the present study, viable hepatocytes were separated from nonviable cells, nonparenchymal cells, and debris by

Table 1. System N and CAT-2A activity in PV, PP, and unenriched hepatocyte preparations

<table>
<thead>
<tr>
<th></th>
<th>System N Activity, pmol·mg protein(^{-1} \cdot 30 \text{ s}^{-1})</th>
<th>Saturable Arginine Transport, pmol·mg protein(^{-1} \cdot \text{min}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP</td>
<td>PV</td>
</tr>
<tr>
<td>Small (&lt;15% tumor burden)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>65 ± 6</td>
<td>116 ± 10(^a)</td>
</tr>
<tr>
<td>TBR</td>
<td>112 ± 10(^b)</td>
<td>190 ± 26(^a,b)</td>
</tr>
<tr>
<td>Large (&gt;15% tumor burden)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>79 ± 14</td>
<td>97 ± 15</td>
</tr>
<tr>
<td>TBR</td>
<td>131 ± 28(^b)</td>
<td>139 ± 23(^b,e)</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 determinations per 5–8 (system N activity) or 3–8 (saturable arginine transport) rats. Tumor burden = tumor/carcass wt \((g)\); TBR, tumor-bearing rat; PP, periporal; PV, perivenous. \(^aP < 0.010\) vs. PP; \(^bP < 0.050\) vs. control; \(^cP < 0.050\) vs. small PV TBR; \(^dP < 0.050\) vs. small PP control; \(^eP < 0.050\) vs. small control.
Percoll gradient centrifugation, as introduced by Bilir and colleagues (3) for the digitonin-collagenase technique. On the basis of recent studies from our group that indicated differences in feeding habits between TBR and pair-fed controls (14), all animals were subjected to an overnight fast before hepatocyte isolation to eliminate influences from prandial status. In contrast, Burger and colleagues (5) utilized animals fed ad libitum and measured histidine uptake for 2 min, a time frame that may not assess initial-rate system N activity. Whether these collective differences sufficiently account for the discrepant results between our two studies remains to be determined.

Although this is the first report of PV-enriched system N activity, its significance was initially unclear and unexpected in the context of PP-enhanced glutaminase and urea synthesis (45, 54). A potential explanation for this observation was provided during the final submission of this manuscript when the gene encoding system N activity was isolated from rat (8) and mouse (23) cDNA libraries. On the basis of immunohistochemical studies in one of those reports (23), system N transporter expression was shown to be enriched in the PV regions of the liver acinus, supporting the results in our study (Table 1). In the other work (8), system N was shown to mediate the bidirectional transport of glutamine under physiological conditions. Its unique mechanism utilizes the countertransport of H⁺ and the transmembrane glutamine gradient to drive glutamine efflux and the Na⁺ gradient to drive uptake. Thus enhanced PV system N activity may be required for efficient glutamine release. Other possible reasons for enhanced PV system N activity were considered as well. Given the pronounced metabolism of glutamine in zone 1, a more robust transport activity may be required to support glutamine-dependent metabolism in zones 2 and 3 in the face of gradually diminishing concentrations of this amino acid along the acinus. This model is based on several independent observations. The affinity of system N for glutamine (Michaelis-Menten constant = 0.6–1 mM) correlates with the physiological levels of this amino acid in the blood. System N transport rates are therefore directly dictated by plasma glutamine levels. Also, studies by Low and colleagues (34) established the quantitative importance of system N activity in regulating intracellular hepatic glutamine metabolism. Therefore, enhanced system N activity may be necessary in zones 2 and 3 to support glutamine-dependent processes such as PV-enriched glutamate dehydrogenase flux (36) at levels equivalent to zone 1 metabolism.

The data in Table 1 show that system N activity is equally stimulated in both regions of the acinus by 9–14% tumor burden, suggesting that this relative distribution of glutamine uptake is maintained and enhanced during clinically relevant tumor growth. This
may represent a response to the diminished circulating arterial glutamine levels associated with the growth of this tumor (10, 41) coupled with an increased glutamine demand for gluconeogenesis and protein synthesis early in tumor growth (39, 53). During the course of these studies, it was determined that a 4% tumor burden was the minimum requirement for stimulation of system N activity, and previous studies in plasma membrane vesicles indicated that this effect is attributable to an increase in maximum transporter velocity (29). Only after supraphysiological tumor burden (26 ± 7%) and associated caloric restriction does this zonal arrangement cease to function (Table 1).

The pan-zonal stimulation of system N activity during cancer must be placed in the context of earlier in vivo work that served as the impetus for the present investigation. Those studies showed that a tumor burden of 6–9% caused the liver to switch to net glutamine output (51), whereas subsequent work showed that this response was secondary to a 35% decrease in GAL and a 43% increase in GS activities (10). A third study implicated transport in the switch to glutamine output, where it was demonstrated that a 7% tumor burden elicited a 2.7-fold increase in Na⁺-independent glutamine transport (system n (42)) and a 24% increase in system N as measured in plasma membrane vesicles (41). The net result is an enhanced ability to release glutamine from hepatocytes, which appeared to be a facilitative (Na⁺-independent) process (17). Those studies must be reevaluated in light of the recent report that system N mediates glutamine uptake and efflux, however (8). Our results indicate that early tumor burden stimulates system N activity in PP and PV hepatocyte populations (Table 1), which makes sense if the function of the liver is to provide glutamine during early tumor growth (51). Under these conditions, enhanced glutamine transport and metabolism in PP hepatocytes would be offset by enhanced GS activity (10) and system N-mediated efflux in the PV region. Given the recent data on system N transport mechanisms (8), modulation of the transmembrane glutamine and proton gradients would serve a regulatory role in determining the net efflux rate of glutamine from the liver. This concept is supported by previous observations (10, 12) that the liver exhibits increased glutamine content and output during early tumor burden (when system N is enhanced in both regions) but decreased glutamine content when the liver switches to net consumption in animals with large (>25%) tumor burdens (when the system N gradient disappears (Table 1)). We speculate that the upregulation of system N in both zones in response to physiologically relevant tumor burden supports hepatic and extrahepatic demands for glutamine and allows the host to survive. Whereas data from whole liver support this paradigm, there are no data on the glutamine content of PP or PV hepatocytes in response to tumor burden, so substantiation of this proposed model awaits further work.

The other significant finding from the present study was the marked saturable arginine transport rates in PP hepatocytes relative to PV or unenriched preparations (Table 1). In contrast to the reciprocal arrangement for glutamine, arginine uptake rates were not stimulated in a statistically significant manner during clinically relevant tumor growth. It is unclear why arginine uptake appears to be markedly accelerated in zone 1. One possibility is that it may help drive the urea cycle in the context of high cytoplasmic glutamine in the extreme PP region (as discussed above), which in turn has been shown to inhibit arginine formation from citrulline in some cells (50). Recent work on this relationship in hepatocytes, however, has shown that glutamine actually enhances argininosuccinate synthase expression and activity through its effects on cell volume (43). A precedent for extracellular arginine driving hepatic ureagenesis was provided in earlier studies with patients afflicted with inborn errors in the urea cycle (4). The hepatic transport of arginine has been ascribed to CAT-2A (11, 35, 55), and our studies confirm the marked expression of this gene in rat hepatocytes (Fig. 4). However, the relative abundance of CAT-2A mRNA does not correlate with enhanced arginine transport activity in PP cells. If CAT-2A is responsible, then significant post-mRNA mechanisms such as enhanced translation or plasma membrane trafficking rates of the protein must underlie this observation. A similar situation exists for the GLUT-1 glucose transporter in the liver, where there are no detectable zonal differences in its mRNA, but only the terminal PV hepatocytes express the protein in the plasma membrane (3).

It is possible that an arginine transporter other than CAT-2A is responsible for the marked PP activity. Previous work from our laboratory in isolated liver plasma membrane vesicles showed that arginine uptake was mediated by high- and low-affinity transporters, both of which were stimulated by tumor burden (29). Clearly, the low-affinity transporter was CAT-2A, but the identity of the high-affinity carrier remains elusive. Northern blot analysis with CAT-1- and CAT-2-specific probes failed to yield detectable signals in the present study, a finding that is consistent with other reports that showed CAT-1 mRNA to be detectable in the liver only if it is induced to regenerate or if animals are injected with specific hormones (33). The possibility exists that CAT-1 may be responsible for enhanced PP arginine transport, but if it is only expressed in the first one or two hepatocytes surrounding the portal inflow, its mRNA may not be detectable in Northern blot analysis of total RNA. This hypothesis is supported by lack of an arginine transport “gradient” [i.e., no intermediate transport velocity value for unenriched hepatocyte preparations (Table 1)] and previous work based on its role as a viral receptor showing that CAT-1 is transiently expressed only around the portal triads during liver regeneration (56). It is unclear whether excessive tumor burden brings the liver to a state of regeneration, but our data also indicate little tumor-specific induction of arginine transport during physiologically relevant tumor burden (Table 1). It is also unclear why the PP-enriched arginine transport
activity disappears only in hepatocytes from pair-fed control animals for the large tumor burden group. Such a response would have to involve the 20–25% decrease in caloric intake (the only variable) in these control animals, while the presence of the tumor somehow preserves PP arginine transport activity under the same conditions in the matched TBR. Clearly, the identity of the PP arginine transporter and its regulation by caloric restriction require further investigation.

In summary, the results presented here represent the first report of reciprocal zonal-enriched glutamine and arginine transport activities and demonstrate the differential effects of clinically relevant tumor growth on each. Although the stated objectives of reporting these effects at the cellular level were met, many new questions were raised based on the findings. This study will therefore serve as the basis for future investigations into the role and regulation of specific amino acid transporters along the liver acinus during cancer and the significance of tumor-influenced hepatic physiology to the survival of the host.

We acknowledge and greatly appreciate the assistance of Dr. David Schoenfeld (Harvard Clinical Research Center) with the statistical analyses in this study. We are grateful to Dr. Jorge Gumucio for helpful discussions regarding technical aspects of the digitonin-collagenase perfusion technique.

This study was supported by National Cancer Institute Grant CA-57690 to W. W. Souba and by National Institute of Diabetes and Digestive and Kidney Diseases Harvard Clinical Nutrition Research Center Grant 1-P30-DK-40561 to B. P. Bode.

REFERENCES


