Brush Border Transport of Glutamine and Other Substrates During Sepsis and Endotoxemia

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The effects of severe infection on luminal transport of amino acids and glucose by the small intestine were investigated. Studies were done in endotoxin-treated rats and in septic patients who underwent resection of otherwise normal small bowel. In rats the kinetics of the brush border glutamine transporter and the glutaminase enzyme were examined. In patients the effects of severe infection on the transport of glutamine, alanine, leucine, and glucose were studied. Transport was measured using small intestinal brush border membrane vesicles that were prepared by Mg^{++} aggregation/differential centrifugation. Uptake of radiolabeled substrate was measured using a rapid mixing/filtration technique. Vesicles demonstrated 15-fold enrichments of enzyme markers, classic overshoots, transport into an osmotically active space, and similar 2-hour equilibrium values. The sodium-dependent pathway accounted for nearly 90% of total carrier-mediated transport. Kinetic studies on rat jejunal glutaminase indicated a decrease in activity as early as 2 hours after endotoxin secondary to a decrease in enzyme affinity for glutamine (K_m = 2.23 ± 0.20 mmol/L [millimolar] in controls *versus* 4.55 ± 0.67 in endotoxin, $p < 0.03$), rather than a change in V_{max}. By 12 hours the decrease in glutaminase activity was due to a decrease in V_{max} (222 ± 36 nmol/mg protein/min in controls *versus* 96 ± 16 in endotoxin, $p < 0.03$) rather than a significant change in K_m. Transport data indicated a decrease in sodium-dependent jejunal glutamine uptake 12 hours after endotoxin secondary to a 35% reduction in maximal transport velocity (V_{max} = 325 ± 12 pmol/mg protein/10 sec in controls *versus* 214 ± 8 in endotoxin, $p < 0.0001$) with no change in K_m (carrier affinity). Sodium-dependent glutamine transport was also decreased in septic patients, both in the jejunum (V_{max} for control jejunum = 786 ± 96 pmol/mg protein/10 sec *versus* 417 ± 43 for septic jejunum, $p < 0.01$) and in the ileum (V_{max} of control ileum = 1126 ± 66 pmol/mg protein/10 sec *versus* 415 ± 24 in septic ileum, $p < 0.001$) The rate of jejunal transport of alanine, leucine, and glucose was also decreased in septic patients by 30% to 50% ($p < 0.01$). These data suggest that there is a generalized down-regulation of sodium-dependent carrier-mediated substrate transport across the brush border during severe infection, which probably occurs secondary to a decrease in transporter synthesis or an increase in the rate of carrier degradation. The decrease in the number of active brush border carriers for glutamine and the decrease in glutaminase activity, in conjunction with diminished consumption of circulating glutamine demonstrated during sepsis in previous studies, may result in inadequate amounts of this key small intestinal substrate.

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THE SMALL INTESTINE plays a key role in the absorption of luminal nutrients. This process not only supplies amino acids for synthetic pathways in the gut mucosa but also provides substrate for the liver and other tissues. Failure to consume adequate amounts of dietary amino acids leads to weight loss, negative nitrogen balance, and erosion of lean body mass. Such consequences may be devastating in critically ill patients who are already catabolic from their underlying disease and who also require greater amounts of dietary protein to maintain nitrogen balance.

Recent studies indicate that the intestinal tract, an organ generally regarded as inactive or quiescent during critical illness, occupies an important physiologic and metabolic position after injury and infection.[1,2] For example the intestinal epithelium functions as a barrier to luminal toxins. This barrier may become threatened or compromised in septic states, leading to an increase in mucosal permeability and subsequent translocation of enteric bacteria and their endotoxins.[3] In addition the gut plays a pivotal role as a processor of circulating substrate, cooperating with the liver and other organs in maintaining amino acid homeostasis. This role in nitrogen metabolism revolves to a considerable extent around the ability of the small intestine to extract large amounts of glutamine from the bloodstream and from the gut lumen.[2,4] Glutamine is the most abundant amino acid in the blood and free amino

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acid pool of the body.[5] Functionally glutamine metabolism by the small intestine (1) provides a major energy source for the gut mucosa to support biosynthetic pathways, (2) provides amide nitrogen that may support nucleotide biosynthesis, and (3) processes nitrogen and carbon from the diet or from the bloodstream for export to other tissues for further metabolism. Glutamine is similarly metabolized whether it enters the mucosal cells across the brush border from the lumen or across the basolateral membrane from the arterial blood, and it is even more important than glucose as an oxidative fuel.[4] This ability of the gut mucosa to extract and metabolize large amounts of glutamine is due to avid transport activity and high levels of the enzyme *glutaminase,* the major enzyme of glutamine metabolism.

We previously reported that glutamine uptake from the bloodstream is diminished in septic patients and in endotoxin-treated rats.[6] Although the consequences of this impairment are unclear, the mechanism by which uptake of circulating glutamine is decreased during sepsis appears to be due primarily to a decrease in glutamine extraction by the bowel and a decrease in the specific activity of mucosal glutaminase, rather than a significant reduction in intestinal blood flow. The changes that occur in brush border substrate transport during septic states are unknown. These changes are important to investigate because certain amino acids, in particular glutamine,[2] may be required for enterocyte growth and function. The purpose of this study was to examine the effects of severe infection on brush border transport of glutamine and other substrates and to study the kinetics of the enzyme *glutaminase* under these circumstances.

Materials and Methods

Studies were done in rodents and in surgical patients. In rats a model of endotoxemia that results in physiologic and physical signs of sepsis was used. In the patients the diagnosis of sepsis was made clinically and confirmed intraoperatively.

Reagents and Chemicals

All chemicals and reagents used were of analytical quality and were purchased from Sigma Chemical Co. (St. Louis, MO). Radiolabeled L-glutamine, L-alanine, L-leucine, and D-glucose were purchased from Amersham (Arlington Heights, IL).

Animal Studies

Male Sprague Dawley rats weighing 250 to 300 g (n = 36, Harlan Sprague Dawley, Indianapolis, IN) were used for the studies. The animals were kept in separate metabolic cages and were fed standard laboratory rat chow (Purina Rodent Chow 5001, Purina Inc., St. Louis, MO) and water *ad libitum.* They were subjected to alternate 12-hour periods of dark and light. Animal care and treatment and study procedures were in accordance with the guidelines of the Animal Care Committee at the University of Florida and the Gainesville Veterans Administration Hospital and the DHEW guide for the care and use of laboratory animals. On the evening before the study, chow was removed from the cages (*ad libitum* intake of water was allowed) and the rats were randomized to receive an intraperitoneal injection of endotoxin (Lipopolysaccharide, *Escherichia coli* 0127:B8, Lot #76F4035, Sigma, 7.5 mg/kg body weight [BW]) or saline vehicle. Batches of six rats were pooled from each group on three separate occasions such that a total of 18 rats were studied in each group. The next morning (12 hours after the saline or endotoxin injection) the animals were anesthetized with ketamine (1 mg/kg BW). The entire length of jejunum was excised, opened along the antimesenteric border, and washed with ice-cold saline (0.9%) solution. The mucosa was then scraped with a glass slide and brush border membrane vesicles (BBMV) were prepared immediately as described below. In separate groups of control and endotoxin-treated rats, jejunal mucosa was obtained 2 and 12 hours after administration of endotoxin or saline. This mucosa was frozen immediately in liquid nitrogen for later determination of glutaminase activity (see below).

Human Studies

Adult surgical patients admitted to the Shands Hospital at the University of Florida of the Gainesville Veterans Administration Hospital were eligible to participate in the study. The studies were approved by the Institutional Review Board at the University of Florida College of Medicine and by the Subcommittee for Clinical Investigation at the Gainesville Veterans Administration Medical Center.

Segments of small intestine were obtained intraoperatively from seven healthy patients and from four septic patients over a 15-month period. The control patients underwent elective abdominal surgery for their primary disease, the treatment of which required resection of small intestine. Four of these patients had a standard right hemicolectomy (two patients with villous adenomas of the cecum, two patients with small carcinomas of the ascending colon). A 10- to 15-centimeter section of ileum was obtained from the surgical specimen and used for transport studies. Portions of jejunum (about 10 cm in length) were obtained from three other patients. One patient had a Roux-en-Y limb of jejunum anastamosed to a pancreatic pseudocyst (for drainage), and a portion of small bowel was obtained at the time the jejunal limb was created. Two other patients had *en bloc* resection of a sigmoid carcinoma and small bowel adherent to the mass.

Ileum was obtained from two septic patients, both of

whom had perforation of the cecum and peritonitis. They were treated with right hemicolectomy and ileotranverse colostomy. Jejunum was obtained from two other septic patients, one of whom had *en bloc* resection of a perforated sigmoid diverticular abscess. The other patient had a perforation of a colon cancer with obvious peritonitis that required resection of an adjacent portion of jejunum.

Care was taken in the control patients to study only small bowel specimens from healthy individuals who had no weight loss, normal intake of food before surgery, and no evidence of organ dysfunction. Criteria for inclusion in the septic group were normotension, no evidence of intestinal ischemia or obstruction, previously healthy patients without weight loss who required an emergency operation based on clinical findings, and evidence of peritonitis intraoperatively. Intestine from patients who were not eating normally before the development of abdominal sepsis (*i.e.*, those being NPO [*nulla per os,* nothing by mouth] for greater than 36 hours) were excluded from study, because food intake may affect transport activity.[7,8] Small intestine from septic patients appeared otherwise normal. All surgical procedures were done by the same surgeon (W. W. Souba), and one of us (R. M. Salloum) was present in the operating room each time a portion of small intestine was submitted for study. The specimen was not devascularized until immediately before passing it off the operating table, at which time it was placed promptly on ice and transported immediately to the Surgical/Metabolism Laboratories. The mucosa was rinsed with 0.9% ice-cold saline, scraped with a glass slide, and stored in liquid nitrogen. Brush border membrane vesicles from human small intestinal mucosa were prepared as described below.

Membrane vesicle preparation. Brush border membrane vesicles (BBMVs) were prepared by a Mg^{++} aggregation/differential centrifugation technique.[9,10] For rat studies, the vesicles were prepared immediately after scraping the mucosa. In the patients, the previously frozen mucosa was first thawed. All steps of the preparation were conducted at 0 to 5 C. Briefly each gram of mucosal scrapings was homogenized in 8 mL of 300 mmol/L mannitol and 1 mmol/L N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-TRIS (pH 7.4) buffer with a Polytron homogenizer (Brinkman, setting #6 for 15 seconds). Homogenates from each group were then treated with 100 mmol/L $MgCl_2$/1 mmol/L HEPES/TRIS, to yield a final concentration of 10 mmol/L $MgCl_2$. After stirring for 20 minutes, the homogenate was centrifuged for 5 minutes at 5000*g*. The supernatant containing brush border material was collected and this step was repeated once. The supernatant was then centrifuged at 45,000*g* for 30 minutes. The brush border membrane pellet was resuspended in 350 mmol/L mannitol/50 mmol/L HEPES/TRIS and centrifuged again at 45,000*g* for 30 minutes. The final pellet was resuspended in the same buffer to yield a final protein concentration of 10 to 15 mg/mL. The brush border enzymes alkaline phosphatase and gamma glutamyl transpeptidase were routinely measured to assay for brush border vesicle purity.[11] Protein concentration was determined by the Biorad protein assay (Bio-Rad Laboratories, Richmond, CA) with gamma globulin as the protein standard.

Transport measurements. The uptake of ^{3}[H]-labeled substrate was measured using a rapid mixing/filtration technique.[9,10] In the BBMVs from rats and human ileal specimens, only L-glutamine transport was studied. In BBMVs from human jejunal specimens, the transport of ^{3}H-L-glutamine (^{3}H-Gln), ^{3}H-L-alanine (^{3}H-Ala), ^{3}H-L-leucine (^{3}H-Leu), and ^{3}H-D-Glucose (^{3}H-Glucose) were investigated. For each uptake measurement, 10 μL of BBMVs and 40 μL of the radioactive uptake buffer were placed separately at the bottom of a 12 × 75 polystyrene tube (Fisher Scientific Inc, Pittsburgh, PA). The uptake buffer components were adjusted so that the final concentration mixture contained initial gradients of 120 mmol/L NaCl or KCl and labeled substrate at 10 to 50 μmol/L (micromolar) concentrations. An electronically controlled device was used to initiate the reaction by rapidly vibrating the tube.[10] After the prescribed reaction period (15 seconds to 2 hours), 1 mL of ice-cold stop buffer (150 mmol/L NaCl/10 mmol/L HEPES/TRIS, pH 7.5) was added to quench the reaction. The quenched reaction mixture was then filtered using a prewetted and chilled 0.45-μm membrane filter (Gelman Scientific product no. 63068 Gn-6). The membranes were washed once with 5 mL of stop buffer and then dissolved in Aquasol Scintillation cocktail (Dupont, NEN Research Products, Boston, MA). The radioactivity trapped by the vesicles was measured by liquid scintillation counting. Values for nonspecific retention of the radioactivity by the filter and the vesicles were obtained from time zero uptakes and were subtracted from the total filter radioactivity. The radioactivity was converted to units of uptake and expressed as picomoles per milligram protein per time. Uptake was measured in the presence and absence of sodium with potassium as the control cation.

Statistical analysis. All data are expressed as mean ± standard error. Data were compared using the unpaired two-tailed Student's t test (MacIntosh Plus Computer, Statview 512 Statistical Program, Apple Computers, Cupertino, CA). A probability value less than 0.05 was considered statistically significant.

Results

Brush Border Purification

The purity of the brush border membranes was ascertained by determining the activities of the brush border marker enzymes alkaline phosphatase and gamma-glutamyl transpeptidase. Both enzymes showed a 14- to 16-

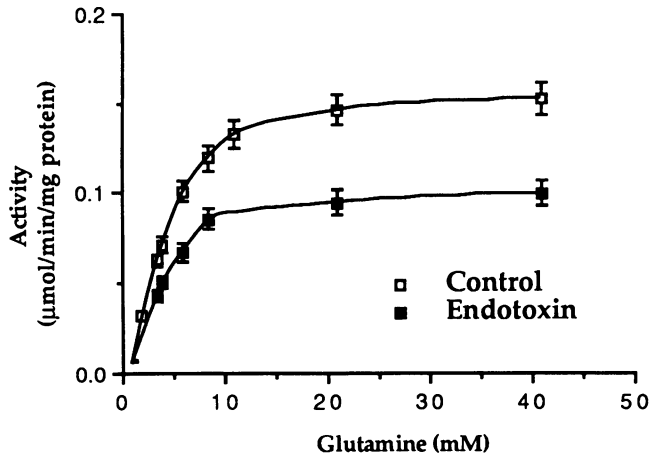

FIG. 1. Saturation plot of jejunal glutaminase activity in control and 12-hour endotoxin-treated rats. The activity of phosphate-dependent glutaminase was measured at various glutamine concentrations ranging from 1 to 40 mmol/L as described in the Methods section. Enzyme activity is plotted *versus* glutamine concentration.

fold enrichment in vesicles from control and endotoxin-treated rats and from normal and septic patients when compared with the crude homogenate (in rat jejunum, 515 ± 32 nmol/mg protein/minute *versus* 7401 ± 676 nmol/mg protein/min in the original homogenate and the BBMV suspension, respectively, and in human jejunum, 416 ± 62 *versus* 6652 ± 598, $p < 0.0001$).

Transport Characteristics

In both human and rat BBMVs, glutamine transport was measured in the presence and absence of NaCl (with KCl substitution). Transport was significantly higher in the presence of sodium and demonstrated classical overshoots. Sodium-dependent carrier-mediated transport accounted for 90% of total carrier-mediated uptake. Hence sodium-dependent glutamine transport data are presented in the results. Sodium-independent transport was not altered by severe infection. Osmolarity studies (data not shown) showed uptake into an osmotically active intravesicular space. Vesicular size was similar in all compared groups, as evidenced by the 2-hour equilibrium values of substrate uptake by the BBMVs.

Rat Studies

Effects of endotoxin. Rats receiving endotoxin developed decreased physical activity, conjuctivitis, and piloerection. Saline-treated animals appeared normal.

Glutaminase kinetic parameters. The activity of phosphate-dependent glutaminase was measured at various glutamine concentrations ranging from 1 to 40 mmol/L, using a fluorometric assay similar to the one described by Pinkus and Windmueller.[12] In rats studied 12 hours after endotoxin treatment, data in the form of a saturation plot (activity *versus* glutamine concentration) is shown in Figure 1. The data were linearized, generating an Eadie-Hofstee plot (Fig. 2), by plotting enzyme activity *versus* activity/glutamine concentration. The K_m (a measure of enzyme affinity for the glutamine) is the negative of the slope of the line and the V_{max} (a measure of the number of active enzyme molecules) is the activity of the enzyme at infinite substrate concentration. This linear relationship is summarized by the following equation:

$$V = V_{max} - K_m V/[Gln]$$

where transport velocity V is measured on the y-axis, V/[GLN] is measured on the x-axis, K_m is the slope of the line, and V_{max} is the y-intercept. At the 12-hour time point, the K_m was not different between both groups, but there was a significant decrease in V_{max} in the endotoxin-treated rats (Fig. 2, Table 1). In contrast, the 2-hour endotoxin-treated rats showed a significant increase in K_m, indicating a decrease in the affinity of the phosphate-dependent glutaminase enzyme for glutamine at this time point (Fig. 3, Table 1).

Glutamine uptake rates. The sodium-dependent timecourse of ^{3}H-Gln by rat jejunal BBMVs is shown in Figure 4. The BBMVs from endotoxin-treated rats had significantly decreased glutamine uptake compared with control rats.

Glutamine transport kinetics. Glutamine transport rates were measured as a function of concentration (10 μmol/L − 10 mmol/L) in the presence of 120 mmol/L NaCl or KCl. Incubations were performed for 10 seconds. Total glutamine uptake velocity (V^{Total}) was resolved into three transport pathways:

$$V^{Total} = V^{D} + V^{Na\text{-}dep} + V^{Na\text{-}indep}$$

$$V^{K} = V^{Na\text{-}indep} + V^{D}$$

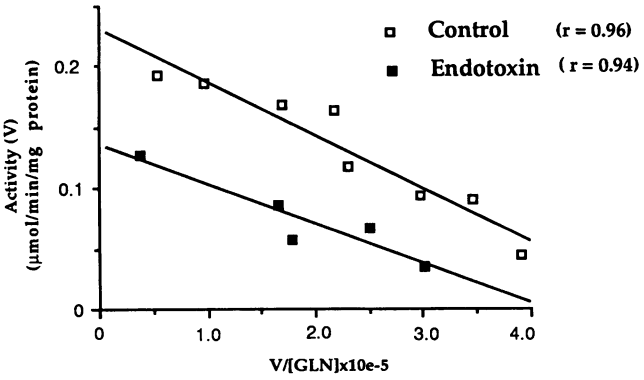

FIG. 2. Eadie-Hofstee plots of saturable glutaminase activity in control and 12-hour endotoxin-treated rats. The activity of phosphate-dependent glutaminase was measured at various glutamine concentrations ranging from 1 to 40 mmol/L as described in the Methods section. Enzyme activity is plotted against activity/[GLN]. Plots shown are representative of data generated from assays performed on three separate control and endotoxin-treated rat mucosal preparations, each done in triplicate. The slopes of the lines indicated a similar K_m, while y-intercepts indicate a decrease in V_{max} in endotoxin-treated rats.

TABLE 1. *Glutaminase Kinetics in Control and Endotoxin-Treated Rats*

Group	2 Hours After Endotoxin		12 Hours After Endotoxin	
	K_m (mmol/L)	V_{max} (nmol/mg/min)	K_m (mmol/L)	V_{max} (nmol/mg/min)
Control	2.23 ± 0.20	146 ± 19	3.67 ± 1.05	222 ± 36
Endotoxin	4.55 ± 0.67*	163 ± 7	2.20 ± 0.50	96 ± 16*

Data = mean ± SEM of three separate experiments done in triplicate. * $p < 0.03$ *versus* control.

such that V^{Total} refers to the total uptake in the sodium buffer, $V^{Na\text{-}dep}$ is the carrier-mediated sodium-dependent uptake, $V^{Na\text{-}indep}$ refers to carrier-mediated sodium-independent uptake, V^D is the passive diffusional uptake, and V^K is the uptake of glutamine in the absence of sodium, using potassium (K^+) as the control cation. The sodium-dependent component of total uptake, which represented nearly 90% of total uptake, is obtained from the following equation:

$$V^{Na\text{-}dep} = V^{Total} - V^K$$

An Eadie-Hofstee plot (Fig. 5) is generated by plotting $V^{Na\text{-}dep}$ *versus* $V^{Na\text{-}dep}$/[Gln] according to the following linear equation:

$$V^{Na\text{-}dep} = V^{max} - K^m V^{Na\text{-}dep}/[Gln]$$

where K_m represents the apparent affinity of the transporter and V_{max} is the maximal uptake velocity. The mean kinetic constants from three separate experiments done in triplicate are shown in Table 2. Endotoxin treatment resulted in a significant decrease in V_{max}, but no change in K_m (Fig. 5).

Human Studies

Glutamine uptake rates. The time course of sodium-dependent jejunal transport is shown in Figure 6. The overshoot phenomenon was observed in both groups of patients and the 2-hour equilibrium values were the same, indicating similar vesicular size. Transport of ^{3}H-Gln by BBMVs was significantly decreased in the septic patients.

Glutamine transport kinetics. Glutamine transport rates were measured as a function of glutamine concentration (10 μmol/L to 10 mmol/L) in the presence of 120 mmol/L NaCl or KCl. Incubations were performed for 10 seconds. Total glutamine uptake velocity (V^{Total}) was resolved into the three transport pathways as described above. Eadie-Hofstee plots for both jejunal and ileal transport were generated by plotting $V^{Na\text{-}dep}$ *versus* $V^{Na\text{-}dep}$/[Gln] to generate the lines shown in Figures 7 and 8. The apparent K_m for the sodium-dependent transporter in both the jejunum and ileum was not altered by the septic state. However the V_{max} of the transporter in septic patients was significantly decreased compared with controls, consistent with a decreased number of active transport molecules. Kinetic data are shown in Table 3.

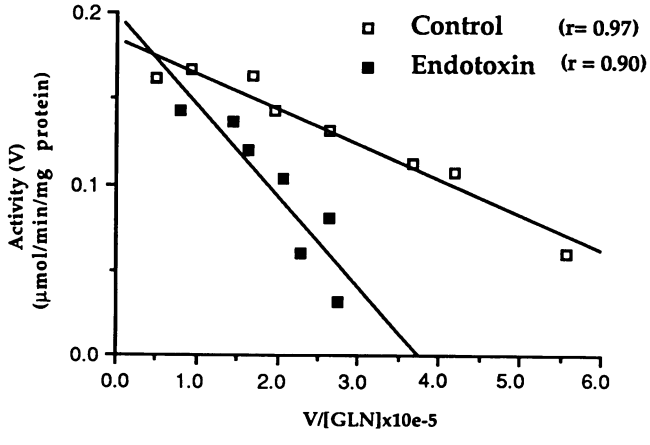

FIG. 3. Eadie-Hofstee plots of saturable glutaminase activity in control and 2-hour endotoxin-treated rats. The activity of phosphate-dependent glutaminase was measured at various glutamine concentrations ranging from 1 to 40 mmol/L as described in Methods. Enzyme activity is plotted against activity/[GLN]. Representative plots are shown. The slopes of the lines show an increase in K_m in endotoxin-treated rats, indicating a decrease in enzyme affinity. The V_{max} (y-intercept) was not affected.

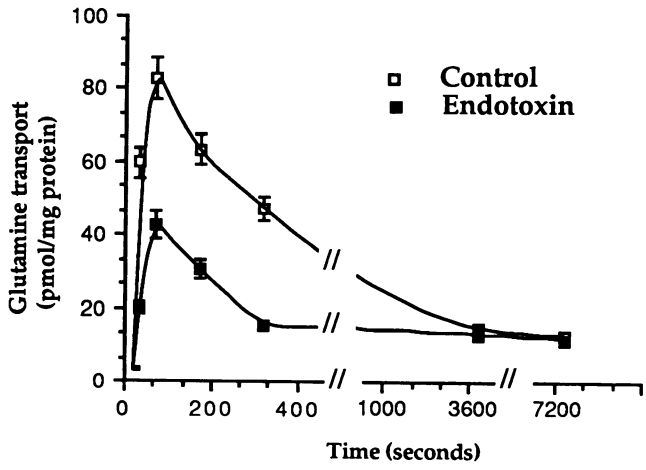

FIG. 4. Time course of glutamine uptake by BBMVs from control and 12-hour endotoxin-treated rats. BBMVs were incubated with 50 μmol/L glutamine uptake buffer as described in Methods. The figure is a representative time course of three assays performed separately from three different BBMVs preparations. Each data point represents mean ± SEM of triplicate measurements. When not shown the error bars are contained within the symbol. BBMV, brush border membrane vesicles.

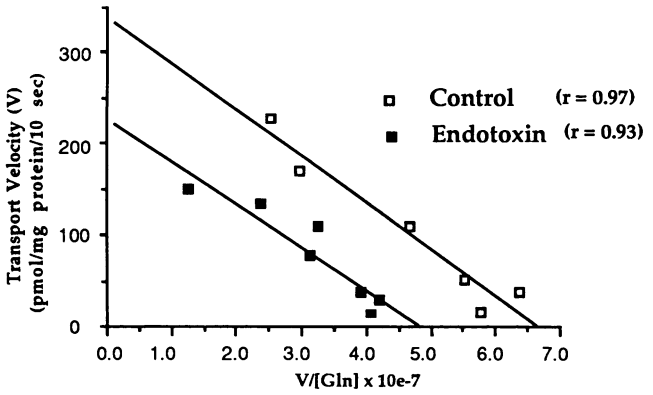

FIG. 5. Eadie-Hofstee plots of glutamine transport in BBMVs from control and 12-hour endotoxin-treated animals. BBMVs were incubated with varying concentrations of glutamine ranging from 10 μmol/L to 10 mmol/L for a period of 10 seconds. Sodium-dependent glutamine uptake was determined as described in Methods and uptake velocity was plotted as a function of velocity/[glutamine]. The y-axis intercept represents maximal transport velocity (V_{max}) and negative slope corresponds to the apparent K_m (transporter affinity). Plots shown are representative of data generated from transport assays performed on three separate control and endotoxin-treated rat BBMV preparations, each done in triplicate.

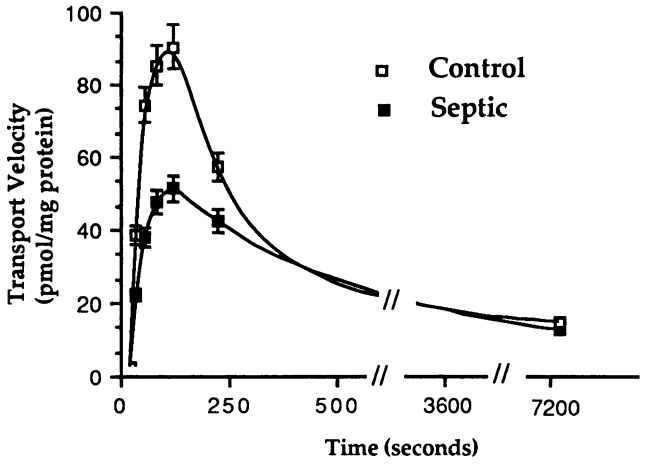

FIG. 6. Time course of glutamine uptake by jejunal BBMVs from control and septic patients. BBMVs were incubated in 50 μmol/L glutamine as described in Methods. The figure is a representative time course of three assays performed separately from three different BBMVs preparations from each of three control and two septic patients. Each data point represents the mean ± SEM of triplicate measurements. When not shown, the error bars are contained within the symbol.

Effects of sepsis on other transporters. To determine the specificity of the effects of sepsis on brush border transport, the total uptake ($V^{Na\text{-}dep} + V^{Na\text{-}indep} + V^{D}$) of ^{3}H-alanine, ^{3}H-leucine, and ^{3}H-glucose by human jejunal BBMVs was also measured. The uptake of all substrates was significantly decreased in the septic patients (Fig. 9, Table 4).

Discussion

Small intestinal brush border transport of glutamine and other substrates was studied in septic patients and in endotoxin-treated rats to gain further knowledge of the changes in gut fuel use that occur during severe infection. The results demonstrate similar decreases in brush border glutamine transport in septic humans and in rodents receiving *E. coli* lipopolysaccharide (LPS). Additional studies in jejunal BBMVs from healthy and septic patients indicated a generalized decrease in amino acid and glucose transport during severe infection.

Endotoxicosis is an accepted laboratory model of sepsis[13]; animal[14] and human[15] studies demonstrate that a single dose of endotoxin evokes many of the physiologic responses observed in severe bacterial infection. The rat endotoxin model we used is a reproducible model that has clinical relevance because septic patients develop the same impairment in gut glutamine metabolism as do entotoxin-treated rats. In addition, the regulation of these transport alterations (*e.g.*, by cytokines) at the molecular level can be studied. Such knowledge of metabolic regulation is essential for treatment strategies to be envisioned and implemented.

Luminal amino acids are transported across the brush border membrane of the small intestine by several well-

TABLE 2. *Jejunal Brush Border Glutamine Transport Kinetics in Control and Endotoxin-Treated Rats*

Group	K_m (μmol/L)	V_{max} (pmol/mg prot/10 s)
Control	492 ± 35	325 ± 12
Endotoxin	488 ± 16	214 ± 8*

Data = mean ± SEM of three separate experiments done in triplicate.
* $p < 0.0001$ *versus* control.

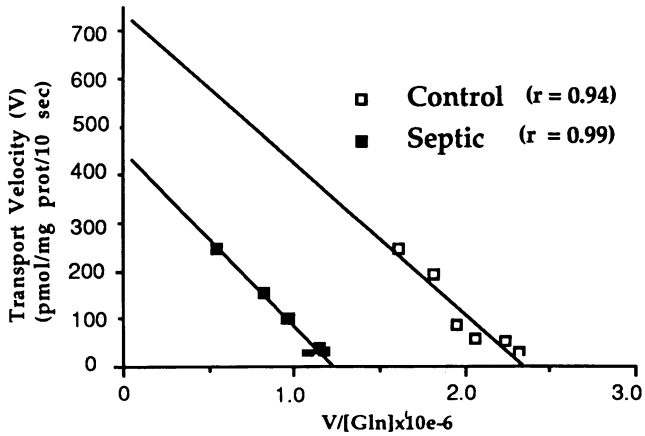

FIG. 7. Representative Eadie-Hofstee plot of glutamine transport in jejunal BBMVs from control and septic patients. BBMVs were incubated as described in Methods. The plot is representative of data generated from three assays performed on three separate control and two septic BBMV preparations, each done in triplicate.

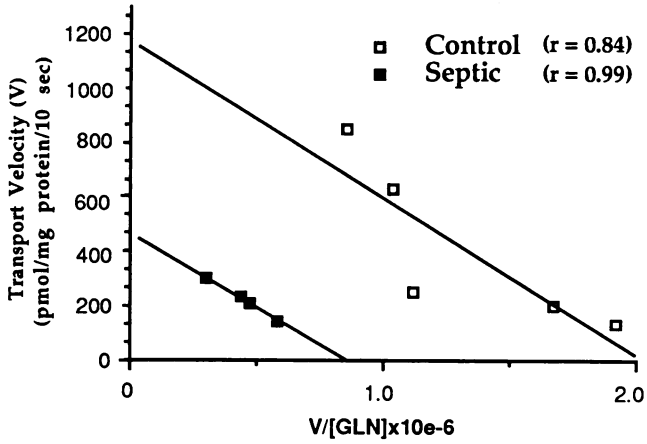

FIG. 8. Representative Eadie-Hofstee plot of glutamine transport in ileal BBMVs from control and septic patients. BBMVs were incubated as described in Methods. The plot is representative of data generated from three assays performed on four separate control and two septic BBMV preparations, each done in triplicate.

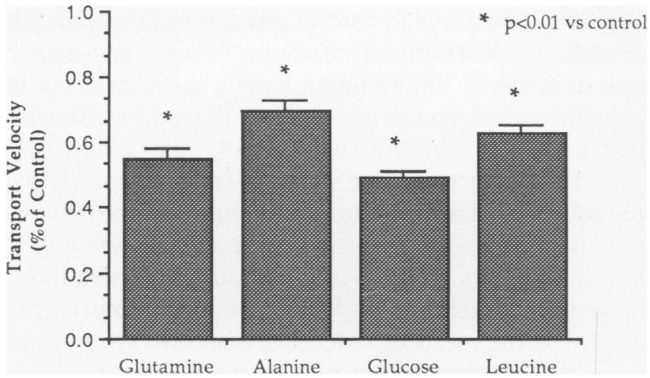

FIG. 9. Total substrate transport in jejunal BBMVs from septic patients expressed as a percentage of transport in jejunal BBMVs from healthy controls. BBMVs were incubated in 50 μmol/L each of glutamine, alanine, glucose, and leucine for a period of 10 seconds as described in Methods. The bar graph is the mean of three assays performed separately in triplicate on BBMVs preparations from each of two control and two septic patients. Each data point represents the mean ± SEM of triplicate measurements.

described transport systems.[16,17] In general translocation of luminal substrates into the cytoplasm of the enterocyte occurs by three separate pathways: a sodium-dependent route, a sodium-independent pathway, and by diffusion, which reflects the permeability of the membrane. The sodium-dependent route is usually the dominant pathway. In the case of glutamine, the jejunal brush border carrier for glutamine has not been fully characterized but may bear some resemblance to system N.[18,19] The carrier for alanine, however, is principally the sodium-dependent system A,[17] whereas leucine is mainly transported by the sodium-independent system L.[17] Glucose uptake from the lumen also occurs through a sodium dependent co-transporter.[20] Under normal circumstances the rate of transporter synthesis is balanced by the rate of carrier degradation such that a steady state exists and the number of copies of transporter molecules on the brush border is constant.

Three principal factors determine the uptake of amino acids and glucose across the brush border. They are the quantity of luminal substrate available to the epithelial cells, the capacity of the individual cells to translocate the compound into the intracellular compartment, and the capacity of the cell to use intracellularly available substrate. In the current investigations, we focussed on the intrinsic activity of the transport machinery as well as on the activity of the glutaminase enzyme. The use of plasma membrane vesicles to assess brush border substrate transport activity offers several advantages over other approaches. Alterations in membrane transport activity are preserved during the preparation of vesicles[9] and transport activity can be evaluated apart from other confounding influences such as metabolism and trans-stimulation/inhibition. The decrease in glutamine transport activity we observed was not the result of nonspecific differential effects of endotoxin on vesicle membrane permeability or transport characteristics, as we did not observe alterations in sodium-independent glutamine uptake (data not shown). In addition, BBMVs from endotoxin-treated rats and septic patients demonstrated enrichments and overshoots, indicating vesicle purity and functionality.

Severe infection did not change the affinity of the Na^+-dependent cotransporter for glutamine (the apparent K_m was unchanged) but the V_{max}^{Na} decreased to about one

TABLE 3. *Brush Border Glutamine Transport Kinetics in Control and Septic Patients*

	Jejunum		Ileum	
Group	K_m (μmol/L)	V_{max} (pmol/mg/10 s)	K_m (μmol/L)	V_{max} (pmol/mg/10 s)
Control	729 ± 84	786 ± 96	513 ± 46	1126 ± 66
Septic	619 ± 92	417 ± 43*	552 ± 10	415 ± 24†

Data = mean ± SEM of two separate experiments done in triplicate.
* $p < 0.01$; † $p < 0.001$ *versus* control.

TABLE 4. *Brush Border Jejunal Substrate Transport in Control and Septic Patients (pmol/mg protein/10 s)*

Group	Glutamine	Alanine	Leucine	Glucose
Control	36.1 ± 1.5	49.5 ± 6.9	83.7 ± 7.8	193.1 ± 9.6
Septic	19.9 ± 1.9*	34.4 ± 1.8*	52.1 ± 2.8*	94.3 ± 3.9*

Data = mean ± SEM of two separate determinations done each in triplicate.
* $p < 0.01$ *versus* control.

half of that observed in control rats and healthy humans. In addition, total transport of alanine, leucine, and glucose was diminished. Interestingly kinetic measurements in animals studied 4 hours after endotoxin treatment showed that glutamine transport was actually increased at this early timepoint (data not shown). Thus it appears that the gut mucosal response to endotoxemia may be biphasic in nature, with an early increase in transport activity followed by a later decrease in the number of active transporters on the cell surface. Whether this change in carrier activity is the result of reduced transporter synthesis or increased transport degradation (or both) is not clear from the present study. Interestingly, recent work by von Allmen and colleagues[21] demonstrated that mucosal protein synthesis is increased 16 hours after induction of fecal peritonitis by cecal ligation and puncture. Protein content of the bowel was unchanged, indicating a concomittant increase in protein breakdown in the mucosa or export of newly synthesized proteins. Thus small intestinal protein synthesis may be reprioritized during severe infection. At a time when energy use is at a premium and protein synthetic requirements must be prioritized, the mucosa may redirect the flow of energy to more acute synthetic needs (such as acute phase protein synthesis) at the expense of transport-related protein synthesis. Simultaneously the gut uses increased amounts of glucose,[22] its alternate fuel. In contrast to the gut, a decrease in the intrinsic glutamine transport activity does not occur in the hepatocyte of endotoxin-treated rats (Souba et al., unpublished observations), and synthesis of the glutamine carrier in pulmonary endothelial cells is actually increased by endotoxin.[23] Thus whether the bowel becomes purposefully 'subservient' to the glutamine needs of the liver and lungs requires further elucidation.

One important question raised by these studies is whether luminal transport activity can be stimulated in septic patients by providing luminal nutrients. Evidence exists that the activity of brush border nutrient transporters can be regulated by their respective substrates.[7] Diamond and Karasov[8] studied the influence of diet on the adaptive regulation of intestinal nutrient transporters. They suggested that fluctuations in transport activity are based on trade-offs among factors such as cost of transporter synthesis and nutrient requirements. Their work indicated that the basal rate of luminal transport can be up-regulated by dietary substrate. This observation may be important in critically ill patients, as it suggests that enteral nutrition may help offset the decrease in luminal transport activity that occurs during severe infection. Maintenance of transport function may also be important in such individuals, as dietary substrate can profoundly influence protein synthesis in the gut mucosa. Furthermore it is clear that enteral feedings improve outcome in critically ill patients,[24] an event that may be related to reprioritization of hepatic protein synthesis.[25] Early enteral nutrition can also blunt the catabolic response to burn injury and help preserve mucosal integrity.[26]

The amino acid glutamine has received considerable attention in the past decade, partly because of new knowledge demonstrating that its metabolism changes markedly during critical illness and also because of studies that suggest that it may be a conditionally essential amino acid. Supplemental glutamine, for example, can improve intestinal morphometrics and improve survival in animals receiving whole abdominal radiation[27,28] or chemotherapy.[29] Thus the relationship between the septic insult and the metabolic derangements may be more than casual. In view of the data presented in this study in conjunction with recent work, one might ask whether the provision of glutamine-enriched diets could stimulate mucosal metabolism and improve gut function during septic states.

We recognize that severe infection may result in changes in the mucosal microcirculation that may directly affect substrate transport by the gut epithelium. Such alterations might be mediated by endothelial cells or inflammatory cells. Certainly, like the changes in permeability that occur during endotoxemia, the regulation of the changes in substrate use that occur is likely to be complex and multifactorial. Regardless of the specific mechanisms involved, it is clear that sepsis results in a marked inability of the bowel to consume glutamine and other nutrients. The consequences of this impairment as well as its natural history with regard to progression and resolution require further study.

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Discussions

DR. EDWIN DEITCH (Shreveport, Louisiana): As you have just heard, the authors have presented data indicating that brush-border glutaminase activity and the transport of glutamine is decreased, and this decrease appears to be due primarily to quantitative decreases in the amount of glutaminase enzyme or glutamine transporters and not due to functional alterations of these systems. That is, what is there works. The problem is that what is there is reduced in number.

This basic work has very important clinical implications because we have information indicating that glutamine has several unique and important functions. We know that glutamine, rather than glucose, is the primary nutrient to the small intestine and that maintenance of intestinal structure and function may depend significantly on glutamine availability.

In addition we know that glutamine is an optimal and essential nutrient that's required for the immune system to function. Thus one clinical implication of these results is that the net effect of these impairments in intestinal glutamine metabolism and transport may result in an inadequate availability of glutamine to support optimally intestinal barrier functions during stress states.

This assumption fits in very well with the previously published data from my laboratory looking at endotoxin and barrier function of the intestine. We have shown, and others have as well, that endotoxin in stress states can lead to gut barrier failure and potential lethal gut origin septic states.

Would the authors comment on the potential therapeutic role of glutamine administration? I ask this question because the second implication of this work is that because the glutaminase and glutamine transporters that are present function normally, it may be possible to meet the cells' glutamine needs by increasing substrate availability.

In other words, it may be possible to compensate for these defects in glutamine transport by increasing the amount of exogenously supplied glutamine.

Because the physiology of glutamine metabolism and transport *in vivo* is incredibly complex and is influenced by more variables than can be addressed by *in vitro* studies, I would like to ask the following questions.

In vivo glutamine is transported to the cells from both the blood *via* the basal lateral membrane as well as to the intestine across the brush border. Thus would you put your data on luminal transport into perspective with the information you have generated on basal lateral transport? This question is potentially important because, especially in a nonfed state, the majority of glutamine transported to the epithelial cells may come from the blood.

There are two glutaminases. A phosphate-independent glutaminase, which is in the brush border, and a membrane-dependent glutaminase, which is in the mitochondria.

Because mitochondria glutaminase activity may be more important physiologically than brush-border glutaminase *in vivo* under certain circumstances, do you have any information on whether mitochondrial glutaminase activity is altered?

This question may be important because, methodologically, when the vesicles are formed from homogenized mucosa, it is possible that it could contain or have been contaminated with mitochondria or mitochondrial fragments.

Thus do you have any data documenting whether brush-border glutaminase was contaminated with mitochondrial glutaminase?

Last because glucose, alanine, and leucine transport also were decreased, it appears that the observations of glutamine transport are not specific. Instead they may reflect a global and nonspecific change in membrane transport. Would you speculate on the mechanisms underlying these changes?

DR. J. W. ALEXANDER (Cincinnati, Ohio): I think it's fairly clear from Dr. Copeland's very lucid presentation what the implications of this paper are. That is why it's so difficult to maintain nutritional status by either the enteral or parenteral route in individuals who are septic.

I'll make my discussion relatively short by focusing on four areas of questions, one of which is methodologic. And that is partly because of my ignorance. It would be interesting for all of us to know whether there could be some methodologic variance of this study because of differences in potential fragility of the vesicles as they are prepared. It could be, as an example, that they are more fragile in the septic state. They could create a methodologic error decreasing the apparent transport.

They have shown clearly that glutamine and glucose and aminoacid uptake is decreased, and I would like to know if there is any evidence of decrease of other alternative fuels for the gut, such as short chain fatty acids, ketoacids, or even lipids.

Next is there anything that might reverse this process? This has tremendous therapeutic implications for the septic patient who is trying to be fed. As we know in normal individuals, there are a variety of things