Glutamine Metabolism in Sepsis and Infection

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ABSTRACT Severe infection causes marked derangements in the flow of glutamine among organs, and these changes are accompanied by significant alterations in regional cell membrane transport and intracellular glutamine metabolism. Skeletal muscle, the major repository of glutamine, exhibits a twofold increase in glutamine release during infection, which is associated with a significant increase in endogenous glutamine biosynthesis. Despite an increase in glutamine synthetase activity in skeletal muscle, the intracellular glutamine pool becomes depleted, indicating that release rates exceed rates of synthesis. Simultaneously, the circulating pool of glutamine does not increase, indicating accelerated uptake by other organs. The liver appears to be the major organ of glutamine uptake in severe infection; studies in endotoxemic rodents have shown net hepatic glutamine uptake to increase by as much as 8- to 10-fold. This increase is due partially to increases in liver blood flow, but also to a three- to fourfold increase in hepatocyte System N activity in the liver. Cytokines and glucocorticoids mediate the increased uptake of glutamine by the liver in septic states as well as other compounds. Sepsis does not appear to induce an increase in System N gene expression, indicating that the increase in hepatic glutamine transport observed during severe infection is probably regulated at the protein level. The bowel displays a decrease in glutamine utilization during sepsis, a response that may be related to the decrease in circulating insulin-like growth factor-1 (IGF-1) levels that is characteristic of sepsis. Recent studies suggest that IGF-1 has a direct effect on stimulating glutamine transport across the gut lumen and thus may represent a therapeutic avenue for improving gut nutrition during severe infection. The cells of the immune system (lymphocytes, macrophages) are also major glutamine consumers during inflammatory states in which cell proliferation is increased. Under these conditions, glutamine availability can become rate limiting for key cell functions, such as phagocytosis and antibody production. J. Nutr. 131: 2535S–2538S, 2001.

KEY WORDS: glutamine, sepsis, glutamine transport, insulin-like growth factor-1

Study approaches

Although regulation of glutamine transport and metabolism eventually resides at the gene or protein level, it is useful to examine the interorgan relationships and regional differences that develop with infection. Interorgan flow of a particular substrate is estimated by comparing the net exchange of the substrate (the difference between uptake by and release from the organ) across individual organ beds; net exchange is calculated as the product of blood flow across an organ and the difference between the substrate arterial concentration and its concentration in the venous effluent from the organ. In aggregate, such estimates give a “global” picture of the flow, and hence metabolism, of specific substrates under a variety of physiologic conditions. In the catabolic state induced by sepsis, the pattern of glutamine flux among organs is altered in both humans and animals.

Once an interorgan model has been developed, the regulation of glutamine at the cellular level and the mediators involved in this control can be appreciated against this larger backdrop. This report will provide an overview of the changes in glutamine handling that occur during severe infection. We will highlight information that has been gathered about sepsis-induced changes that occur at the level of the organ, cell, and in some cases, the gene (Fig. 1).

Models of sepsis

Studies in humans are, of necessity, limited in scope and usually provide information only at the level of the organ or...
Sepsis and glutamine metabolism: models used, measurements made and tissues studied. CLP, cecal ligation and puncture.

**Fig. 1** Sepsis and glutamine metabolism: models used, measurements made and tissues studied. CLP, cecal ligation and puncture.

Sepsis-induced changes in glutamine handling

Endotoxin initiates marked changes in interorgan glutamine exchange, but the responses appear to be mediated primarily by cytokines and the counterregulatory hormones, rather than LPS itself (Fig. 2). Prior nutritional status, dietary composition, and the magnitude and severity of the illness can also influence the alterations. Only when the septic process resolves does glutamine homeostasis return to normal, and this assumes that no permanent organ damage was sustained.

**Skeletal muscle.** Given its key role as a repository for glutamine, it is not surprising that skeletal muscle displays dramatic changes in glutamine metabolism during sepsis. Many of the changes are similar to those observed in other catabolic disease states, such as advanced malignant disease and postoperative stress. As a general rule, the glutamine depletion that commonly develops during sepsis tends to be more severe and of longer duration, particularly if the septic insult is persistent or untreated. Patients in the surgical intensive care unit who have repeated bouts of sepsis, often of several etiologies (gut, lung, urosepsis), are often those who become profoundly glutamine depleted. In these individuals, the availability of glutamine may become rate limiting for certain cells.

In vivo studies using a hindquarter rat model have demonstrated that endotoxin accelerates muscle glutamine release despite a small reduction in hindquarter blood flow (Austgen et al. 1992). Simultaneously, the activity of glutamine synthetase (GS) nearly doubles, which helps prevent the muscle glutamine pool from being completely depleted in a short period of time. This increase in GS activity is preceded by a several-fold increase in GS mRNA levels in skeletal muscle but no change in glutaminase activity, suggesting that endogenous glutamine hydrolysis is not increased. The development of muscle glutamine depletion, which has been shown to be profound in some septic patients, suggests that the increase in GS expression cannot fully compensate for the accelerated glutamine release by muscle.

Hypothesizing that the endotoxin-induced increase in GS in muscle was adrenal gland dependent, Lukaszewicz et al. (1997b) studied the expression of GS in normal and adrenalectomized rats after administration of LPS. The increase in muscle GS mRNA observed in normal rats in response to LPS was abrogated in adrenalectomized rats at 3 h after high dose LPS treatment and markedly attenuated at 5.5 h after low dose LPS administration. These findings implicate the glucocorticoid hormones as pivotal, but not exclusive regulators of muscle GS expression during infection.

**Lung.** The lungs are a logical site of glutamine metabolism because of the high flow of blood through the pulmonary circulation and because they contain the necessary prerequisite machinery, GS, to catalyze de novo glutamine biosynthesis. Studies in rats indicate that lungs release glutamine very readily and are capable of releasing it in conjunction with skeletal muscle to help maintain the circulating glutamine pool (Welbourne 1987). Much of the total glutamine amide and amino nitrogen detected in the lung can be accounted for by the uptake of precursor nitrogen in the form of ammonia and glutamate.

The role of the glucocorticoid hormones in regulating GS expression appears to be similar to that observed in muscle. In vivo studies have demonstrated that dexamethasone accelerates lung glutamine release twofold, a response that is due to an increase in the fractional release rate of glutamine by the lungs and an associated increase in GS activity, rather than change in pulmonary blood flow (Souba et al. 1990b). Furthermore, similar to skeletal muscle, the GS gene in lung is induced during sepsis. Although adrenalectomy attenuated the expression of lung GS in response to LPS, the lungs of adrenalectomized animals do respond to endotoxin by upregulating GS (Lukaszewicz et al. 1997a). In addition, plasma from septic animals can induce GS expression in lung cells in the presence of an effective dose of a glucocorticoid receptor antagonist. These findings indicate that a mediator(s) other than glucocorticoids is capable of enhancing GS expression in the lung during sepsis.

**Bowel.** The observation that sepsis results in marked shifts in splanchic glutamine redistribution was first reported by Austgen et al. (1991c). Gut uptake of circulating glutamine is diminished in endotoxemic rats (Austgen et al. 1991c), and this is associated with a fall in luminal glutamine transport activity and mucosal glutaminase activity (Souba et al. 1990a). LPS decreases glutamine transport across the brush border of endotoxemic rats and septic humans (Salloum et al. 1991, Souba et al. 1990a). These changes are in contrast to the augmented glutamine uptake that occurs in postoperative and steroid-treated animals. Interleukin (IL)-1 treatment also diminishes intestinal uptake of circulating glutamine (Austgen et al. 1991a), whereas lymphocyte glutaminase activity in mesenteric lymph nodes is increased in septic rats (Sarantos et al. 1993). Given that glutamine utilization is 10-fold greater in proliferating lymphocytes compared with resting cells (Brand et al. 1986), the reduced gut uptake of glutamine that occurs during sepsis may occur primarily in the mucosal cells, as opposed to lymphatic tissues, which may actually consume more glutamine during stress states.

Using an intraperitoneal fecal pellet model, Pan and colleagues in the Department of Surgery at Penn State's College of Medicine are studying the effect of insulin-like growth factor-1 (IGF-1) on mucosal glutamine transport in a cultured intestinal cell model. Their investigations were stimulated by observations that circulating IGF-1 levels were reduced by 50% in rodent 5 d after intraperitoneal implantation with a
fetal pellet (personal communication, Thomas Vary, Penn State College of Medicine). Preliminary studies indicate that IGF-1 treatment of cultured Caco-2 cells augments glutamine transport by 50%. These findings have therapeutic implications in septic patients who often develop feeding intolerance.

Although the studies are limited, it appears that changes in renal glutamine metabolism also occur after endotoxin administration. In one study, endotoxin treatment caused the kidney to change from an organ of slight glutamine uptake in controls to an organ of net glutamine release (Austgen et al. 1991b). This “switch” was associated with a 50% increase in glutamine synthetase activity and a fall in urinary ammonium excretion. These changes took place when there was evidence of renal damage, suggesting that the early renal failure associated with sepsis may impair the kidney’s ability to maintain acid/base homeostasis by altering renal glutamine metabolism.

Liver. The fall in intestinal glutamine uptake that develops during endotoxemia is accompanied by a large increase in glutamine uptake by the liver. Under these circumstances, the liver becomes the major glutamine consumer (Austgen et al. 1991c). Hepatic glutamine uptake increases nearly 10-fold after endotoxin treatment secondary to increases in hepatic blood flow and in transhepatocellular membrane transport. Inoue et al. (1993) examined the effects of in vivo LPS administration on glutamine transport activity (System N) in rat hepatic plasma membrane vesicles. Endotoxemia resulted in a time-dependent two- to threefold increase in Na⁺-dependent glutamine transport activity in vesicles secondary to an increase in the transport $V_{\text{max}}$ (with no change in transporter affinity, $K_m$), consistent with the appearance of increased numbers of corresponding transporter proteins in the hepatocyte plasma membrane.

To further investigate these findings, Fischer et al. (1996a) used isolated hepatocytes to test the hypothesis that nutrient starvation and endotoxemia would act synergistically to augment hepatocyte glutamine transport activity. Starvation increased hepatocyte glutamine transport 16-fold, whereas LPS treatment of fed rats increased transport by 2.6-fold. Of interest was that treatment of food-deprived animals with LPS induced a sixfold increase in glutamine uptake. These studies indicate that starvation and endotoxin regulate hepatocyte glutamine transport independently and coordinately.

Several compounds, including tumor necrosis factor (TNF)-α, glucocorticoids and prostaglandins mediate the endotoxin-induced increase in hepatic glutamine transport. Pretreatment of endotoxemic rats with an anti-TNF-α monoclonal antibody diminished the endotoxin-induced enhancement in transport activity by >50% by decreasing carrier maximum velocity (Inoue et al. 1994). In contrast, when the antibody was given after LPS challenge, transport activity was not attenuated. In vivo administration of TNF-α to rats did not alter sodium uptake but resulted in time- and dose-dependent 50% maximal increases in System N activity secondary to an increase in the transport $V_{\text{max}}$ (Pacitti et al. 1993). Similar to endotoxin treatment, maximal increases in transport were observed 4 h after exposure to TNF-α and had returned to basal levels within 24 h. Pretreatment of animals with the glucocorticoid receptor antagonist RU 38486 attenuated the TNF-α-induced enhancement in transport activity by 50%. These data indicate that the marked increase in System N transport activity stimulated by TNF-α is mediated in part by the glucocorticoid hormones and represents an important mechanism underlying the accelerated hepatic amino acid uptake that occurs during critical illness.

These investigations indicate that inflammatory-mediated increases in the activity of hepatic System N may play a major role in redirecting glutamine flow during sepsis and support glutamine utilizing processes in the liver. However, it is not possible from these aforementioned studies to distinguish whether endotoxins or cytokines administered in vivo exert their effects directly or indirectly on transport. To test for a direct effect, Fischer et al. (1996b) studied the effects of IL-6 and TNF-α on glutamine transport in isolated human hepatocytes. They reported that both cytokines exerted a small stimulatory effect on glutamine transport. Dexamethasone alone did not alter transport, but pretreatment of the cultured human hepatocytes augmented the effects of both cytokines on carrier-mediated glutamine transport. Dexamethasone pretreatment and a combination of IL-6 and TNF-α resulted in a more than twofold increase in transport activity. Fluorescent-activated cell sorter analysis demonstrated that dexamethasone induced a threefold increase in the expression of high affinity IL-6 receptors.

Recent studies have demonstrated that the increase in
hepatic glutamine transport that occurs during sepsis is not transcriptionally regulated (Karinch and Souba, unpublished observations). Endotoxicemic rats do not display an increase in System N gene expression despite a several fold increase in transport activity. By Western blot analysis, the System N protein level in hepatic plasma membrane vesicles prepared from LPS-treated rats appears to be increased by ~50%. Studies are in progress to learn more about the regulation of this response.

Previous studies in other cells have shown that endotoxin and cytokines modulate glutamine transport. For example, endotoxin (Herskowitz et al. 1991), TNF-α and IL-1 (Souba et al. 1991) stimulate glutamine uptake by endothelial cells, whereas high doses of TNF-α diminish glutamine transport in fibroblasts (Dudrick et al. 1992). In cultured Caco-2 cells, γ-interferon decreases glutamine transport across the brush border, whereas TNF-α and IL-1 do not alter transport (Souba and Copeland 1992). Here again we see that the effects of cytokines, whether direct or indirect, are diverse and tissue specific. This is in keeping with the concept that the body responds to prevailing metabolic pressures to redirect the flow of glutamine during critical illnesses and this is often accomplished at the plasma membrane level. It appears that hepatocytes, lymphocytes and endothelial cells rank high on the body’s list of cells that must have adequate amounts of glutamine after a septic insult. Although sepsis causes marked increases in both net glutamine consumption and in net glutamine production, tissue requirements outstrip the body’s ability to produce glutamine and, with time, tissue and plasma levels fall.

SUMMARY

Severe infection results in marked changes in interorgan glutamine flow and in glutamine depletion. The resulting alterations in glutamine transport and metabolism are regulated by a number of mediators, including cytokines and glucocorticoids. What are the consequences of this altered interorgan glutamine shuttle that develops in sepsis, especially if it results in severe and long-standing glutamine depletion? Under these circumstances, glutamine availability could become rate limiting and result in inadequate amounts of this key substrate for certain cells (Newsholme and Parry-Billings 1990). Moreover, such critically ill patients often have a gastrointestinal tract that is unusable or unused; hence, they are nourished with total parenteral nutrition, which currently does not contain glutamine. In these situations, the administration of exogenous glutamine in conjunction with anabolic agents that promote nutrient uptake may be beneficial.

LITERATURE CITED


