Regulation of the Cellular and Physiological Effects of Glutamine

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Abstract: Glutamine is the most abundant amino acid in humans and possesses many functions in the body. It is the major transporter of amino-nitrogen between cells and an important fuel source for rapidly dividing cells such as cells of the immune and gastrointestinal systems. It is important in the synthesis of nucleic acids, glutathione, citrulline, arginine, gamma aminobutyric acid, and glucose. It is important for growth, gastrointestinal integrity, acid-base homeostasis, and optimal immune function. The regulation of glutamine levels in cells *via* glutaminase and glutamine synthetase is discussed. The cellular and physiologic effects of glutamine upon the central nervous system, gastrointestinal function, during metabolic support, and following tissue injury and critical illness is also discussed.

INTRODUCTION

The goal of this article is to explore the current understanding of regulatory mechanisms that govern glutamine homeostasis in the body. As an overview, glutamine is the most abundant amino acid in humans and accounts for approximately 60% of the free amino acid pool in muscle and approximately 20% of the amino acid pool in plasma [1]. Muscle synthetic rates of glutamine are high, and glutamine is the major transporter of amino-nitrogen from muscle and lung. In the brain, glutamine functions as a nitrogen transporter, which allows for the removal of excess ammonia to help preserve neuron-astrocyte interchange of amino acids and monoamines and, thus, protect central nervous system (CNS) functional integrity [2]. Glutamine exhibits very rapid turnover rates and it serves as an important metabolic and energy substrate in a wide variety of cellular reactions throughout the body. It is an important anapleurotic substrate and may be particularly valuable as an energy source during states involving oxygen deprivation. Glutamine is an important substrate for protein synthesis and both hepatic and renal gluconeogenesis. It donates nitrogen for nucleotide and amino sugar synthesis, and it participates in free-radical scavenging by supporting glutathione homeostasis. It helps control acid-base homeostasis by facilitating renal ammoniagenesis and contributes to hepatic ureagenesis and nitric oxide metabolism by serving as a substrate for citrulline and arginine syntheses. It participates in neurotransmitter homeostasis by acting as a precursor for gamma aminobutyric acid (GABA) and as a shuttle for glutamate. Glutamine is important in cell growth, especially in rapidly replicating cell populations such as lymphocytes, fibroblasts, and enterocytes, which use it as a preferred fuel source. Although the exact mechanism of action is not known, glutamine (and glutamate) appear to be necessary for optimal growth, as diets deprived of these substrates, either completely [3] or in lesser proportion to essential amino acids [4, 5], have been observed to be growth-limiting in both animal and human infant study models.

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GLUTAMINE CATABOLISM

Glutamine and glutamate share a number of functions that can be fulfilled by either substrate, since they are metabolically interconvertable [6]. Because most tissues are capable of glutamine synthesis, it is considered to be a non-essential amino acid under normal metabolic conditions. However, during acute injury states, characterized by catabolism and negative nitrogen balance, glutamine may become conditionally essential if endogenous synthesis rates cannot meet metabolic utilization requirements [7].

The control of glutamine homeostasis is based, in large part, on the balance between glutamine formation and breakdown. The enzymes responsible for these two processes are glutamine synthetase (GS) and glutaminase, respectively. Glutamine synthetase is a ligase, which catalyzes the synthesis of glutamine from glutamate and ammonia in the cytoplasm.

Due to the relative cytoplasmic abundance of these two precursor substrates, the control of glutamine production is GS-dependent. Intracellular concentrations of this enzyme are directly related to the transcription of glutamine synthetase mRNA and the subsequent production of GS protein. The expression of glutamine synthetase is regulated by the stability of GS protein in response to intracellular glutamine concentration [8] and increased GS mRNA transcription in response to glucocorticoid [9]. This transcriptional response to stress-induced steroid production has been demonstrated in a rat model to be tissue-specific (lung and muscle) [10] and glucocorticoid receptor-dependent [11]. Using rat L2 cells (clonal isolation from rat alveolar type II pneumocytes), investigators have been able to show that (a) decreasing glutamine concentrations in the culture medium are associated with increased (post-transcriptional) GS protein production; (b) in combination, the effects of low glutamine concentration and glucocorticoid-induced increases in GS protein production are synergistic; (c) the addition of glutamine to the culture medium results in decreased GS protein concentrations (product feedback); and, (d) this effect

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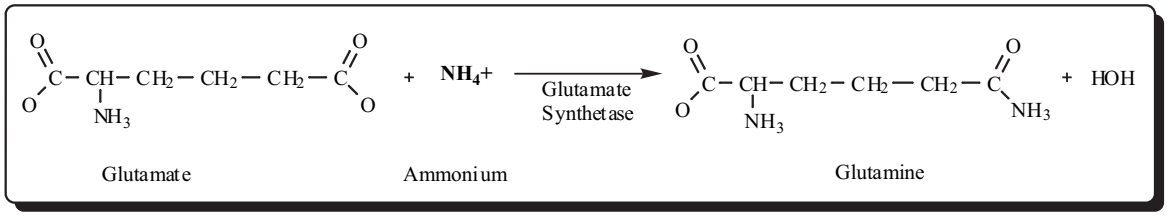


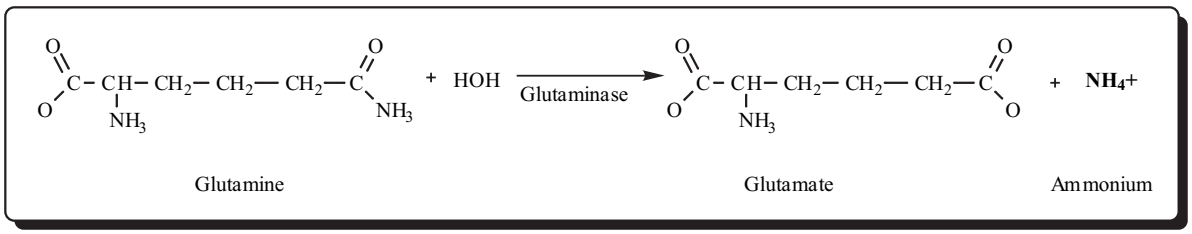
(Glutamate + NH_4^+ + ATP → Glutamine + ADP + Pi + H^+)

is almost completely blocked by 26S proteosome inhibition [12]. The synergistic response relative to GS protein production is consistent with independent effects of glucocorticoid (transcriptional) and low intracellular glutamine (post-transcriptional) concentrations on GS gene expression. This combined regulatory mechanism helps to establish a homeostatic balance between glutamine supply and demand.

Glutamine breakdown can result in the formation of glutamate and ammonia, but can also occur during a variety of deamidation reactions during cellular metabolism. A number of transamidases are capable of catalyzing this reaction in the cytoplasm of a variety of cells, using the γ-amido nitrogen of glutamine [13]. In contrast, glutaminase acts in the mitochondrion to catalyze the hydrolysis of glutamine (also at the γ-amido nitrogen location), yielding glutamate and ammonia.

Glutaminase has been demonstrated in the liver and kidney, and the expression of at least three non-hepatic isoforms of the enzyme has been determined using tissue-specific alternative splicing [14]. The hepatic isoform is expressed exclusively in the mitochondria of periportal hepatocytes of the post-natal liver. The regulation of the activity of the enzyme can reflect the function of the organ in which it exists. In the liver, glutaminase expression is increased as a result of increased hepatic glutaminase gene transcription in association with processes resulting in protein catabolism, such as starvation or diabetes [15, 16], during which hepatic gluconeogenesis and ureagenesis increase. Furthermore, when hypoinsulinemia or hyperglucagonemia are reversed by exogenous insulin or glucose administration, elevated glutaminase concentrations decrease to normal levels [17]. Both insulin and glucagon alter mitochondrial cAMP concentrations, and a cAMP-responsive component has been identified in the promoter region of the hepatic glutaminase gene in rats [18]. Epinephrine-induced activation of hepatic glutaminase also occurs in conjunction with increased cAMP concentrations [19]. In the kidney, increased glutaminase gene expression has been described in response to metabolic acidosis [15], where increased renal ammoniagenesis resulting from the hydrolysis of glutamine is known to facilitate acid-base homeostasis (see below). In concert, these findings indicate that the regulation of glutamine/glutamate homeostasis involves the control of tissue-specific glutamine synthetase and glutaminase gene expression in response to variations in hormonal signals and alterations in glutamine concentration, and results in the maintenance of relatively constant glutamine levels during a variety of metabolic conditions.

In regard to tissue specificity related to the regulation of glutamine homeostasis, the partition of metabolic functions to specific anatomic intraorgan and intracellular compartments may play an important role in the regulation of glutamine physiology. As an example, in the liver, glutaminase activity is higher in the hepatocytes of the periportal region, where glutamine uptake from the hepatic circulation occurs [20]. In contrast, hepatic synthesis and release of glutamine occurs primarily in the perivenous region, where hepatocyte concentrations of glutamine synthetase are higher [21, 22]. It has been suggested that this anatomic separation of sites, where glutamine breakdown and synthesis occur, facilitates the hepatic removal of excess ammonia from the circulation [23]. The majority of ammonia uptake is thought to occur in periportal cells where ammonia, along with glutamine and other amino acids, is used as substrate for urea synthesis. However, some ammonia escapes detoxification in this manner, due to a relatively low affinity of urea cycle enzymes for ammonia. In contrast, glutamine synthetase has a relative high affinity for ammonia, allowing for the perivenous cells to take up and utilize this excess ammonia in the synthesis of glutamine. In the brain, neurotransmitter function is facilitated by glutamate-glutamine compartmentation, which allows glutamate, removed by astrocytes from the synaptic cleft, to be returned to the pre-synaptic neuron for further signaling [24]. In comparison, glutamine metabolism in intestinal mucosal cells (enterocytes) is separated within the cell on the basis of biochemical processes into cytoplasmic and mitochondrial compartments. The synthesis of purines, pyrimidines, and amino sugars using the amide nitrogen of glutamine takes place in the cytoplasm, whereas glutamine carbon skeleton catabolism, initiated with α-amino group



(Glutamine + H_2O → Glutamate + NH_3)

deamination occurs in the mitochondria through the action of mitochondrial phosphate-dependent glutaminase [25]. Another potential feature of biochemical regulation involves the concept of tight channeling, a biochemical phenomenon in which two or more sequential enzymes in a given pathway interact to directly transfer a metabolic substrate intermediate from one enzyme to a second enzyme without diffusion of the intermediate into bulk solvent. Channeling of this sort is known to occur with urea cycle enzymes [26]. Speculation has been advanced, on theoretical grounds, that glutaminase and carbamoyl phosphate synthetase (the mitochondrial enzyme that incorporates the first nitrogen into the urea cycle) might also be capable of this type of interaction [27], thus providing a mechanism by which glutamine could participate in the regulation of urea synthesis.

GLUTAMINE FUNCTIONS AS NEUROTRANSMITTER

Brain function is partially mediated by glutamate, the principal excitatory neurotransmitter in the mammalian central nervous system (CNS), and by gamma-aminobutyrate (GABA), the principal inhibitory neurotransmitter. Both of these compounds are produced from glutamine. Intercellular metabolism of glutamine between neurons and astrocytes participates in the regulation of brain function. The process is facilitated by the cerebral glutamate-glutamine cycle [28], in which glutamate, released from the pre-synaptic neuron during neurotransmission, is cleared from the synaptic cleft and transported to astrocytes, where it is converted to glutamine. This glutamine is then transported from the astrocyte back to neurons, where it is converted to glutamate and, once again, can participate in neurotransmission. A number of enzymes and specialized protein transporters that facilitate this process have been identified, and the compartmentalization of these proteins within the cerebral tissue is thought to contribute to functional efficiency. First, a number of sodium ion-dependent protein transporters, including specific glutamate transporter isoforms, excitatory amino acid transporters, astroglial transporters, and a predominantly neuron-specific transporter, have been identified in cerebral tissue. These protein transporters have a high binding affinity for glutamate. They facilitate glutamate clearance from the synapse and glutamate transport to astroglia [29]. Once in the astrocyte, glutamate is converted to glutamine by an astroglia-specific glutamine synthetase [30]. Glutamine transport back to the neuron from the astrocyte is facilitated by specific sodium and hydrogen ion-dependent astroglial and neuronal glutamine transporters [31, 32]. Within the neuron, glutamine is converted back to glutamate by a phosphate-activated mitochondrial glutaminase (PAG) [33]. Within the neuron, glutamate can also be converted to GABA by glutamate decarboxylase (GAD). Since the accumulation of excessive excitatory amino acids is thought to cause neuronal death resulting from excitotoxicity (due to high intracellular calcium concentrations), an important role of the glutamate-glutamine cycle is to keep CNS glutamate levels within appropriate ranges, particularly during periods of physiological stress. For example, exposure of rat pheochromocytoma (PC12) cells to hypoxia results in increased expression of glutamine synthetase mRNA and protein, with increased glutamine synthetase activity, in association with decreased PAG mRNA and protein expression, and decreased PAG activity [34]. Hypoxia also results in increased concentrations of GAD protein and GAD activity, as well as increased concentrations of several of the glutamate transporters noted above. Together, these effects help to maintain CNS glutamate levels within a safe range so as to protect against excitotoxic neuronal damage.

GLUTAMINE REGULATES RENAL ACID-BASE HOMEOSTASIS

Glutamine serves as a substrate for gluconeogenesis. In the normal post-absorptive state, approximately 5% of the glucose generated in the body derives from glutamine, 75% of which is generated in the proximal tubules of the kidney [35]. Glutamine also serves an important function in renal acid-base homeostasis. Two ammonia ions, which act as cations to facilitate urinary acid excretion, and α-ketoglutarate are generated from the deamination (by renal glutaminase) and the oxidative deamination (by glutamate dehydrogenase) of each glutamine molecule. In addition, two bicarbonate ions are generated from the further breakdown of α-ketoglutarate, which can enter the circulation to serve as acid buffers. The renal catabolism of glutamine increases during metabolic acidosis. During this period, renal extraction of plasma glutamine, derived primarily from the muscle glutamine pool [36], increases to about 30%. The regulation of this process has been studied primarily in the rat kidney (both in vivo and *in vitro*), where increased expression of genes that encode renal glutaminase, phosphoenolpyruvate decarboxylase (PEPCK), and glutamate dehydrogenase, as well as protein transporters for mitochondrial glutamine, a basolateral bicarbonate co-transporter, and an apical Na^+/H^+ exchanger have all been observed to increase in response to induced acidosis [37]. Associated increases in the glutamine mitochondrial transporter [38] and Na^+/H^+ exchanger [39] activity facilitate mitochondrial glutamine catabolism and acidification of the urine to promote removal of cellular ammonium ions, respectively. In addition, results using a specialized renal cell line that retains pH-responsive adaptations and has gluconeogenic capability suggest that the regulatory mechanism of renal glutamine homeostasis depends on the pH-responsive increases in renal glutaminase and PEPCK mRNAs [40, 41]. A pH-induced increase in glutamate dehydrogenase mRNA has also been demonstrated in this cell line, further supporting the role of glutamine in the adaptive response of the kidney to acidosis [42]. While increased PEPCK activity is due to increased transcription of the PEPCK gene [43], it has been suggested that increased glutaminase activity results from pH-responsive stabilization of glutaminase mRNA [44, 45]. Induction of PEPCK is thought to occur as a result of decreasing intracellular pH, triggering activation of a stress-activated protein kinase resulting in phosphorylation (activation) of a specific transcription factor that binds to the PEPCK promoter, thus, enhancing transcription [37]. In contrast, increased renal glutaminase activity is thought to be related to glutaminase mRNA stabilization, due to the binding of a ζ-crystallin NADPH:quinone reductase to an eight-base pH-responsive

element located in the 3'-untranslated region of glutaminase mRNA [44, 45]. The signal transduction mechanism for these two processes (stabilization of glutaminase mRNA and transcription of the PEPCK gene) is likely to be the same, since both occur in concert in the same area of the renal proximal tubules [37].

GLUTAMINE AS FUEL AND GROWTH PROMOTING AGENT

Glutamine is an important fuel source and nucleic acid precursor (nitrogen donor) for cells that proliferate rapidly and require readily available energy for metabolic support. These populations include cells such as intestinal mucosal cells (enterocytes) and stimulated immune cells. For example, glutamine is the preferred respiratory fuel source for lymphocytes, where its utilization is four times higher than that of glucose [46]. As previously discussed, the principal source of this glutamine is skeletal muscle and, to a lesser degree, the lung. Although glutamine has been shown to promote a variety of functions in immune cell populations (phagocytosis, cytokine production, bacteriocidal effect, cell growth and proliferation), the mechanisms governing glutamine action in these processes remain largely unknown. The fact that neutrophils, lymphocytes, and macrophages take up and metabolize glutamine at high rates *in vitro*, and that glutaminase activity is also high in these cells has led to the speculation that the amount of glutamine available to the cell might have a regulatory effect on cell proliferation or function. For instance, the proliferation of stimulated lymphocytes has been observed to increase in a linear fashion dependent upon increasing concentrations of glutamine added to cultures [47]. Conversely, the reduction of extracellular glutamine concentrations has been shown to result in a 40% reduction in MHC class II expression in stimulated human monocytes [48]. Studies in stimulated murine macrophages and human monocytes, demonstrate that cytokine production is dependent on the concentration of glutamine available in the medium [49].

In the bowel, glutamine serves as an important energy substrate for oxidative metabolism in enterocytes and gut-associated lymphocytes, as a precursor for the synthesis of nucleotides, amino sugars, and other amino acids (such as arginine, urea cycle intermediates, and proline), and as a stimulus to increase intestinal blood-flow [50]. Glutamine is the preferred respiratory fuel source for enterocytes [51]. The presence and activity of glutamine synthetase and glutaminase has been established throughout the mucosa of the adult human gastrointestinal tract on the basis of biopsy analysis [52]. Glutamine synthetase activity was found to be substantial in the stomach, but was low in the small and large intestine and in the esophagus. In contrast, glutaminase activity was high in the small and large bowel (highest in the duodenum) and low in the stomach and esophagus. These data support the concept that the intestine is capable of metabolizing glutamine. The intestine (with the exception of the stomach) utilizes vascular glutamine (derived from other tissues or delivered exogenously) and dietary glutamine (derived from the gut lumen). Glutamine metabolism may differ during infancy. Study of rat small intestine shows that glutamine synthetase activity in the small intestine increases during gestation, peaks in infancy, and then is decreased in adults [53]. Furthermore, inhibition of glutamine synthesis *in vitro* is associated with diminished enterocyte growth and differentiation [54]. Glutamine may regulate gut growth by stimulating mitogen-activated protein kinases (MAPK). Using rat intestinal crypt (IEC-6) cells, investigators have shown that MAPK activity, thymidine incorporation, and cell number increase after addition of glutamine to the medium [55]. This effect is substantially greater in glutamine-starved versus glutamine-sufficient (unstarved) IEC-6 cells. The mitogenic effect is totally inhibited by cAMP in unstarved cells and partially inhibited in starved cells. Since cAMP is known to block extracellular signal-related kinases (ERK), these data suggest that glutamine regulates enterocyte mitogeny *via* this signaling pathway when intracellular glutamine concentration is sufficient. In glutamine-starved cells, where the mitogenic effect was only partially inhibited, glutamine was found to activate a second MAPK signaling pathway, c Jun nuclear kinase (stress-activated protein kinase), which was not blocked by cAMP.

GLUTAMINE ROLE IN CRITICAL ILLNESS

The response of the host organism to tissue injury (such as trauma, sepsis, and intense radiotherapy) is stereotypic and depends on the magnitude and duration of the insult. The injury response is characterized by the release of cytokines, counter-regulatory hormones, and other inflammatory mediators, resulting in the catabolism of endogenous substrate resources in order to provide energy and metabolic precursors to fuel the response process. During the injury response, the interorgan flow of glutamine is altered. Under normal physiological conditions, a moderate amount of glutamine (which is largely produced in muscle and lung) flows primarily to the gut and, to a lesser degree, to the immune cell pool and the liver. In contrast, glutamine production in muscle and lung substantially increases following tissue injury, and flow is redirected primarily to the liver and immune cell pool with decreased flow to the gut. It is believed that glutamine becomes a conditionally essential amino acid when glutamine synthesis cannot keep up with catabolism and accelerated export from muscle (and lung) [56]. In these circumstances, glutamine (or precursor glutamate) availability can become a rate-limiting feature of glutamine metabolism [57].

Several mechanisms are thought to play a regulatory role in the redirection of the flow of glutamine between tissues during critical illness [58]. Muscle catabolism is stimulated by metabolic acidosis, insulin deficiency, and glucocorticoids [59]. These signals stimulate transcription of genes that encode ubiquitin (UbA, UbB, and UbC), ubiquitin-conjugating enzymes, and proteosome subunits. The ubiquitin-proteasome enzyme complex is a major proteolytic system in many cells. A human UbC promoter region with potential binding sites for several transcription factors (including Sp1) has been identified [60]. Using glucocorticoid-stimulated L6 muscle cells, investigators have demonstrated that increased ubiquitin (UbC) gene expression occurs in association with the activation of Sp1 and mitogen-activated protein kinase (MEK1) signaling pathways [61]. Muscle glutamine release increases

substantially in conjunction with increased expression of glutamine synthetase (GS) mRNA and increased activity of glutamine synthetase following endotoxin challenge in rats [62]. Similar stimulation of gene expression and enzyme activity were observed in rats administered glucocorticoids. However, when glutamine was infused instead of saline (control), glutamine synthetase expression was inhibited, though muscle glutamine concentration remained constant, suggesting additional regulatory control mechanism(s) [63].

Glutamine synthetase expression in muscle is regulated by both transcriptional and post-transcriptional mechanisms [9]. Glucocorticoids substantially increase muscle GS mRNA concentrations, without an equivalent increase in GS protein concentrations. This discrepancy is thought to be due to GS protein feedback destabilization by newly synthesized glutamine. GS activity is diminished in the presence of increasing concentrations of glutamine [64]. Inhibition of this effect results in proportional increases in both GS gene expression and enzyme activity levels [9]. The expression of GS in lung tissue is also up-regulated in response to tissue injury, and, similar to muscle, this up-regulation is due to a direct glucocorticoid-mediated process. Furthermore, as in muscle, glutamine destabilization of GS activity is felt to be an important post-transcriptional control mechanism regulating GS gene expression [8].

In addition to the hormonal factors influencing glutamine synthetase and glutaminase expression and activity in the liver, hepatocytes are known to possess novel plasma-membrane glutamine transporter systems [65]. A Na^+-dependent System *N* transports glutamine into the hepatocyte, and a Na^+-independent System n transports glutamine out of the hepatocyte. Endotoxin administration in rats has been found to increase System N transporter activity two- to threefold while System N activity remains unchanged [66]. In addition to endotoxemia, starvation also increases hepatocytic transport-mediated glutamine uptake, and both starvation and endotoxin act synergistically in this regard [67]. Furthermore, TNF-α, IL-6, glucocorticoids, and prostaglandins have been shown to mediate this endotoxin-induced increase in System N glutamine transport into hepatocytes [58]. In contrast, glutamine uptake by the gut following endotoxin challenge in rats is diminished in association with decreased glutamine transport across the brush border and decreased enterocyte glutaminase activity [68, 69], though lymphocyte glutaminase activity in mesenteric lymph nodes is substantially increased [70]. In concert, these results help to explain the alterations in glutamine interorgan flow, which occur in response to tissue injury.

CONCLUSION

In summary, the regulation of glutamine homeostasis involves a variety of mechanisms and includes mechanisms that regulate the expression and activity of glutamine synthetase and glutaminase isoforms, often on a tissue-specific basis, in response to systemic changes in hormonal concentrations and other metabolic parameters. In some cases, regulation also appears to depend on the expression and activity of additional enzymes associated with glutamine metabolism and tissue-specific protein transporters that facilitate transcellular or intracellular glutamine commerce. Insufficient glutamine availability (during severe catabolic states) or glutamine excess (through feedback destabilization of glutamine synthetase) may both play regulatory roles. Furthermore, mechanisms involving the activation of specific protein kinase signaling pathways, mRNA stabilization, intraorgan and intracellular partition of glutamine metabolism, and unique biochemical relationships (such as tight channeling) have been linked to the regulation of glutamine metabolism. In all likelihood, these mechanisms paint a very preliminary and largely incomplete picture of the processes governing glutamine metabolism, but they also provide an important foundation upon which a comprehensive and accurate understanding may be built. In final analysis, the value of these data will ultimately be determined by the improvements they bring to patient care.

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