Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes

ITZHAK NISSIM
Division of Child Development and Rehabilitation, Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4318

Nissim, Itzhak. Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. Am. J. Physiol. 277 (Renal Physiol. 46): F493–F497, 1999.—This review focuses on the role of acute pH changes in the regulation of Gln/Glu metabolism in the kidney, liver, and brain. Alterations of proton concentration ([H⁺]) profoundly affect flux through phosphate-dependent glutaminase (PDG) or glutamate dehydrogenase (GDH), the primary enzymes responsible for mitochondrial metabolism of glutamine and glutamate, respectively. In the kidney, acute acidosis stimulates Gln uptake and its metabolism via the PDG pathway. The Glu formed from Gln can be removed via 1) oxidative deamination through the GDH reaction, 2) transamination reactions, and 3) transport of Glu from intracellular to extracellular compartment, thereby diminishing the intramitochondrial pool of glutamate sufficiently to stimulate flux through the PDG pathway. Converse changes may occur with increased pH. In the liver, acidosis diminishes the rate of Gln and Glu metabolism via the PDG and GDH pathways, but stimulates glutamine synthesis (i.e., glutamine recycling). Alkalosis has little effect. Hepatic Gln metabolism via the PDG pathway has a central role in ureagenesis via 1) supplementation of nitrogen for the synthesis of carbamyl phosphate, and 2) providing glutamate for N-acetylglutamate synthesis. In the brain, Gln/Glu metabolism links ammonia detoxification and energy metabolism via 1) detoxification of ammonia and excess glutamate by glutamine synthesis in astrocytes, 2) formation and export of glutamine to neurons where it is metabolized to glutamate and GABA, and 3) production of α-ketoglutarate and lactate from Glu and their transport to neurons. Changes in intracellular pH associated with changes in cellular [K⁺] may have a key role in the regulation of these processes of glial-neuronal metabolism of Gln/Glu metabolism.

investigations of the regulation by pH of glutamine and glutamate (Gln/Glu) metabolism are attracting widespread attention not only because of their nutritional relevance in normal and pathological state, but also because of the role of Gln/Glu in numerous physiological and biochemical processes (3, 4, 15, 18, 23). Gln/Glu has a crucial role in transporting excess systemic ammonia into its detoxification via hepatic ureagenesis (2–4, 10, 11, 14) or via its urinary excretion following renal ammoniagenesis (3, 8, 18, 19). Both hepatic ureagenesis and renal ammoniagenesis have a key role in maintaining acid-base homeostasis and whole body nitrogen balance (4, 18). In addition, synthesis of Gln from Glu and ammonia in the brain is an important, albeit temporary, alternative pathway for detoxification of ammonia in cases of hepatic failure associated with hyperammonemia (4, 15). Glutamine synthesis in astrocytes is also important in maintaining glutamate homeostasis in the brain, thereby protecting the brain from excitotoxicity (15, 17, 23). Glutamate is the major excitatory neurotransmitter of the mammalian central nervous system and the major source of GABA (20).

At physiological pH Gln has no net charge, whereas, glutamate has a negative charge due to two carboxyl groups. Because cellular uptake of amino acids is dependent on their chemical-physical characteristics, alteration of systemic pH such as in acute or chronic acidosis or alkalosis may regulate the transport of Gln/Glu across cell membranes, and hence, their metabolism. This review focuses on the role of acute pH
changes in the regulation of Gln/Glu metabolism in the kidney, liver, and brain. Acute pH changes reflect the direct effect of pH on the existing transporter systems and the metabolic pathway in question. Furthermore, alteration of intracellular pH (pHi) has profound effects on the gene(s) expression encoding for synthesis of phosphate-dependent glutaminase (PDG) or glutamate dehydrogenase (GDH), the primary enzymes responsible for mitochondrial metabolism of glutamine and glutamate, respectively (3, 8). Although a variety of hormones and cellular modifiers (such as, Ca\(^{2+}\), K\(^{+}\), cAMP, cGMP) may have important roles in the regulation of Gln/Glu metabolism, their action seems to be subsequent to alteration of pH (13, 18, 19).

An Overview of Gln/ Glu Metabolism

As illustrated in Fig. 1, Gln is either taken up from an extracellular source or synthesized in the cytosol via the glutamine synthetase (GS) pathway (especially in skeletal muscle and adipose tissue), whereas, the mitochondrial PDG and GDH are the immediate pathways by which Gln and Glu are metabolized (3, 8–19). The hepatic PDG is made up of different isozymes with different kinetic properties and protein structure from that in the kidney or the brain (3). Gln may also be metabolized via the membrane-bound \(\gamma\)-glutamyl transpeptidase (\(\gamma\)-GT) and the cytosolic glutamine-amino transferase-\(\omega\)-amidase pathway (Gln-II) (3, 7–9, 11, 21). In addition, metabolism of Gln/Glu intersects with the transamination reactions, the \(\gamma\)-glutamyl cycle (synthesis of GSH), purine nucleotide cycle (PNC), the tricarboxylic acid (TCA) cycle, and the hepatic urea cycle (2, 8, 9, 13, 14, 23). Except for the urea cycle, which is exclusively hepatic, the kidney, brain, and liver possess all metabolic pathways and the enzyme machinery outlined in Fig. 1. Thus alteration or perturbation of a metabolic pathway or site which seems remote from the site or pathway in question might well affect Gln/Glu metabolism. Hence, a fuller understanding of Gln/Glu metabolism would require the demonstration of the extent to which their nitrogens are utilized for formation of other amino acids, GSH, ammonia, and/or urea, and the extent to which their carbon skeleton, i.e., \(\alpha\)-ketoglutarate (\(\alpha\)KG), is oxidized in the TCA cycle. Therefore, a tracer kinetic study with isotopes of nitrogen or carbon is essential. To that end, we have employed a stable isotope, i.e., \(^{15}\)N, as metabolic probe and gas chromatography-mass spectrometry (GC-MS) for determination of \(^{15}\)N-labeled Gln/Glu metabolism and of N flux in metabolic intermediates (2, 8–14, 19, 22, 23). Furthermore, this methodological approach allows in vitro experiments to be done with an incubation medium containing a physiological mixture of amino acids, and only Gln or Glu is labeled with \(^{15}\)N (10). This is an extremely important consideration, since exposure of tissue preparation (liver, kidney, or brain) to a single substrate in the absence of a physiological mixture of amino acids might well affect the transport and the metabolism of the test substrate, and thereby confound the results and conclusions to be obtained.

pH Regulation of Gln/ Glu Metabolism

Recent views regarding the pH regulation of the Gln/Glu metabolism in the kidney, liver, and brain are summarized as follows.

Renal Gln/ Glu metabolism. The mechanism(s) by which acute pH changes regulate renal Gln/Glu metabo-

---

**Fig. 1.** Schematic illustration of glutamine and glutamate metabolism and their interaction with the tricarboxylic acid (TCA) cycle, urea cycle, \(\gamma\)-glutamyl cycle (formation of GSH), and transamination reactions. AT, aminotransferase reactions; CPS-I, carbamoyl-phosphate synthetase-I; \(\gamma\)-GT, \(\gamma\)-glutamyl transpeptidase; GL-II, glutamine amino transferase pathway; GS, glutamine synthetase; GDH, glutamate dehydrogenase; NAG, N-acetylglutamate; PC, pyruvate carboxylase; PDG, phosphate-dependent glutaminase; and PDH, pyruvate dehydrogenase; "*" indicates activation.
Glutamate transport and metabolism has posed one of the more complex problems in metabolic regulation. In normal acid-base homeostasis, the kidney can be considered as an organ that supplies ammonia to the body pools (4). However, in case of acidosis, the kidney is the major organ in clearing ammonia from the body via generation of ammonia from glutamine and its excretion in the urine (3, 4, 18, 19). Dependent on the severity of acidosis, the observations have demonstrated that the flux through both PDG and GDH pathways are stimulated in response to acute acidosis (pH 6.8) and inhibited in alkalosis (pH 7.6) (8, 9, 12, 13, 19). Approximately 60% of ammonia is derived from the amido-N of glutamine via the flux through the PDG pathway vs. 15–25% from the amino-N via the GDH reaction (8, 11, 12, 19). In addition, both Gln-II and γ-GT pathways are increased at pH 6.8 and decreased at pH 7.6 compared with pH 7.4. Recent observations using 15N-labeled Gln and cultured LLC-PK1-F495 cells indicate that γ-GT may have a major regulatory role in renal Gln metabolism (unpublished observations). However, previous studies using cultured human kidney cells suggest that both Gln II and γ-GT pathways have a minor role in renal ammoniagenesis from glutamine (8, 9). Furthermore, recycling of glutamine (e.g., formation of Gln from Glu and ammonia via the GS pathway), is negligible in the kidney, especially in acidosis.

What is the mechanism(s) by which acute acidosis augments renal Gln metabolism via the PDG pathway? Two conditions should be met to stimulate the flux through the PDG pathway. First, glutamine uptake must be stimulated, and second, the Glu formed via the PDG pathway must be removed, because of its inhibitory effect on the renal PDG pathway. It has been well documented that acidosis stimulates glutamine uptake by renal mitochondria (3, 18, 19). In addition, experiments with 15N-labeled glutamine and cultured proximal tubule cells suggest that acute acidosis elicits stimulation of Gln uptake and its metabolism via the PDG pathway (13). The glutamate formed from Gln in the mitochondria via the PDG pathway can be removed via 1) the flux through the GDH reaction, 2) transamination reactions, and 3) the transport of Glu from intracellular to extracellular compartment. With [2-15N]glutamine, the data have demonstrated that regardless of whether isolated renal tubules or cultured proximal tubules from different species (human, rat, OK, or LLC-PK1 cells) were used as a model system, the GDH reaction is significantly stimulated at pH 6.8 and inhibited at pH 7.6 compared with pH 7.4 (8, 9, 12, 13, 19). Furthermore, the severity of acidosis (pH 7.0 vs. 6.8) further stimulated the flux through the GDH reaction (12). Similarly, both the conversion of glutamate to alanine and aspartate via transamination reactions and the translocation of glutamate and aspartate from intracellular to extracellular compartment are stimulated by acute acidosis with little effect at acute alkalosis compared with physiological pH (8, 12, 13). Acute acidosis is also associated with decreased intracellular αKG levels following the stimulation of the αKG dehydroge-nase in the TCA cycle (8, 13, 18). Thus decreased αKG level would be expected to favor the oxidative deamination of glutamate via stimulation of the GDH reaction, thereby diminishing the intramitochondrial pool of glutamate sufficiently to stimulate flux through the PDG pathway as we have indicated (13). The opposite may occur with increased pH. The notion that depletion of intracellular Glu in acidosis is directly responsible for stimulation of the flux through the PDG pathway is further supported by recent studies of Welbourne and Mu (7, 21) suggesting that the diminished uptake of extracellular Glu contributes to depletion of intracellular Glu and, thereby, deinhibition of the PDG pathway (7). The observation indicated that in metabolic acidosis, ~50% of the intracellular Glu fall is attributed to reduced production of extracellular Glu via the membrane-bound γ-GT activity and its inward transport (7, 21).

An additional support for the mechanism indicated above can also be obtained from studies with other cellular modifiers of renal Gln/Glu metabolism. For instance, parathyroid hormone (PTH) mimics the effect of acute acidosis on the flux through the PDG and GDH pathways, depletes the intracellular levels of Glu and αKG, and stimulates uptake of glutamine and the export of intracellular glutamate to extracellular compartment (13). Numerous investigations have indicated that PTH inhibited the luminal Na+/H+ exchanger and decreased pHi (19). Hence, the effect of PTH on Gln/Glu metabolism is likely mediated via decreased pHi. However, the opposite was observed in the presence of PGF2α or 12-O-tetradecanoylphorbol-13-acetate (TPA), an activator of protein kinase C (19). Both PGF2α and TPA decreased renal Gln/Glu metabolism mediated by stimulation of Na+/H+ antipporter activity, increased pHi, and reversed the acute acidosis-induced depletion of αKG level (19).

Hepatic Gln/Glu metabolism. As in the kidney, hepatic glutamine metabolism is mediated mainly via the flux through the PDG pathway. PDG is located in the mitochondria of periportal hepatocytes and is sensitive to small changes in pH, [NH4]+, and glucagon (3, 14). Contrary to the kidney glutaminase, the hepatic type has a pH optimum between 7.8–8.2, and the enzyme is not inhibited by glutamate (3). With 15N-labeled glutamine and isolated hepatocytes, the results indicate several site(s) of pH regulation of hepatic Gln/Glu metabolism, including 1) diminished metabolism via the PDG and GDH pathways in acidosis (pH 6.8) and increased alkalosis (pH 7.6), 2) enhancement in acidosis and diminishing alkalosis of the flux through the Gln-II pathway, and 3) stimulation of glutamine synthesis in acidosis (i.e., glutamine recycling) with little effect in alkalosis (11, 14). As in the kidney, at acute acidosis (pH 6.8), there is a remarkable conversion of Glu-N to alanine via transamination and outward transport of alanine to the extracellular compartment (11, 14). It is possible that hepatic alanine production and its removal from hepatocytes serves as an acceptor of excess amino-N in cases of perturbed activity of the urea cycle. At physiological acid-base homeostasis Gln
is the chief precursor for urea-N, whereas in acidosis, the liver is a major donor of GIN for renal ammoniagenesis (4, 11). The periportal Gln metabolism via the PDG pathway is coupled with the perivenous GS pathway, thereby constituting a hepatic glutamine cycle, in which Gln metabolized to ammonia and glutamate in periportal hepatocytes is resynthesized in perivenous cells (2, 4), especially in acidosis (11). It has been suggested that conditions resulting in increased hepatic ureagenesis are associated with stimulated Na-dependent transport of Gln and Glu into liver cells, especially transport of Glu into perivenous cells (6). This phenomenon is consistent with the periportal Gln metabolism and the perivenous Gln synthesis from Glu and ammonia.

The hepatic GDH reaction has an insignificant role in providing N for ureagenesis, and in the liver the GDH reaction is mainly in the direction of reductive amination of αKG to form glutamate required for Gln synthesis (2, 10, 11, 14). The mitochondrial glutamate formed via the PDG or the GDH pathway may have an important role in formation of N-acetylglutamate (NAG), an allosteric activator of carbamoyl-phosphate synthetase-I (CPS-I). NAG is synthesized in the mitochondria from acetyl-CoA and glutamate (14). Therefore, as illustrated in Fig. 1, hepatic Gln metabolism via the PDG pathway has a central role in hepatic ureagenesis via 1) supplementation of nitrogen for the synthesis of carbamoyl phosphate and 2) providing glutamate for NAG synthesis. The observation suggests a linkage between the PDG pathway, CPS-I, and NAG synthesis. These three pathways are located in the mitochondria, are stimulated by glucagon or increased pH, and are decreased by insulin or acute acidosis (14).

Brain Gln/Glu metabolism. Understanding the role of pH in the regulation of the brain Gln/Glu metabolism is of special importance in cases of liver disease and hyperammonemia (4, 15). The blood-brain barrier is freely permeable to ammonia; therefore elevated blood ammonia following liver failure would then lead to increased brain ammonia uptake and ammonia toxicity. Hence, glutamine synthesis from ammonia and glutamate in astrocytes is an important process for brain ammonia detoxification (4, 15, 22, 23). Most of the brain GS activity is localized in astrocytes, and glutaminase activity is mainly in neurons (15, 16, 22, 23). Therefore, a "glutamine-glutamate cycle" has been introduced, in which neuronal Glu is transported to astrocytes and converted to Gln via the GS pathway (15, 17, 22, 23). This "cycle," which metabolically links neurons and astrocytes, may resemble the coupling between the hepatic periportal Gln metabolism via the PDG pathway and its synthesis via the perivenous GS pathway (2).

Similar to the liver and kidney, Gln/Glu metabolism in the brain is highly pH sensitive. Increased pH enhances transport of Glu and decreases formation and export of Gln (4, 15). However, K⁺-induced alkalinization reverses Glu-induced inhibition of the GS pathway (1). Therefore, K⁺ in conjunction with elevated pH is a signal that regulates astrocyte Glu/Gln metabolism. However, the stimulation of the GS pathway by increased pH may be associated with astrocyte swelling rather than with net release of glutamine from astrocytes, thus challenging the importance of the "glutamine-glutamate cycle" in the brain.

Gln, which is taken up by neurons, is metabolized to glutamate and ammonia via the PDG pathway (15, 16, 23). In neurons, Glu is the primary source for GABA formation via the glutamate decarboxylase reaction (20). Therefore, the GS pathway in astrocytes detoxifies ammonia and regulates brain excitability by inactivating glutamate, thereby protecting the brain from excitotoxicity. However, observations obtained in experiments using 15N-labeled glutamine have demonstrated a cycling of glutamate in astrocytes, i.e., astrocytes metabolized Gln via the PDG pathway almost at the same rate (~10 nmol·min⁻¹·mg protein⁻¹) as its synthesis via the GS pathway (22). In addition, astrocytes possess high activity of aminotransferase reactions (16, 17, 22, 23). Thus the combined actions of the GS, PDG, and aminotransferase pathways may determine the fate of Gln/Glu metabolism in the brain.

In vivo studies with 15NH₃ have indicated that with a blood ammonia level of 0.9 μmol/g, the GS approaches enzyme-saturating level (5). Thus the brain may benefit from the GS pathway until the enzyme-saturating activity is reached. Similarly, in cultured astrocytes the fraction of Glu that is converted to Gln is decreased with increased Glu concentration in the incubation medium (17). Thus the excess glutamate beyond the GS saturating activity must be metabolized by other means to prevent glutamate toxicity in the brain.

It has been suggested that the energy demand may determine the metabolic fate of glutamate in astrocytes (15–17). In addition to the GS pathway, Glu can be metabolized via 1) the oxidative deamination through the GDH pathway forming ammonia and αKG and 2) transamination reactions (16, 17). In astrocytes, GDH reaction is mainly in the direction of oxidative deamination to provide αKG. The αKG so formed is oxidized in the TCA cycle to supply energy and lactate (16). The latter is an important transferable energy source required by neurons. Neurons lack the capacity to synthesize de novo TCA cycle intermediates due to the absence of pyruvate carboxylase (16). However, neurons have high-affinity uptake for αKG and lactate (16, 17, 20). Thus Gln/Glu metabolism in astrocytes links ammonia detoxification and energy metabolism via 1) detoxification of ammonia by the GS pathway; 2) formation and export of glutamine to neurons where it is metabolized to glutamate, and thereby, GABA production; and 3) formation of αKG and lactate from Glu and their transport to neurons. Changes in pH, associated with changes in cellular [K⁺] may have a key role in the...
regulation of these processes of glial-neuronal metabolism of Gln/Glu metabolism.

Summary

In the kidney, lowering extracellular pH stimulates inward transport of Gln and outward transport of Glu as well as Gln/Glu metabolism. In the liver, lowering pH stimulates the perivenous Gln synthesis and inhibits the periportal Gln metabolism and ureagenesis, whereas the opposite occurs with elevated pH. In astrocytes, increased pH stimulates Glu uptake and the GS pathway without net stimulation of glutamine release. The $[H^+]_i$ is a key signal that determines the metabolic fate of Gln/Glu in the kidney, liver, and brain. Thus alteration of systemic pH may have a significant role in the regulation of nitrogen shuttling and economy in the body.

I thank Dr. M. Yudkoff for careful review and helpful comments. This work was supported by National Institutes of Health Grants DK-53761 and NS-34900.

Address for reprint requests and other correspondence: I. Nissim, Division of Child Development, Abramson Pediatric Research Ctr. Rm. 510C, 34th St. and Civic Center Boulevard, Philadelphia, PA 19104-4318.

REFERENCES