The Glutamine/Glutamate Couplet and Cellular Function

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All cells require glutamine as a nitrogen donor as well as an energy source for cell-specific functions. Understanding how glutamine utilization is metered to these demands is fundamental to basic cell processes as well as to therapeutic manipulation of regulatory mechanisms. The regulatory role of the glutamine/glutamate couplet in cellular function is illustrated for acid-base homeostasis and for production of the extracellular matrix.

Glutamine (Fig. 1) is the major source of nitrogen for protein synthesis and is an important oxidizable fuel present in plasma and in culture media. In contrast, glutamate (Fig. 1) is far less prevalent than glutamine, at a plasma concentration only 1/50 of glutamine. This static disparity, however, belies the dynamic relationship of glutamine conversion to glutamate and glutamate’s primary role in regulating and fueling cellular processes. The development of this concept and its use in understanding cellular and organ responses to physiological challenges is our thesis.

Development of the concept

The conversion of glutamine to glutamate occurs in both the extracellular and intracellular compartments in many cell types and organs (Fig. 1). These site-specific rates can be monitored by following the formation of glutamate from glutamine either labeled in the amino nitrogen position with ¹⁵N (8) or on the carbon skeleton with ¹⁴C (6). In proximal tubule-like epithelial cells obtained from pig kidney (LLC-PK₁/F⁺; Fig. 2), the extracellular glutaminase [phosphate-independent glutaminase (PIG)] is localized on the external surface of the plasma membrane in close association with the intrinsic membrane high-affinity glutamate transporters (X₅₆ sorts EAAC1 and GLT1; Fig. 2). Although glutamate does not normally accumulate in the media of cells expressing PIG, its contribution to glutamate uptake can readily be demonstrated after the high-affinity glutamate uptake is blocked and following the conversion of uniformly ¹⁴C-labeled glutamine to [U-¹⁴C]glutamate; extracellular glutamate then accumulates, and this accumulation of radiolabeled glutamate can be eliminated by inhibiting PIG with acivicin (6).

Glutamine is also converted to glutamate by the intracellular phosphate-dependent glutaminase (PDG), whose functional activity is expressed on the cytosolic surface of the inner mitochondrial membrane, as first demonstrated by Kvamme et al. (4). Using isolated mitochondria from pig kidney (Fig. 3), they monitored the appearance of [U-¹⁴C]glutamate formed from [U-¹⁴C]glutamine in the media and mitochondrial matrix compartments with time and showed that glutamate enrichment in the former preceded and greatly exceeded that of the matrix compartment. Importantly, they also showed that this functional glutaminase was competitively inhibited by glutamate present in the cytosol (12). The presence of the functional glutaminase activity within the cytosolic compartment and inhibited by glutamate, a product of the extracellular glutaminase, suggested that the formation and uptake of extracellular glutamate may regulate the intracellular couplet conversion rate.

Using the proximal tubule-like pig kidney cells, we eliminated extracellular glutamate uptake either by blocking the high-affinity uptake with D-aspartate or by inhibiting PIG with acivicin. Both of these maneuvers produced a 40–50% reduction in the intracellular glutamate content without reducing the extracellular glutamate content (18). Measurement of the intracellular hydrolysis of [¹⁴C]glutamine to [¹⁴C]glutamate showed a two- to threefold increase in the conversion rate (18), whereas a tenfold increase in media [¹⁵N]glutamate from [2-¹⁵N]glutamine under these conditions demonstrated the close proximity of the functional glutaminase to the plasma membrane transporters responsible for the efflux (20). Thus extracellular conversion of glutamine to glutamate coupled to uptake functions as a unit (17) in modulating the intracellular conversion of glutamine to glutamate.

This functional unit can be rendered inoperative by “knockout” either the EAAC1 or GLT1 glutamate transporter gene in mice. With the EAAC1 gene knockout the intraluminal conversion of glutamine to glutamate results in a large glutamate to NH₄⁺ excretion (11); with the GLT1 gene knockout (16) antiluminal glutamate uptake is reduced, lowering the cytosolic concentration and activating the intracellular glutaminase, resulting in an increased NH₄⁺ excretion (K. Tanaka and T. Welbourne, unpublished observations). Pharmacological knockout of PIG by using acivicin in rats in vivo lowers kidney glutamate content and enhances renal glutamine uptake and NH₄⁺ production (19). These findings are consistent with the functional unit playing a regulatory role in modulating intracellular glutaminase flux in vivo (17).

The fate of glutamate formed intracellularly from glutamine labeled with ¹⁵N in the amino position can be studied after removing the functional unit control by using X₅₆ blockers and/or acivicin. The appearance of [¹⁵N]alanine reflects the transamination of [¹⁵N]glutamate with pyruvate catalyzed by
alanine aminotransferase (ALT), predominantly a cytoplasmic activity, whereas the $^{15}$N in $\text{NH}_4^+$ reflects the deamination of glutamate catalyzed by glutamate dehydrogenase (GDH) present within the matrix space of the mitochondrion (Fig. 2; Refs. 9 and 14). In pig kidney cells, as in most cells, both GDH and ALT pathways are expressed, but the glutamate flux through the cytosolic ALT pathway is normally far higher than the flux through the intramitochondrial GDH pathway (20). However, if the cytosolic pH is reduced by using the acid-loading modality of the $X_{\text{GC}}$ activity (6), cytosolic glutamate is channeled into the mitochondrial GDH pathway via the proton-driven inner membrane glutamate transporter (14). Consequently, the $^{15}$N label from glutamate is recovered in $^{15}$NH$_4^+$ (20). Thus the balance between glutamate’s amino nitrogen recovered as alanine versus that recovered as NH$_4^+$ provides a window into the prevailing cytosolic acid/base conditions (10, 20).

**Pivotal role of glutamate formed in the cytosol**

The glutamate formed in the cytosol is a pivotal point for dedicated cellular processes, and its fate is determined as depicted in Fig. 4. If glutamate enters the deamination pathway, base and energy (ATP) are produced, as opposed to the transamination pathway, which provides amino acids and energy (ATP) for biosynthetic processes. The cytosolic ALT flux is dependent on the availability of glutamate, pyruvate, and ATP as well as the amount of ALT protein. The mitochondrial GDH flux is dependent on the inner membrane glutamate/Na$^+$ transporter (14), H$^+$ and glutamate in the cytosol, and the activity of GDH. Note that the combined flux through both pathways determines the ATP generated from glutamine oxidation, and this in turn is set by the level of the PDG activity. In addition to the cytosolic glutamate concentration, the PDG is dependent on the amount of PDG protein, which in turn is influenced by the acid-base status (1) and growth factors. Hormones and cytokines that upregulate gene expression of key proteins in each pathway, such as glucocorticoids (Gc) for the catabolic pathway and growth factors for the anabolic pathway, play integral and predominantly opposing roles in the adaptive responses of cellular processes detailed below.

**Response of glutamine/glutamate to metabolic acidosis**

As shown in Fig. 2, in vivo, proximal tubule cells of the kidney respond to a reduction in the body fluid alkaline reserves by increasing glutamate uptake and glutaminase flux coupled specifically to the intramitochondrial GDH pathway. This results in two molecules of NH$_4^+$ formed from glutamine.

![Diagram](image-url)

**FIGURE 1.** The glutamine/glutamate couplet plus amide-derived NH$_4^+$ Dynamic conversion of glutamine to glutamate can be monitored by using uniformly $^{14}$C-labeled (*) glutamate or amido-labeled $[^{15}$N]glutamine (*).

![Diagram](image-url)

**FIGURE 2.** Extracellular and intracellular glutamine conversion to glutamate and regulation of cellular functions. Glutamine labeled in either the carbon (●) or in the amino nitrogen (*) is converted to glutamate outside the cell by phosphate-independent glutaminase (PIG; 1), a γ-glutamyltranspeptidase-glutaminase reaction (Y), with labeled glutamate measured in the media after uptake by $X_{\text{GC}}$ is blocked (2). Extracellular glutamate transported by $X_{\text{GC}}$ (EAAC-1 on apical and GLUT-1 on basal surface) and concentrated in the cytosol inhibits intracellular glutamine conversion to glutamate catalyzed by phosphate-dependent glutaminase (PDG; 3). Inhibiting the extracellular glutamate uptake reduces cytosolic glutamate and allows extracellular $[^{14}$C]glutamine uptake via ASC-like transporter (4) to be converted to $[^{14}$C]glutamate in the cytosol. Using amino-labeled glutamine allows tracing of the amino nitrogen through the cytosolic transamination pathway (6) with recovery in alanine or the mitochondrial glutamate dehydrogenase pathway (5) and recovery in NH$_4^+$. Both amide- and amino-derived NH$_4^+$ molecules are secreted into urine by the apical Na$^+$/H$^+$ exchanger (7) with HCO$_3^-$ derived from oxidation of α-ketoglutarate (AKG$^2-$) via the Krebs cycle (10) being transported out the basolateral surface by the Na$^+$/HCO$_3^-$ symporter (8). Biosynthetic cell functions such as protein synthesis (9), as well as base generation, require regulatory mechanisms based on the glutamine/glutamate couplet.

![Diagram](image-url)

**FIGURE 3.** Evidence for the functional PDG activity at the cytosolic surface of the inner mitochondrial membrane. Intact mitochondria incubated with $[^{14}$C]glutamine produce $[^{14}$C]glutamate (label indicated by ●), with a far greater specific activity in the cytosol (media) than in the matrix (4). The conversion rate is slowed by increasing the media glutamate concentration, which competes with glutamine (12). If amino-labeled $[^{15}$N]glutamine (*) is used (20), $[^{15}$N]glutamate is transaminated with pyruvate to form $[^{15}$N]alanine (ALA) and AKG$^2-$, AKG$^2-$ is then transported into the mitochondrion to support ATP formation. Cytosolic glutamate can be transported via a proton-driven inner membrane carrier (14) into the matrix space and can be deaminated to $^{15}$NH$_3$ and AKG$^2-$.

![Diagram](image-url)

**FIGURE 4.** Cytosolic glutamate metabolism depends on a balance of proton (H$^+$) and hormonal interactions [glucocorticoids (Gc) favor the catabolic pathway, whereas growth factors (GF) favor the anabolic pathway]. The overall flux ([the sum of glutamate dehydrogenase (GDH) and alanine aminotransferase (ALT) fluxes]) depends on PDG activity, which is also dependent on proton and growth factors.
with complete oxidation of α-ketoglutarate, two molecules of HCO$_3$ are formed from labeled glutamate derived from glutamine (9). The amide and amino nitrogens are secreted as NH$_4^+$ at the cell’s apical surface Na$^+$/H$^+$ (NH$_4^+$) exchanger and are excreted in the urine; HCO$_3$ is transported out of the cell at the basolateral Na$^+$/HCO$_3^-$ cotransporter into the renal vein for a net gain of two molecules of base (Fig. 2). In vitro, pig kidney proximal tubule-like cells also respond to metabolic acidosis with an increased NH$_4^+$ production and glutamine conversion to glutamate via the intracellular glutaminase (1, 7). Because the extracellular PIG is HCO$_3^-$ and glutamine concentration-dependent, lowering both the alkaline reserves and plasma glutamine levels serves as a signal-reducing extracellular glutamine conversion to glutamate, limiting glutamate available for uptake and removing the functional unit’s inhibitory influence on the intracellular glutaminase. Simultaneously, the intracellular acidosis drives glutamate into the mitochondrial GDH pathway (9) and thereby reinforces the reduction in cytosolic glutamate (7). The lowered intracellular glutamate content combined with an increased NH$_4^+$ production and glutamine conversion to glutamate, limiting glutamate available for uptake and removing the functional unit’s inhibitory influence on the intracellular glutaminase. Simultaneously, the intracellular acidosis drives glutamate into the mitochondrial GDH pathway (9) and thereby reinforces the reduction in cytosolic glutamate (7). The lowered intracellular glutamate content combined with an increased glutamine content as the cytosolic pH has occurred as the result of troglitazone exposure or absence of 20 μM troglitazone (13). Since the glutamine/glutamate couplet shows regulatory properties, it is asked whether the effect of troglitazone on matrix and, specifically, laminin synthesis might be mediated through the couplet. To test this, the sum of $^{15}$NH$_4^+$ and $[^{15}$N]alanine fluxes, respectively, in pig kidney cells incubated in the presence or absence of 20 μM troglitazone. As shown in Fig. 5, the predominant glutamate flux is normally through the biosynthetic transamination pathway (see Fig. 2). However, in the presence of troglitazone the transamination flux is sharply curtailed, and this reduction is paralleled by a proportional drop in the assayable ALT activity (Fig. 5B). The $^{15}$NH$_4^+$ flux from labeled glutamate is increased, shifting the pivotal glutamate flux balance far toward the GDH flux (Fig. 4). Since this NH$_4^+$ is formed from labeled glutamate derived from glutamine within the cytosolic compartment, one may infer that a drop in cytosolic pH has occurred as the result of troglitazone exposure since the assayable GDH activity did not increase (Fig. 5B). Furthermore, the overall glutaminase flux (the sum of $^{15}$NH$_4^+$ and $[^{15}$N]alanine) decreases, indicating a reduction in

Response of glutamine/glutamate to troglitazone

Mesangial and proximal tubule cells elaborate an extracellular matrix, the overexpression of which during inflammatory diseases results in glomerulosclerosis and interstitial nephritis (15). The synthesis of extracellular matrix proteins, e.g., collagen and laminin, requires an adequate supply of precursor amino acids dependent in large part on glutamine’s amino nitrogen and the transamination pathways coupled to protein synthesis (Fig. 2). The thiazolidinedione troglitazone has been shown to halt mesangium expansion in animal models of type 2 diabetes (5) and to act directly on rat mesangial cells by reducing collagen type I and laminin synthesis (13). Since the glutamine/glutamate couplet shows regulatory properties, it is asked whether the effect of troglitazone on matrix and, specifically, laminin synthesis might be mediated through the couplet. To test this, the sum of $^{15}$NH$_4^+$ and $[^{15}$N]alanine fluxes, respectively, in pig kidney cells incubated in the presence or absence of 20 μM troglitazone. As shown in Fig. 5, the predominant glutamate flux is normally through the biosynthetic transamination pathway (see Fig. 2). However, in the presence of troglitazone the transamination flux is sharply curtailed, and this reduction is paralleled by a proportional drop in the assayable ALT activity (Fig. 5B). The $^{15}$NH$_4^+$ flux from labeled glutamate is increased, shifting the pivotal glutamate flux balance far toward the GDH flux (Fig. 4). Since this NH$_4^+$ is formed from labeled glutamate derived from glutamine within the cytosolic compartment, one may infer that a drop in cytosolic pH has occurred as the result of troglitazone exposure since the assayable GDH activity did not increase (Fig. 5B). Furthermore, the overall glutaminase flux (the sum of $^{15}$NH$_4^+$ and $[^{15}$N]alanine) decreases, indicating a reduction in

FIGURE 5. Troglitazone’s action on the glutamine/glutamate couplet and cellular responses. A: shift of cytosolic glutamate derived from $[^{15}$N]glutamine (99 atom % excess) from alanine (ALA) synthetase into NH$_4^+$ formation in LLC-PK$_1$-F$^+$ monolayers incubated in DMEM or DMEM plus 20 μM troglitazone for 16 h. Over PDG flux as the sum of $[^{15}$N]ALA + $^{15}$NH$_4^+$ was decreased by 16% (774 ± 9 to 654 ± 11 nmol/mg; P < 0.01). Monolayer glutamate content increased from 76 to 98 ± 3 nmol/mg protein (P < 0.03). Results are means ± SE; n = 3. B: assayable GDH and ALT activity in the above monolayers of LLC-PK$_1$-F$^+$ after incubation for 16 h under the above conditions. C: laminin content of LLC-PK$_1$-F$^+$ cells after incubation under the above conditions. Laminin content was assayed in 50 μg protein aliquots of cell lysates as previously described (13).
the glutamine conversion to glutamate (Fig. 2). The measured intracellular glutamate concentration increases by 26%, which is consistent with glutamate from the inhibited ALT pathway backing up in the cytosol and slowing the PDG flux. This in turn limits the ATP available from the oxidation of glutamate's carbon skeleton (Fig. 2) (14). These responses allocate the cells’ nitrogen resources away from the biosynthetic pathway and into the base generating pathway, as reflected by the inverse relationship between $15\text{NH}_4^+$ production (Fig. 5A) and cellular laminin content (Fig. 5C). Thus the regulatory role of glutamate in the functional glutaminase flux can be demonstrated both in the antegrade direction in response to acid-base disturbances and in the retrograde direction in the response to troglitazone.

Perspectives

The glutamine/glutamate couplet offers a novel perspective on how growth factors such as transforming growth factor (TGF)-β regulate the extracellular matrix expansion (in addition to their well-established effects on matrix protein gene expression; Ref. 15). For example, TGF-β may upregulate the PDG and Na/H+ exchanger 3 (NHE3) gene expression and downregulate the PIG gene expression so that the intracellular glutaminase is accelerated with the glutamate that is formed coupled to the transamination pathway. Of course, increasing matrix protein gene expression is necessary for matrix expansion, but synthesis of matrix protein will not proceed unless there are adequate precursor amino acids made available via the glutamine/glutamate couplet. Thus it is reasonable that effects on PDG expression and transamination pathways should accompany these recognized actions of TGF-β.

Troglitazone has been shown to interdict extracellular matrix expansion in experimental diabetes associated with a decrease in levels of TGF-β and extracellular matrix protein mRNA (2). However, by inhibiting the transamination pathway, shifting the nitrogen into the catabolic GDH pathway, and at the same time slowing the PDG flux, troglitazone effectively “starves” the extracellular matrix expansion of precursors. Mechanistically, this shift in glutamate’s fate can be mimicked by lowering the cytosolic pH, suggesting that one of troglitazone’s actions is to impair the cell’s ability to extrude protons. Possibly, troglitazone blocks activation of protein kinase C (PKC) by increasing diacylglycerol (DAG) kinase activity (2)

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References


