Glutamate transport and renal function

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Welbourne, Tomas C., and James C. Matthews. Glutamate transport and renal function. Am. J. Physiol. 277 (Renal Physiol. 46): F501-F505, 1999.—Brush border γ-glutamyltransferase-glutaminase activity and the high-affinity glutamate transporter EAAC1 function as a unit in generating and transporting extracellular glutamate into proximal tubules as a signal that modulates intracellular glutamine/glutamate metabolism, paracellular permeability, and urinary acidification. The reported presence of a second glutamate transporter, GLT1, on the antiluminal tubule surface points to specific functional roles for each subtype in physiological and pathophysiological processes.

γ-glutamyltransferase; EAAC1; GLT1; d-glutamate; phosphate-dependent glutaminase; paracellular permeability; urinary acidification

THE ECTOENZYME γ-glutamyltransferase, phosphate-independent glutaminase (PIG), and the apical membrane glutamate transporter form a unit that functions in regulating both glutamine metabolism and paracellular permeability (Fig. 1, A and B). PIG generates glutamate from extracellular glutamine (and glutathione when available), which is transported across the apical border by the Na+-dependent, high-affinity EAAC1 transporter subtype (18). Mechanistically, this “functional unit” produces three intracellular signals that modulate cellular energy production and paracellular permeability. One signal is the intracellular glutamate concentration (Fig. 1A), set by the rate of extracellular glutamate formation and transport into the cell. Because cellular glutamate competes with glutamine (17) for the cytosolic reactive site of the mitochondrial glutaminase (phosphate-dependent glutaminase, PDG) (9), increasing or decreasing the functional unit activity results in suppression (Fig. 1A, left) or acceleration (Fig. 1A, right) of glutamine utilization by this rate-limiting reaction and, hence, ATP and base formation by this pathway. The glutamate transporter turnover (movement of 3Na+ in and 1K+ out; see the accompanying article by Fairman and Amara, Ref. 5) generates a second signal in the form of a localized increase in ADP, which stimulates glycolysis and ATP production; this ATP is distributed to the plasma membrane Na+-K+-ATPase and the F-actin, maintaining the junctions tight (Fig. 1B, left). Reducing the glutamate transport therefore slows glycolysis resulting in dispersion of the F-actin and an opening of the tight junction. Finally, a third signal is the rise in intracellular H+ concentration resulting from the transport of glutamate and acid into the cell (see accompanying article by Hediger, Ref. 8). As a result of cellular acidification, oxidative deamination of glutamate is accelerated producing NH4+ and α-ketoglutarate (see accompanying article by Nissim, Ref. 15). Therefore, the consequence of high functional unit activity is extracellularly generated glutamate (PIG pathway) being substituted for intracellularly formed glutamate (PDG pathway) as the major fuel, sparing cellular glutamine for biosynthetic pathways and, at the same time, maintaining tight cell-to-cell junctions. Conversely, diminishing the functional unit activity releases the powerful glutaminase, with the intracellularly produced glutamate diverted to the default transamination pathway (alanine formation) as the tight junctions open (Fig. 1B, middle cell). High functional unit activity is characteristic of differentiated cell monolayers in contrast to a low activity in their rapidly proliferative phase.

Evidence in support of the functional unit concept was obtained in vitro, from cells grown as monolayers on plastic dishes (Fig. 1A) (12), or porous supports (Fig. 1B) (22), as well as in vivo (1, 2). The in vitro studies were performed primarily, but not exclusively, using LLC-PK1-F− cells, which were selected (7) from the parental LLC-PK1 cell line for growth in the absence of glucose, relying upon glutamine as their metabolic fuel. Because growing these proximal tubule-like LLC-PK1-F− monolayers on plastic dishes is the simplest approach for demonstrating the role of the functional unit in glutamine metabolism, evidence for the associ-
ated function of PIG and apical glutamate transport using this experimental model will be summarized first.

**In Vitro Evidence: LLC-PK₁-F₁ Monolayers Grown on Plastic**

The ontologic expression of the functional unit in cultured LLC-PK₁-F₁ cells parallels PIG activity and tight junction formation in the developing kidney (3, 6). The PIG activity is initially low after splitting monolayers and then increases. However, PIG still generates less glutamate than the transporter’s capacity in this phase of rapid proliferation; with differentiation (5–10 days), rates of extracellular glutamate production and uptake converge, marked by dome formation and transepithelial fluid movement; finally (after 10 days), the two activities diverge as PIG continues to rise as transporter activity declines with aging (13). Cellular glutamate concentration reflects the developmental changes in the functional unit; cellular glutamate concentration rises in parallel with the PIG activity up through differentiation and then levels off at ~175 nmol/mg (35 mM) as transporter activity declines, leading to an accumulation of extracellular glutamate in the media. Inhibiting the PIG activity eliminates this extracellular glutamate accumulation (13). The inverse relationship between the two functional unit components is consistent with the hypothesis that the glutamate transporter is regulated by the cellular glutamate content (see accompanying article by McGivan and Nicholson, Ref. 11) resulting in the autoregulation of the functional unit’s activity.

From this perspective, monolayers must be studied at a time when both PIG and transporter activity are high, to best demonstrate the role of the functional unit in metabolic regulation. With monolayers grown 8–10 days, postsplitting and acutely inhibiting PIG with acivicin (AT-125) or blocking glutamate uptake with D-aspartate (D-ASP) (Fig. 1A, right cell) results in a 15–20% reduction in cellular glutamate concentration within 1 h (12). Associated with this was a 30–50% increase in glutaminase flux with formed intracellular glutamate coupled to alanine formation (acivicin) or ammoniagenesis (D-aspartate). These results are consistent with the functional unit modulating glutaminase flux coupled to either alanine aminotransferase or glutamate dehydrogenase (GDH) dependent upon the transporter turnover (acid loading).

**In Vitro Evidence: LLC-PK₁-F₁ Monolayers Grown on Porous supports**

These cells show a markedly different phenotype from those grown on plastic, being densely packed columnar cells expressing well-developed brush borders and tight junctions and having structural characteristics of the in situ proximal tubule (21). Also, unlike the cells grown on plastic, cells grown on porous supports express a second glutamate transporter on the basal cell surface, which functions in the regulation of the basolateral glutamate concentration (Fig. 1B). Net glutamate efflux across this cell surface diminishes the intracellular glutamate concentration to a level ~40% below that for cells grown on plastic (20 mM) despite a comparable apical glutamate uptake. In fact, inhibiting the apical glutamate formation with acivicin and lowering the intracellular glutamate also eliminates basal...
efflux associated with a 40% decrease in cellular glutamate. Furthermore, basal efflux can be regulated as shown by the action of acidogenic hormones, i.e., growth hormone, which primarily accelerate basal glutamate efflux, lowering cellular glutamate (also contributed to by an increased metabolic removal via GDH; see accompanying article by Nissim, Ref. 15) and accelerating glutaminase flux (21). Although more than 80% of the cell’s glutamate uptake capacity is present on the apical surface, the reverse is true for the glutamine uptake capacity. Consequently, the cellular glutamine-to-glutamate concentration ratio is set higher in these monolayers (Fig. 1B) than those grown on plastic (Fig. 1A), as is the dependent glutaminase flux. Nevertheless, when either PIG or glutamate uptake were eliminated with acivicin or d-aspartate, cellular glutamate concentration fell 40–50% with a two- to threefold acceleration of the glutaminase flux (22) coupled to either deamination or transamination, depending upon whether the transporter turnover was maintained (d-aspartate) or not (acivicin). Thus the role of the functional unit in regulating cellular glutamine metabolism can also be demonstrated in monolayers grown such that both surfaces are exposed.

An additional advantage of studying monolayers grown on porous supports is the opportunity to observe changes in paracellular permeability after eliminating the functional unit (Fig. 1, middle cell). With two independent measures of paracellular permeability, diffusion of radiolabeled L-[14C]glucose and transepithelial electrical resistance (TER) and acivicin or DL-threo-3-hydroxy-aspartate (THA) to reduce apical glutamate uptake, monolayer “tightness” was shown to be modulated by the functional unit activity (20). After 2 h, THA (0.5 mM) resulted in a 2.6-fold increase in the L-glucose diffusion rate from the outer to inner well and a 50% drop in TER. Subsequent studies demonstrated that transporter turnover rather than the cellular glutamate concentration was the effective signal (19). Consequently, acute reduction of the functional unit activity should open up the paracellular pathway. At the same time, energy formed from glutamine oxidation for transport would be enhanced, enabling the cells to withstand a less efficient transepithelial transport while generating base.
In Vivo: Role of the Functional Unit in the Functioning Rat Kidney

The functional unit as a regulatory mechanism elucidated in vitro would carry more relevance if it could be demonstrated to function in vivo. In this regard, both PIG and glutamate transporter EAAC1 are codistributed along the apical brush border, increasing in density from early to the late proximal tubule (4, 18) and indicating that both components are present at the appropriate sites. The intraluminal generation of glutamate from secreted glutamine along the proximal tubule would depend upon the prevailing plasma glutamine concentration and PIG activity. Importantly, metabolic acidosis lowers the plasma glutamine by 20–40%. Hence, substrate is reduced along with the acutely reduced PIG activity (14), curtailing the extra-cellular glutamate generation; consequently, cellular glutamate would subsequently decrease accelerating the intracellular glutaminase flux. Conversely, restoring the plasma glutamine concentration should return the cellular glutamate and suppress the glutaminase flux. To test this hypothesis, rats were maintained on an elemental diet devoid of glutamine or fed an elemental diet supplemented with glutamine (23). After 3 days, their arterial plasma glutamine concentration, renal cortical glutamate content, and glutamine utilization were measured. The glutamine-fed animals had an 18% rise in arterial plasma glutamine concentration, a proportional rise in cellular glutamate, and a marked reduction in glutamine utilization, consistent with suppression of the intracellular glutaminase flux. To confirm the role of the functional unit in this response, acivicin was administered intravenously to “knockout” PIG activity, and the above parameters were reevaluated. Despite a slightly elevated arterial glutamine concentration, renal cortical glutamate dropped 40% with a twofold increase in renal glutamine utilization. These in vivo results are consistent with the hypothesis that the functional unit generates a glutamate flux that acts as a governor on intracellular glutaminase flux. Evidence that the functional unit also modulates paracellular permeability may be seen in the urinary amino acid profile shown in Fig. 2. Control (Fig. 2B) and acivicin-treated animals (Fig. 2C) had similar urine flow rates, yet the concentrations of glutathione, aspartate, glutamine, and alanine, but not glutamate, were higher with PIG inhibited. The rise in glutathione excretion (Fig. 2C) was not surprising, given that it is freely filtered with subsequent absorption dependent on intraluminal breakdown initiated by the PIG. However, the rise in glutamine (and alanine) excretion is surprising, because proximal transport removes virtually all of the filtered glutamine, consistent with movement (secretion) from plasma to lumen via the paracellular pathway. Of course, this leak would normally be self-correcting as intraluminal glutamine concentration rises, driving the glutamate flux of the functional unit (Fig. 1B, right). Evidence consistent with the generation of intraluminal glutamate from secreted glutamine may also be seen in EAAC1 knockout mice (16), in which urinary glutamate excretion was increased 1,400-fold above control compared with only 10-fold for aspartate; this 140-fold greater increase in glutamate is consistent with leaky tight junctions and backflux of plasma glutamine resulting in increased PIG hydrolysis.

One can also disrupt the functional unit glutamate signal by displacing L-glutamate from the apical surface transporter using D-aspartate while retaining the proton signal, since D-aspartate is effectively reabsorbed (1). As a consequence of infusing D-aspartate, renal cortical glutamate content decreased and was associated with a two- to threefold increase in glutamate utilization and ammonium formation (1). Because the rat proximal convoluted tubule lacks the transamination pathway, the ratio of NH₃ produced to glutamine utilized approximated a value of 2, which is consistent with the activation of both the glutaminase and GDH fluxes as the consequence of the reduced cellular glutamate and pH, respectively, similar to the in vitro responses (12, 22). On the other hand, the D-isomer of glutamate does not inhibit apical L-glutamate transport, but, rather, reduces the blood side L-glutamate uptake and activates glutamine utilization and ammonium formation with the ratio approaching a value of 2 (2). These findings point to asymmetrically distributed glutamate transporter subtypes: EAAC1 on the apical border and a second transporter subtype (GLT1?) on the basal border. To test for the expression of another glutamate transporter in rat kidney, total RNA was isolated from rat renal cortex and LLC-PK₁, MDCK, and LLC-PK₁ cells and probed for the presence of EAAC1 and GLT1 mRNA (Fig. 3). Dominant bands at around 4 kb for EAAC1 and 11 kb for GLT1 could be detected in both the rat renal cortex and the cultured kidney cell lines. An example of a separate subtype of glutamate transporter that is localized to the basal membrane surface and that may express novel stereospecificity has been demonstrated in rat placenta (10). This emphasizes the importance of studying glutamate transport regulation of cellular processes in a physiological context. Further studies are obviously necessary to characterize this curious transporter asymmetry and the regulatory role played in glutamine and glutamate metabolism as well as in transepithelial transport.

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