Shuttling glucose across brain microvessels, with a little help from GLUT1 and AMP kinase. Focus on “AMP kinase regulation of sugar transport in brain capillary endothelial cells during acute metabolic stress”

**Warren L. Lee** and **Amira Klip**

1Keenan Research Centre, Li Ka Shing Knowledge Institute at St. Michael’s Hospital, Toronto, Ontario, Canada; and 2Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada

The Blood-Brain Barrier: a Gatekeeper of Brain “Individuality”

The blood-brain barrier (BBB) maintains the individuality of brain fluid, while allowing it to selectively import nutrients and process toxic products. Its major constituent is a single layer of continuous, nonfenestrated endothelium lining the microvessels, whose unique features account in large part for the integrity of the barrier. The endothelial cell monolayer is the major determinant, but other cells in the central nervous system (e.g., pericytes and astrocytes) contribute to the integrity of the BBB. The interendothelial tight junctions of the BBB are one of its signature elements that greatly restrict paracellular permeability to solutes, fluid, and transmigrating cells. This is markedly distinct from the leakier, discontinuous, and fenestrated endothelium of liver microvessels (sinusoids). For example, the tight junctions of the cerebral endothelium (pial vessels) confer an electrical resistance (1,300 Ω·cm²) far higher than other endothelia (6). Therefore, communication between blood and the brain interstitial fluid requires individually tailored molecular mechanisms.

Transcytosis is the movement of molecules through a cellular barrier. Whereas proteins can traverse through vesicular carrier processes that could carry solutes through fluid phase endocytosis, the brain microvasculature is uniquely poor in carrier processes that could carry solutes through fluid phase endocytosis. The authors show that glucose dephosphorylation by hexokinase might contribute to hexose uptake.

3-OMG avoids the confounding that phosphorylation by hexokinase and AMP kinase.

Deprivation and Cellular Energy Demand

The prevailing view is that GLUT1 is predominantly at the plasma membrane. Despite the “stationary” view of GLUT1, it translocates from intracellular to plasma membranes in muscle and adipose cells (3, 11), emulating the well-known response of GLUT4 to insulin, muscle contraction, and mitochondrial compromise (14, 15, 18). Further, prolonged energy/oxygen challenges trigger GLUT1 biosynthesis (4).

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Glucose Uptake and GLUT1 Regulation by Glucose Deprivation and Cellular Energy Demand

In this issue of *American Journal of Physiology-Cell Physiology*, Cura and Carruthers (8) report that GLUT1 is regulated in a brain microvascular cell line, bEnd.3, such that its availability at the cell surface rapidly increases upon challenges to the cells’ energy stores (Fig. 1). These investigators previously showed that extracellular glucose depletion, a mitochondrial uncoupler (carbonyl cyanide p-trifluoromethoxyphenylhydrazone, FCCP) or a metabolic poison (KCN), rapidly drop cellular ATP levels, accompanied by a rapid rise in 3-O-methyl glucose (3-OMG) uptake without changing total GLUT1 protein levels (7). Measuring uptake of the nonmetabolizable 3-OMG avoids the confounding that phosphorylation by hexokinase might contribute to hexose uptake.

Cura and Carruthers used biotinylation of exposed GLUT1 protein residues coupled to pull-down with bead-associated avidin, followed by immunodetection of the sedimented proteins with anti-GLUT1 antibodies (recognizing a cytosolic, COOH-terminal epitope). The authors show that glucose depletion or KCN increases GLUT1 levels exposed to the medium by two to threefold within 10–30 min of energy challenge. Since these cells also express GLUT8 and GLUT9 (7),
it will be interesting to know whether these transporters are also regulated.

What is the signal for this increase in plasma membrane GLUT1? Reasoning that the cue must be energy depletion, and since AMP-dependent kinase (AMPK) is a sensor of the energy charge (ATP/AMP + ADP) in other tissues (10), Cura and Carruthers show the following: 1) Stimulating AMPK with the small molecule AICAR rapidly increases surface GLUT1 and 3-OMG uptake. AICAR is converted intracellularly to ZMP, which binds to the activating AMP-binding site on AMPK. 2) Silencing AMPK expression prevents the increase in 3-OMG uptake caused by AICAR, glucose-depletion, KCN, or FCCP. Intriguingly, AMPK phosphorylation (a measure of its activation) was unevenly affected by AMPK silencing in each case, a finding that will require future exploration. 3) Compound C (CC), a chemical inhibitor of AMPK, inhibited the stimulation of 3-OMG uptake by all stimuli. The key finding is that CC prevents the GLUT1 increase at bEnd.3 cell membranes caused by challenges to cellular energy levels, and that AMPK silencing similarly inhibits the response to KCN. An interesting spin-off of this study is the authors’ interpretation to the paradoxical observation that CC elevated 3-OMG uptake in otherwise unchallenged cells, that CC may displace cytosolic ATP from GLUT1, alleviating ATP’s inhibition on GLUT1 intrinsic activity (5).

Questions Arising

This study opens the door for future questions regarding glucose transfer to the brain.

Silencing either AMPKα1 or α2 subunits abolished the stimulation of 3-OMG uptake in response to glucose depletion or KCN. This reflects a requirement for each isoform but how this occurs requires investigation. What proportion of active AMPK is left after knocking down individual isoforms? How does AMPK signal to the GLUT1 traffic machinery? What is the GLUT1 traffic machinery and does it share common elements with molecules mobilizing GLUT4 in muscle and fat cells? In addition to AMPK, could sirtuins, which sense NADH levels, regulate GLUT1?

Is GLUT1 similarly regulated in microvessels? AMPK is stimulated by hypoxia and oxygen-glucose deprivation in primary brain microvascular endothelial cells (21), strengthening the concept that AMPK is a relevant energy sensor at the BBB. Are bEnd.3 cells polarized, as are the endothelial cells in microvessels? Is the response to glucose depletion equally sensitive and productive if the challenge is abluminal (originating from neuronal demand) or luminal (originating from low glucose supply)? What proportion of GLUT1 faces the luminal vs. the abluminal side of microvessels, and how is this changed by energy deprivation?

This paper further shows that bEnd.3 cells express both 48-kDa and 55-kDa forms of GLUT1, in contrast to the predominant 55-kDa form of rat and bovine brain microvessels. Intriguingly, while only the 55-kDa form increased at the membrane of AICAR-treated bEnd.3 cells, glucose depletion/KCN enhanced expression of both 55-kDa and 48-kDa forms. The functional consequence of these changes will be interesting to define.

Is GLUT1 exocytic movement stimulated by energy deprivation, or could energy deprivation cues reduce GLUT1 internalization from the membrane? Indeed, energy deprivation causes GLUT4 retention at the surface of myoblasts (2) and cardiomyocytes (23). Interestingly, the regulated endocytic route carrying GLUT4 requires membranes, cholesterol and dynamin, but not clathrin (2), inviting the question of how is GLUT1 internalized? Ultimately, it makes energetic “sense” that under conditions of energy deprivation, cells would reduce endocytosis (saving energy) rather than stimulating energetically-costly exocytosis.

The results of Cura and Carruthers are good news for patients with GLUT1DS. This disease is currently treated by replacing all dietary carbohydrate to promote ketosis, as ketone bodies freely cross the BBB to be utilized by neurons (13). While bypassing the hurdle, the process is inefficient and costly to patients’ quality of life.

Regulating nutrient passage is a major function of the BBB endothelium. Cura and Carruthers’ work highlights that energy demand within cells—or low glucose in their surroundings—orchestrates a response that activates AMPK, driving GLUT1 to the surface to enact directional glucose transfer (Fig. 1). Failure of this response system may result in syndromes akin to GLUT1DS, and conversely, strategies to increase AMPK, judiciously administered, may promote glucose delivery to the brain “upon demand.”

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AUTHOR CONTRIBUTIONS

W.L.L. and A.K. interpreted the results of the experiments; A.K. analyzed the data; A.K. prepared the figure; A.K. drafted the manuscript; W.L.L. and A.K. edited and revised the manuscript; W.L.L. and A.K. approved the final version of the manuscript.

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