AMP kinase regulation of sugar transport in brain capillary endothelial cells during acute metabolic stress

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Cura AJ, Carruthers A. AMP kinase regulation of sugar transport in brain capillary endothelial cells during acute metabolic stress. Am J Physiol Cell Physiol 303: C806–C814, 2012. First published July 3, 2012; doi:10.1152/ajpcell.00437.2011.—AMP-dependent kinase (AMPK) and GLUT1-mediated sugar transport in brain-blood barrier endothelial cells are activated during acute cellular metabolic stress. Using murine brain microvasculature endothelium bEnd.3 cells, we show that AMPK phosphorylation and stimulation of 3-O-methylglucose transport by the AMPK agonist AICAR are inhibited in a dose-dependent manner by the AMPK antagonist Compound C. AMPK α1- or AMPK ε2-knockdown by RNA interference or AMPK inhibition by Compound C reduces AMPK phosphorylation and 3-O-methylglucose transport stimulation induced by cellular glucose-depletion, by potassium cyanide (KCN), or by carbonyl cyanide-p-trifluoromethoxy-phenylhydrazone (FCCP). Cell surface biotinylation studies reveal that plasma membrane GLUT1 levels are increased two- to threefold by cellular glucose depletion, AICAR or KCN treatment, and that these increases are prevented by Compound C and by AMPK α1- or ε2-knockdown. These results support the hypothesis that AMPK activation in blood-brain barrier-derived endothelial cells directs the trafficking of GLUT1 intracellular pools to the plasma membrane, thereby increasing endothelial sugar transport capacity.

blood brain barrier; endothelial cells; glucose transport; membrane; metabolism

THE ADULT MAMMALIAN BRAIN USES glucose as its primary energy source. While brain function and development require a constant glucose supply, diffusional exchange of glucose between blood and brain is severely restricted by the blood-brain barrier. This barrier comprises a nonfenestrated, endothelium formed by capillary endothelial cells connected by tight junctions. Glucose that enters the brain must, therefore, cross the endothelium by protein-mediated trans-cellular transport. In mammalian brain, this is catalyzed by the glucose transport protein GLUT1, which is expressed in luminal and abluminal membranes of microvasculature endothelial cells and is essential for brain metabolic homeostasis (13, 15, 30, 36, 43).

Under resting conditions and at physiologic blood glucose levels, GLUT1-mediated glucose transport across the blood-brain barrier barely exceeds brain glucose utilization (42). Under conditions where glucose consumption could exceed glucose supply, for example during hypoglycemia, hypoxia, or intense neuronal activation, blood-brain barrier glucose transport capacity is increased by two different mechanisms. During chronic metabolic stress [e.g., hypoxia (24) and hypoglycemia (4, 33)], endothelial cell GLUT1 gene and protein expression increases in vitro (3) and in vivo (5), thereby increasing blood-brain barrier sugar transport. In contrast, acute metabolic stress [e.g., short-lived hypoglycemia, hypoxia, metabolic poisoning (14), or seizures (10, 35)] is without effect on GLUT1 expression but promotes recruitment of intracellular GLUT1 to the plasma membrane (16), thereby increasing blood-brain barrier sugar transport (14). The signals that regulate blood-brain barrier sugar transport during acute metabolic stress are not known.

Phosphorylated AMP-activated kinase (AMPK) plays a key role in maintaining energy homeostasis in skeletal muscle (19, 45), heart (12, 32), brain (29, 37, 40), and endothelial cells (17) by switching cellular metabolism to lowered ATP consumption and increased ATP production. AMPK regulates glycolysis, fatty acid oxidation, and glucose transport (11, 22, 23), the activity of cell surface GLUT1 (1, 2) in cultured rat liver Clone-9 cells and translocation of the insulin-sensitive glucose transporter GLUT4 to the plasma membrane in heart and muscle (25).

Aglycemia, hypoxia, and oxygen glucose deprivation activate AMPK in primary bovine brain microvascular endothelial cells (44). Several forms of acute metabolic stress, including glucose starvation, KCN or FCCP treatment induce AMPK phosphorylation and sugar transport stimulation (14) in the cultured brain microvascular endothelial cell line bEnd.3 (34). These observations establish that immortalized and primary cultures of cerebral microvasculature endothelial cells share a common response to cellular metabolic stress but do not directly address the role of AMPK activation in controlling GLUT1-mediated sugar uptake. In the present study, we employ AMPK agonists and antagonists and AMPK small interfering (si)RNAs to perturb AMPK signaling. Our results strongly suggest that AMPK directs the trafficking of GLUT1 between endothelial cell membrane and intracellular pools and thereby regulates blood-brain barrier endothelial cell sugar transport in response to altered cellular metabolic status.

EXPERIMENTAL PROCEDURES

Tissue culture. bEnd.3 cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) from Gibco supplemented with 10% fetal bovine serum (FBS) from Hyclone and 1% penicillin-streptomycin (Pen-Strep) solution (Gibco) at 37°C in a humidified 5% CO2 incubator as described previously (14).

Antibodies. A custom, affinity-purified rabbit polyclonal antibody raised against a synthetic peptide corresponding to GLUT1 amino acids 480–492 was produced by New England Peptide. Rabbit polyclonal and monoclonal antibodies against AMPK and phosphorylated

1 This article is the topic of an Editorial Focus by Warren L. Lee and Amira Klip (26a).
AMPK (P-Thr172), and phosphorylated acetyl-CoA carboxylase (ACC) (P-Ser79) were obtained from Cell Signaling Technology, and an antibody against cyclophilin B was obtained from Abcam. Horseradish peroxidase- conjugated goat anti-rabbit secondary antibody was obtained from Jackson ImmunoResearch.

Buffers. Cell lysis buffer consisted of 5 mM HEPES, 5 mM MgCl₂, 150 mM NaCl, 50 μM EDTA, and 1% SDS. Uptake stop solution included 10 μM cytochalasin B (Sigma) and 100 μM phloretin (Sigma) in Dulbecco’s phosphate-buffered saline (DPBS). TBS was composed of 20 mM Tris base and 135 mM NaCl, pH 7.6. TBST comprised TBS buffer with 0.2% Tween 20. Biotin Quench solution was composed of 250 mM Trizma Base. Biotin lysis buffer contained TBS with 0.5% Triton X-100.

Western blotting of bEnd.3 cells. Confluent 100 mm dishes of bEnd.3 cells were treated with 2 mM AICAR (ThermoFisher) or 2 mM AICAR plus 1, 2, 5, 10, or 20 μM Compound C for 1.5 h followed by serum-free DMEM plus 2 mM AICAR ± 10 μM Compound C for 2 h, 2 mM AICAR ± 10 μM Compound C for 1.5 h followed by serum-free, glucose-free DMEM ± 10 μM Compound C for 30 min, or 5 mM KCN ± 10 μM Compound C for 10 min at 37°C before proceeding with biotinylation. We therefore asked whether AICAR stimulation of transport is suppressed by Compound C (44). To analyze Compound C inhibition of AMPK activity in bEnd.3 cells, we treated cells with 2 mM AICAR to activate AMPK in the absence or presence of increasing concentrations of Compound C. Thr172 phosphorylation was analyzed by using an AMPK-phospho-Thr172-specific antibody (Fig. 1A). AICAR-stimulated AMPK phosphorylation decreases with increasing concentrations of Compound C. AMPK phosphorylation at 2 mM AICAR plus 10 μM Compound C is indistinguishable from AMPK phosphorylation in the absence of AICAR. The apparent inhibitory constant [K_i(app); 5.5 ± 2.2 μM] for Compound C inhibition of AMPK phosphorylation was computed by nonlinear regression analysis of the concentration dependence of Compound C inhibition of AICAR-dependent AMPK phosphorylation by assuming simple Michaelis-Menten inhibition (Fig. 1B).

RESULTS

Compound C inhibits AICAR-dependent AMPK phosphorylation. AMPK is activated by phosphorylation of AMPK threonine 172 (22) and this phosphorylation is inhibited in bovine brain microvascular endothelial cells by Compound C (44). To analyze Compound C inhibition of AMPK activity in bEnd.3 cells, we treated cells with 2 mM AICAR to activate AMPK in the absence or presence of increasing concentrations of Compound C. Thr172 phosphorylation was analyzed by using an AMPK-phospho-Thr172-specific antibody (Fig. 1A). AICAR-stimulated AMPK phosphorylation decreases with increasing concentrations of Compound C. AMPK phosphorylation at 2 mM AICAR plus 10 μM Compound C is indistinguishable from AMPK phosphorylation in the absence of AICAR. The apparent inhibitory constant [K_i(app); 5.5 ± 2.2 μM] for Compound C inhibition of AMPK phosphorylation was computed by nonlinear regression analysis of the concentration dependence of Compound C inhibition of AICAR-dependent AMPK phosphorylation by assuming simple Michaelis-Menten inhibition (Fig. 1B).

We also examined phosphorylation of ACC, a direct downstream target of AMPK (21). Using an antibody specific to phosphorylated Ser79 on ACC, we measured AICAR-stimulated ACC phosphorylation in the presence of increasing Compound C concentration ([Compound C]) (Fig. 1, C and D). Compound C reduces ACC phosphorylation in a dose-dependent manner with K_i(app) = 2.9 ± 1.2 μM. Both AMPK and ACC are phosphorylated in the absence of AICAR, suggesting either that untreated bEnd.3 cells experience a low, but measurable level of metabolic stress or that there is a low level of AMPK and ACC phosphorylation independent of metabolic stress.

Compound C inhibits AICAR-stimulated 3-OMG uptake. The facilitative glucose transport protein GLUT1 mediates sugar transport in control and AICAR-treated bEnd.3 cells where AICAR increases V_{max} for 3-OMG net uptake by twofold (14). We therefore asked whether AICAR stimulation of transport is suppressed by Compound C.

bEnd.3 cells were treated with 2 mM AICAR plus increasing concentrations of Compound C, and uptake of 20 mM 3-OMG was measured over 30 s at 4°C (Fig. 2). 3-OMG is a nonmetabolizable GLUT1 substrate whose net uptake proceeds until intracellular [3-OMG] = extracellular [3-OMG] and unidire-
tional 3-OMG uptake and exit are quantitatively identical. Net uptake at 30 s achieves only 9% and 20% equilibration in control and FCCP-treated cells, respectively (14), indicating that transport determinations at 30 s underestimate steady-state transport rates by <7%. In the present study, AICAR stimulates 3-OMG uptake approximately 2.5-fold over control cells. Addition of Compound C inhibits bEnd.3 sugar uptake in a dose-dependent manner. $K_{i(app)}$ for Compound C inhibition of AICAR-activated sugar uptake is 1.1 ± 0.2 μM. The 95% confidence interval for $K_{i(app)}$ for transport inhibition (0.1–2.1 μM, Fig. 1B). Transport in the absence of AICAR is increased by 1.4-fold by Compound C, demonstrating that inhibition of AICAR-stimulated transport does not result from a direct inhibition of GLUT1 by Compound C. We address Compound C stimulation of transport in the DISCUSSION.

Acute metabolic stress-induced AMPK phosphorylation is reduced with Compound C treatment and AMPK knockdown. Treatment of bEnd.3 cells with siRNA oligonucleotides targeting mouse AMPK subunits α1 plus α2 reduces AMPK expression by 2.2-fold. Treatment with cyclophilin B siRNAs and a pool of nonsilencing siRNAs is without effect on bEnd.3 AMPK expression (Fig. 3, A–D). The use of isoform-specific AMPK siRNAs indicates that message knockdown by AMPK α1 or α2 siRNA is specific and is not accompanied by upregulation of the nontargeted AMPK isoform (Fig. 4, A and B).

Pretreatment of cells with AMPK-targeted siRNAs for 48 h reduces acute stress-induced AMPK phosphorylation. Cells were treated with AICAR, 5 mM KCN, 8 μg/ml FCCP, 10 μM Compound C, or glucose starved, and assayed for AMPK phosphorylation by Western blot analysis (Fig. 5A). Quantitation of band densities (Fig. 5B) reveals that basal AMPK phosphorylation is reduced by 72% following siRNA treatment. The responses to glucose deprivation, AICAR, Compound C, KCN, and FCCP treatments are reduced by 25%, 70%, 71%, 53%, and 46%, respectively, following siRNA treatment. AMPK knockdown is incomplete (Figs. 3A and 5A) and the question remains as to whether the residual AMPK.
which continues to respond to metabolic stress (Fig. 5, A and B), is sufficient to activate glucose transport.

Compound C inhibits acute metabolic stress-induced AMPK activation in endothelial cells. We subjected bEnd.3 cells to 2 mM AICAR (10 min), glucose starvation (30 min), 5 mM KCN (10 min), or 8 μM FCCP (10 min) in the absence or presence of Compound C, and assayed AMPK phosphorylation by Western blot analysis. An untreated control was measured for basal AMPK phosphorylation (Fig. 5C). Quantitation of phosphorylation (Fig. 5D) indicates that all stress conditions and AICAR treatment increase AMPK phosphorylation by 4- to 10-fold. Cells treated with only Compound C show no significant increase in phosphorylation over controls. Compound C treatment reduces stress-induced AMPK phosphorylation by 36% in glucose-starved cells, 56% in AICAR-treated cells,
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**Fig. 5. Compound C and AMPK siRNA inhibition of AMPK phosphorylation during metabolic stress.** A: representative Western blot of whole cell bEnd.3 lysates from cells pretreated for 48 h with or without AMPK siRNAs followed by 15 min pretreatment at 37°C with or without 5 mM glucose (Glc), 5 mM KCN, 8 μg/ml FCCP, 10 μM Compound C (Comp C), or for 2 h with 2 mM AICAR. Cell lysate (30 μg total protein) was loaded into each lane and probed with AMPK P-Thr172 antibody and band volumes were quantitated. B: ordinate, relative band volume (%); abscissa, experimental condition. Data represent means ± SE of three separate experiments. *P < 0.05, significantly different from control condition. §P < 0.05, AMPK knockdown significantly reduces AMPK phosphoprotein levels relative to the test condition. C: representative Western blot of whole cell bEnd.3 lysates in the absence or presence of glucose, KCN, FCCP, AICAR, and Compound C. Before lysis, cells were treated for 15 min at 37°C with 5 mM glucose (control), 0 glucose, 5 mM KCN, 8 μg/ml FCCP, or for 2 h with 2 mM AICAR in the absence or presence of 10 μM Compound C. Cell lysate (30 μg total protein) was loaded into each lane and probed with AMPK P-Thr172 antibody and band volumes were quantitated. D: ordinate, relative band volume (%); abscissa, experimental condition. Data represent means ± SE of three separate experiments. *P < 0.05, significantly different from control condition. §P < 0.05, Compound C significantly reduces AMPK phosphoprotein levels relative to the test condition.

69% in KCN-treated cells, and 61% in FCCP-treated cells. Although AMPK phosphorylation was reduced in the presence of Compound C, complete phosphorylation inhibition was not observed.

**Compound C and AMPK knockdown inhibit metabolic stress-induced 3-OMG uptake stimulation.** We next asked whether inhibition of AMPK phosphorylation suppresses sugar uptake stimulation during metabolic stress. 3-OMG uptake was measured in bEnd.3 cells in the absence and presence of 10 μM Compound C in cells that were glucose starved, treated with 2 mM AICAR, treated with 5 mM KCN, or treated with 8 μg/ml FCCP as described above. 3-OMG uptake (Fig. 6A) is stimulated 2.6-fold in glucose-starved cells, 2.6-fold in AICAR-treated cells, 2.9-fold in KCN-treated cells, and 3.0-fold in FCCP-treated cells. Glucose transport in control cells is also stimulated 1.9-fold by 10 μM Compound C. Compound C inhibits 3-OMG uptake by 40% in glucose-starved cells, 42% in AICAR-treated cells, by 54% in KCN-treated cells, and by 64% in FCCP-treated cells (Fig. 6A).

In cells where AMPK is knocked down before measurement of 3-OMG uptake, stress-induced stimulation of uptake is also reversed (Fig. 6B). Mock-transfected and AMPK knockdown cells not subjected to stress show no appreciable increase in 3-OMG uptake over control cells. Nontransfected cells treated with AICAR, KCN, FCCP, or cells subjected to glucose starvation all show a two- to threefold increase in 3-OMG uptake. This stimulation of sugar uptake is reversed to near-control levels in AMPK knockdown cells. AMPK knockdown inhibits 3-OMG uptake by 48% in glucose-starved cells, by 41% in AICAR-treated cells, by 50% in KCN-treated cells, and by 43% in FCCP-treated cells (Fig. 6B). Compound C stimulates 3-OMG uptake 1.3-fold, but AMPK knockdown has no effect on this stimulation. The use of nonsilencing siRNAs or mock transfection protocols are without effect on basal sugar uptake or KCN-stimulated sugar uptake (Fig. 6, A and B). All subsequent AMPK siRNA experiments therefore used mock transfection as the appropriate control.

The use of AMPK α1 or α2 siRNAs specifically reduces AMPK α1 or α2 message expression, respectively (Fig. 4). We therefore asked which AMPK isoform is required for stress-induced sugar transport stimulation in bEnd.3 cells. Transport stimulation by glucose deprivation or by KCN treatment is blocked by AMPK α1 knockdown, by AMPK α2 knockdown, and by knockdown of AMPK α1 plus α2 (Fig. 6C). We therefore conclude that both AMPK α1 and α2 are required for sugar transport stimulation during metabolic stress.

**Compound C and AMPK knockdown inhibit GLUT1 recruitment during acute metabolic stress.** bEnd.3 sugar transport stimulation during acute metabolic stress is mediated by recruitment of intracellular GLUT1 to the plasma membrane (14). The role of AMPK in this process was evaluated by examining the effects of Compound C on GLUT1 recruitment. Cells were treated with 2 mM AICAR, glucose starved, or KCN treated as described above in the absence or presence of 10 μM Compound C. Cells were then washed, cooled to 4°C, and treated with the membrane-impermeant Sulfo-NHS-SS-Biotin to label exposed primary amines (lysine side-chains) as described previously (14). The reaction was quenched, the cells were lysed in detergent-containing lysis buffer, and biotinylated proteins were precipitated with streptavidin beads. Precipitated proteins were analyzed by Western blotting using anti-

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GLUT1 COOH-terminal IgGs (Fig. 7, A, C, and E) and band densities were quantitated (Fig. 7, B, D, and F). As recently reported (14), biotinylated, COOH-terminal-reactive GLUT1 is revealed as a 48-kDa species plus a broadly mobile 55-kDa species. Occasionally, a 42-kDa species is also observed (Fig. 7, C and E). Treatment of membranes with peptide:N-glycosidase F causes all species to collapse to a 40- to 42-kDa GLUT1 species. Occasionally, a 42-kDa species is also observed (Fig. 7, C and E). Treatment of membranes with peptide:N-glycosidase F causes all species to collapse to a 40- to 42-kDa GLUT1 species (14). Cell surface expression of the 48-kDa GLUT1 COOH-terminal antibody reactive species is unchanged by AICAR, whereas expression of the 55-kDa species is increased by 2- to 3-fold (Fig. 7, B and F). Glucose depletion and KCN appear to enhance cell surface expression of the 48- and 55-kDa species by 2- to 4-fold. AICAR treatment increases total plasma membrane GLUT1 by approximately 2.1-fold, glucose depletion by approximately 3.0-fold, and KCN treatment by approximately 1.8- to 2.4-fold over controls. These results mirror the increases in 3-OMG transport capacity produced by metabolic poisons. In contrast, Compound C-treated cells show no significant change in plasma membrane GLUT1 (Fig. 7, B, D, and F).

AMPK knockdown before KCN treatment also prevents GLUT1 recruitment to the plasma membrane. KCN treatment increases GLUT1 at the plasma membrane 1.8- to 2.4-fold over mock transfected cells, but this recruitment is blocked in AMPK knockdown cells treated with KCN. Basal plasma membrane [GLUT1] is unaffected by AMPK knockdown (Fig. 7, G and H). These data are consistent with the results obtained by Compound C inhibition of AMPK in Fig. 7, E and F. Biotinylation of cell surface GLUT1 is increased 2.2-fold following glucose deprivation of mock-transfected cells but is unchanged following glucose deprivation of cells previously transfected with AMPK siRNA (data not shown).

DISCUSSION

This study examines the hypothesis that AMPK modulates GLUT1-mediated sugar uptake in brain microvascular endothelial cells by regulating plasma membrane GLUT1 levels.
during acute metabolic stress. We show that endothelial cell AMPK is phosphorylated during metabolic stress and that this is inhibited in a dose-dependent manner by the AMPK antagonist, Compound C. AMPK activation by the AMPK agonist AICAR or by metabolic stress is associated with stimulation of GLUT1-mediated sugar uptake; but transport stimulation is inhibited by AMPK knockdown and in a dose-dependent manner by Compound C. Transport stimulation appears to result from recruitment of intracellular GLUT1 to the cell surface because Compound C and AMPK knockdown block AICAR- and metabolic stress-induced GLUT1 recruitment.

Compound C is a high-affinity ligand that competes with AMP and ATP for binding to AMPK (47). ATP- and Compound C-ligated AMPK is catalytically inactive, but AMP-binding promotes AMPK phosphorylation, resulting in activation (22, 23). ZMP, an AICAR metabolite, also binds at the AMP-binding site to activate the kinase (22). Compound C and ZMP binding are thus mutually exclusive, thereby explaining
Compound C inhibition of AMPK activation by AICAR. Our studies confirm that AMPK phosphorylation in b.End.3 cells is blocked by Compound C in a dose-dependent manner. The observed \( K_{i(app)} \) (1–5 \( \mu M \)) is significantly greater than the reported \( K_d(\text{Compound C}) \) (120 nM) for Compound C interaction with AMPK (20). This discrepancy most likely results from competition between Compound C and intracellular ZMP for binding to AMPK. At \([\text{ZMP}] \leq 2 \text{ mM and } K_d(\text{ZMP})\) for ZMP binding to AMPK = 90 \( \mu M \) (38), \( K_{i(app)} \) for Compound C inhibition of AMPK \( K_d(\text{Compound C}) (1 + [\text{ZMP}]/K_d(\text{ZMP})) \leq 2.8 \setminus \mu M.\)

Our previous work shows that AICAR application to b.End.3 cells and ATP depletion-induced acute metabolic stress promote AMPK phosphorylation and increased sugar uptake (14). While inferring a link between AMPK activation and sugar transport stimulation, these findings do not establish causality. The present study demonstrates that the AMPK inhibitor Compound C inhibits AMPK activation and sugar transport stimulation. While pharmacological inhibition of a target protein can produce unforeseen side effects, the observation that AMPK knockdown also prevents metabolic stress-induced sugar transport stimulation validates the use of Compound C as an effective AMPK inhibitor. The concordance between the results of pharmacologic and knockdown approaches further implicates AMPK as the mediator of GLUT1 translocation to the plasma membrane during acute stress. Metabolic stress-induced AMPK phosphorylation (particularly that promoted by KCN and FCCP) is never completely ablated by Compound C treatment or AMPK knockdown. Nonetheless, Compound C or AMPK knockdown inhibits KCN- and FCCP-induced 3-OMG uptake stimulation. This result implies that there is a threshold of AMPK activation below which phosphorylation of AMPK is not sufficient to stimulate GLUT1 recruitment to the plasma membrane.

Compound C does not directly inhibit GLUT1-mediated b.End.3 cell sugar transport. In fact, 3-OMG uptake is stimulated 1.3- to 1.9-fold by Compound C. This may result from a previously well-characterized, independent regulatory mechanism (6–8, 27, 28) in which GLUT1-adenine nucleotide interactions allosterically modify sugar transport activity. ATP binding to GLUT1 reduces \( V_{\text{max}} \) and \( K_a \) for sugar uptake, while AMP displaces ATP from GLUT1, converting the protein to a high-capacity low-affinity transporter. Compound C may compete with intracellular ATP for binding to GLUT1, thereby reversing allosteric inhibition of transport and increasing sugar uptake. If this interpretation of Compound C-stimulation of basal sugar transport is correct, this suggests that basal sugar transport in endothelial cells is subject to tonic, allosteric inhibition by cytoplasmic ATP. The lack of effect of AMPK knockdown on basal sugar transport and on Compound C-stimulated sugar transport reinforces the view that Compound C stimulation of transport is AMPK independent and suggests that basal glucose transport in cultured b.End.3 cells is not activated by basal AMPK phosphorylation. Compound C and AMPK knockdown significantly attenuate stimulation of sugar uptake and AMPK phosphorylation by metabolic stress. Cell surface GLUT1 recruitment is completely blocked by Compound C and AMPK knockdown, while AMPK knockdown in the absence of stress has no appreciable effect on GLUT1 localization. These data, in conjunction with our previous findings (14), strengthen the hypothesis that AMPK activation mediates b.End.3 cell sugar transport stimulation during metabolic stress.

Chronic metabolic stress causes increased GLUT1 expression and increased sugar transport in brain microvascular endothelial cells (4, 5, 24, 26, 33, 41). In contrast, acute metabolic stress is without effect on GLUT1 expression but increases cellular sugar transport capacity (9, 10, 14). The present work supports the hypothesis that activation of AMPK (the primary sensor in cellular energy homeostasis (22, 23)) stimulates sugar transport by rapidly enhancing GLUT1 trafficking to the plasma membrane. AMPK also regulates GLUT4-mediated sugar transport in muscle and adipose by chronic control of gene expression and by acute regulation of protein trafficking to the plasma membrane (1, 18, 25, 46). AMPK therefore plays a dual role in each tissue by initiating short-term responses to acute changes in metabolic state and long-term responses to chronic alterations in cellular metabolism.

Glucose is a primary energy source for the brain, and its transport across the blood-brain barrier is rate-limiting for cerebral glucose utilization (31, 42). When a region of the brain becomes acutely activated or when blood glucose levels rapidly fall, local blood flow and glucose uptake are activated to maintain brain glucose availability (4, 5, 26, 33, 39, 41). The present study supports the hypothesis that elevated intracellular AMP promotes endothelial cell AMPK phosphorylation, which leads to intracellular GLUT1 translocation to luminal and abluminal membranes. The net effect is increased glucose transport across the blood-brain barrier and enhanced glucose delivery to astrocytes and neurons.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
A.J.C. and A.C. conception and design of the research; A.J.C. performed the experiments; A.J.C. analyzed the data; A.J.C. and A.C. interpreted the results of the experiments; A.J.C. and A.C. prepared the figures; A.J.C. and A.C. drafted the manuscript; A.J.C. and A.C. edited and revised the manuscript; A.J.C. and A.C. approved the final version of the manuscript.

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