Brief Communications

Fast and Reversible Stimulation of Astrocytic Glycolysis by K^+ and a Delayed and Persistent Effect of Glutamate

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Synaptic activity is followed within seconds by a local surge in lactate concentration, a phenomenon that underlies functional magnetic resonance imaging and whose causal mechanisms are unclear, partly because of the limited spatiotemporal resolution of standard measurement techniques. Using a novel Förster resonance energy transfer-based method that allows real-time measurement of the glycolytic rate in single cells, we have studied mouse astrocytes in search for the mechanisms responsible for the lactate surge. Consistent with previous measurements with isotopic 2-deoxyglucose, glutamate was observed to stimulate glycolysis in cultured astrocytes, but the response appeared only after a lag period of several minutes. Na^+ overloads elicited by engagement of the Na^+-glutamate cotransporter with D-aspartate or application of the Na^+ ionophore gramicidin also failed to stimulate glycolysis in the short term. In marked contrast, K^+ stimulated astrocytic glycolysis by fourfold within seconds, an effect that was observed at low millimolar concentrations and was also present in organotypic hippocampal slices. After removal of the agonists, the stimulation by K^+ ended immediately but the stimulation by glutamate persisted unabated for >20 min. Both stimulations required an active Na^+/K^+ ATPase pump. By showing that small rises in extracellular K^+ mediate short-term, reversible modulation of astrocytic glycolysis and that glutamate plays a long-term effect and leaves a metabolic trace, these results support the view that astrocytes contribute to the lactate surge that accompanies synaptic activity and underscore the role of these cells in neurometabolic and neurovascular coupling.

Introduction

Early during neural activation, there is an acute fall in glucose concentration and a concurrent increase in lactate concentration in brain tissue (Prichard et al., 1991; Silver and Erecińska, 1994; Hu and Wilson, 1997a,b; McNay et al., 2000; Mangia et al., 2003; Caesar et al., 2008). The lactate surge is thought to play roles in neurometabolic (Pellerin and Magistretti, 1994) and neurovascular (Gordon et al., 2008) coupling, but its source has remained controversial (discussed by Barros and Deitmer, 2010). The lactate surge develops in seconds, so it follows that if astrocytes were involved, their rate of glycolysis should also react to one or more neuronal signals within seconds, a hypothesis that has not been tested because of limited resolution of current methods based on isotopic deoxyglucose. In the present study, we have addressed this question with a Förster resonance energy transfer (FRET)-based methodology recently developed in our laboratory that allows measurement of the glycolytic rate in single cells with high temporal resolution (Bittner et al., 2010).

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Materials and Methods

Reagents. Standard chemicals and tissue culture reagents were from Sigma. Threo-β-benzyloxyaspartate (TBOA), (S)-3,5-dihydroxyphenylglycine (DHPG), and 2-methyl-6-(phenylethynyl)-pyridine (MPEP) were from Tocris Bioscience. SBFI AM, Fura2 AM, and calcein AM were obtained from Invitrogen. Constructs coding for the sensors FLIPglu600$\mu\Delta$11 and FLII^{12}Pglu600$\mu\Delta$6 have been described previously (Deuschle et al., 2005; Takanaga et al., 2008). Plasmids are available through www.addgene.org. Adenoviral vectors were custom made by Vector Biolabs.

Cell culture, brain slices, and experimental measurements. Most measurements were performed in astrocytes in mixed neuronal glial cultures or organotypical hippocampal slices, cultured from mixed F1 male mice (C57BL/6J × CBA/J), as described previously (Porras et al., 2004; Bittner et al., 2010). All experiments were approved by the Centro de Estudios Científicos Animal Care and Use Committee. Glucose, Na^+, Ca^{2+}, and volume measurements were done at room temperature (22–25°C) in a 5% CO_2-gassed Krebs–Ringer buffer containing 24 mM bicarbonate, 1 mM lactate, and 1–2 mM glucose as detailed (Loaiza et al., 2003; Bittner et al., 2010). For some selected experiments, cells were kept at 36°C by heating the perfusion line. Lactate release from pure astrocytic subcultures at 36°C was estimated enzymatically using 5 mM hydrazine as a pyruvate sink (Rosenberg and Rush, 1966). Data are presented as means ± SEM. Differences between experimental groups were assessed with Student's *t* test or ANOVA followed by Tukey-Kramer's *post hoc* test. *p* values <0.05 were considered significant and are indicated with an asterisk (*). *p* values >0.05 were considered nonsignificant (NS).

Results

Long-term stimulation of glycolysis by glutamate

The glycolytic rate was measured using the inhibitor transport method (ITM). Briefly, cells were exposed to cytochalasin B, a glucose transporter blocker, while glucose concentration was being measured in real time with a DNA-encoded FRET glucose nanosensor. The rate at which the concentration of glucose decreases after glucose transport inhibition is equal to the rate of hexokinase, i.e., the rate of glycolysis at its entry point (Bittner et al., 2010). Using this technique in astrocytes in mixed cortical cultures, we first investigated possible acute effects of glutamate. Figure 1*A* shows that 50 μM glutamate caused a slight glycolytic inhibition of 18 ± 9% ($n = 20$ cells in five experiments, $p < 0.05$). Functional Na^+-glutamate cotransport was confirmed by an increase in intracellular Na^+ (Fig. 1*B*). Independent engagement of the transporter with D-aspartate or of the metabotropic glutamate receptor with DHPG (Porras et al., 2008) also failed to affect glycolysis (supplemental Fig. S1*A*,*B*, available at www.jneurosci.org as supplemental material). In brain tissue, glutamate transporters and metabotropic glutamate receptors may segregate in different astrocytic subtypes, but in culture they can be present in the same cell. It is therefore possible in principle that the absence of a stimulatory effect of glutamate in culture conditions had been due to an inhibitory cross talk between transporters and receptors. However, such a scenario seems unlikely, considering that glutamate did not stimulate glycolysis in the presence of TBOA, a glutamate transporter blocker, or in the presence of MPEP, a metabotropic glutamate receptor blocker (supplemental Fig. S1*C*,*D*, available at www.jneurosci.org as supplemental material).

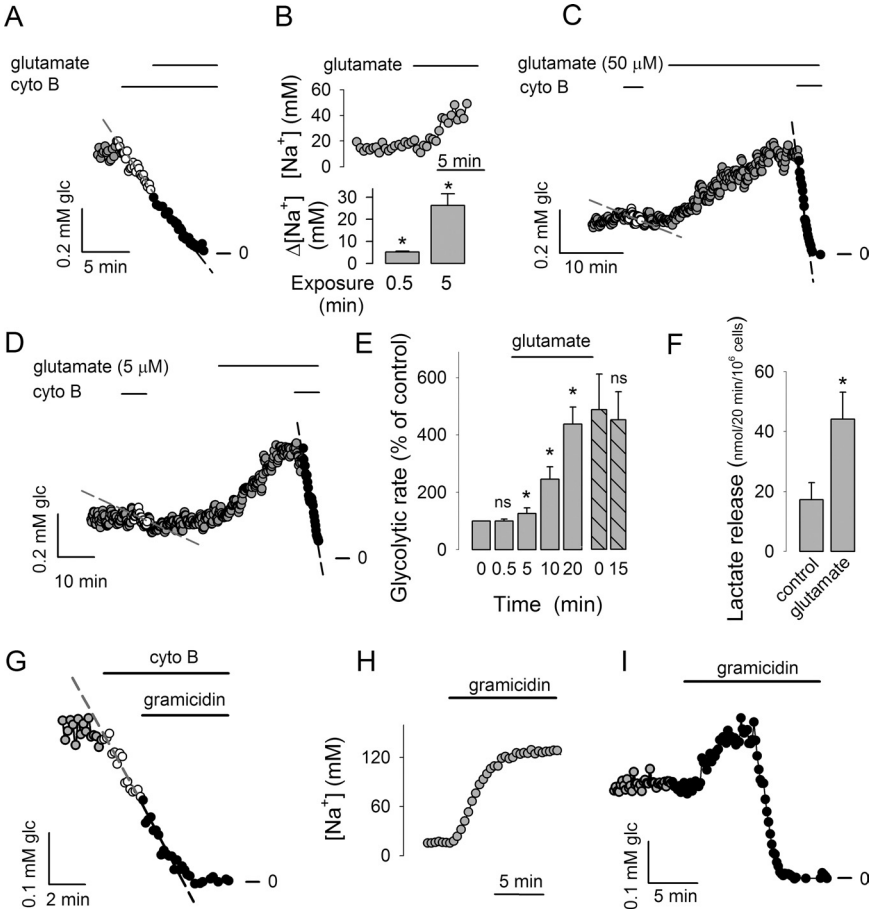

Figure 1. Delayed stimulation of astrocytic glycolysis by glutamate. ***A***, Exposure of an astrocyte to 20 μM cytochalasin B resulted in a linear decrease in glucose concentration at a rate of 0.8 μM/s (open symbols), which decreased slightly to 0.6 μM/s after application of 50 μM glutamate (closed symbols). ***B***, Top, Application of 50 μM glutamate to an astrocyte caused an increase in intracellular Na^+. Bottom, Average Na^+ increase measured after 0.5 and 5 min of exposure to 50 μM glutamate ($n = 30$ cells in three experiments). ***C***, The glycolytic rate of a single astrocyte was measured using 20 μM cytochalasin B before (open symbols; 0.11 μM/s) and after (closed symbols; 1.45 μM/s) a 20 min exposure to 50 μM glutamate. ***D***, An experiment similar to that shown in ***C*** but with 5 μM glutamate. Control and glutamate-stimulated rates were 0.23 μM/s and 1.61 μM/s, respectively. ***E***, Rates were measured before (0) and after exposure to 5 μM glutamate for either 0.5, 5, 10 or 20 min (gray bars; $n = 15$–40 cells in 2–5 experiments). Hatched bars represent a separate set of experiments in which rates were measured after 20 min exposure to 5 μM glutamate and 15 min after glutamate washout. Rates are relative to a control measurement before glutamate exposure ($n = 16$ cells in three experiments). ***F***, Lactate release by pure astrocyte cultures was measured over a period of 20 min in the absence and presence of 50 μM glutamate ($n = 3$ experiments paired with Fig. 2*G*). ***G***, No significant change was observed in the rate of glycolysis upon exposure to 20 μg/ml gramicidin. ***H***, Gramicidin caused a rapid increase in intracellular Na^+, representative of three separate experiments. ***I***, Biphasic effect of gramicidin on intracellular glucose; similar data were obtained in two separate experiments.

Next, a protocol was devised to detect longer-term effects of glutamate. Measurement of the rate of glycolysis with ITM can be done in reproducible manner several times, allowing the design of before-and-after type experiments (Bittner et al., 2010). Using this kind of protocol, we saw that a 20 min exposure to 50 μM glutamate caused strong stimulation of glycolysis (Fig. 1*C*), with respective rates of 0.19 ± 0.05 μM/s and 0.71 ± 0.15 μM/s ($n = 26$ cells in four experiments; $p < 0.05$), a stimulation of almost fourfold. A similar response was observed at 5 μM glutamate (Fig. 1*D*). Figure 1*E* summarizes the results obtained at increasing times of exposure, showing that glutamate required at least 5 min of continuous exposure to elicit sizable glycolysis stimulation. Worthy of note is the observation that the cells remained stimulated after neurotransmitter withdrawal. As reported previously (Pellerin and Magistretti, 1994), glutamate also stimulated the release of lactate by astrocytes (Fig. 1*F*). Glutamate caused a rise in cytosolic glucose (Fig. 1*C*,*D*), as expected from the acute stimulatory effect of the neurotransmitter on the astrocytic glucose transporter GLUT1 (Loaiza et al., 2003; Chuquet et al., 2010). As with glutamate and D-aspartate, the Na^+ ionophore gramicidin failed to affect the glycolytic rate in the short term, with a nonsignificant inhibition of 13% ($n = 11$ cells in four experiments) (Fig. 1*G*) despite causing a substantial increase in intracellular Na^+ (Fig. 1*H*). Gramicidin did affect glucose metabolism after a few minutes, with a glucose rise consistent with GLUT1 activation (Porras et al., 2008), followed by severe glucose depletion, consistent with strong glycolytic activation (Fig. 1*I*). Failure of gramicidin to increase the concentration of glucose during the first seconds may be due to the obligatory requirement of a coincident Ca^{2+} increase in the stimulation of GLUT1 by intracellular Na^+ (Porras et al., 2008).

Short-term stimulation of glycolysis by K^+

Searching for a short-term neuronal signal for astrocytic glycolysis, we focused on K^+. The rate of glycolysis was first monitored

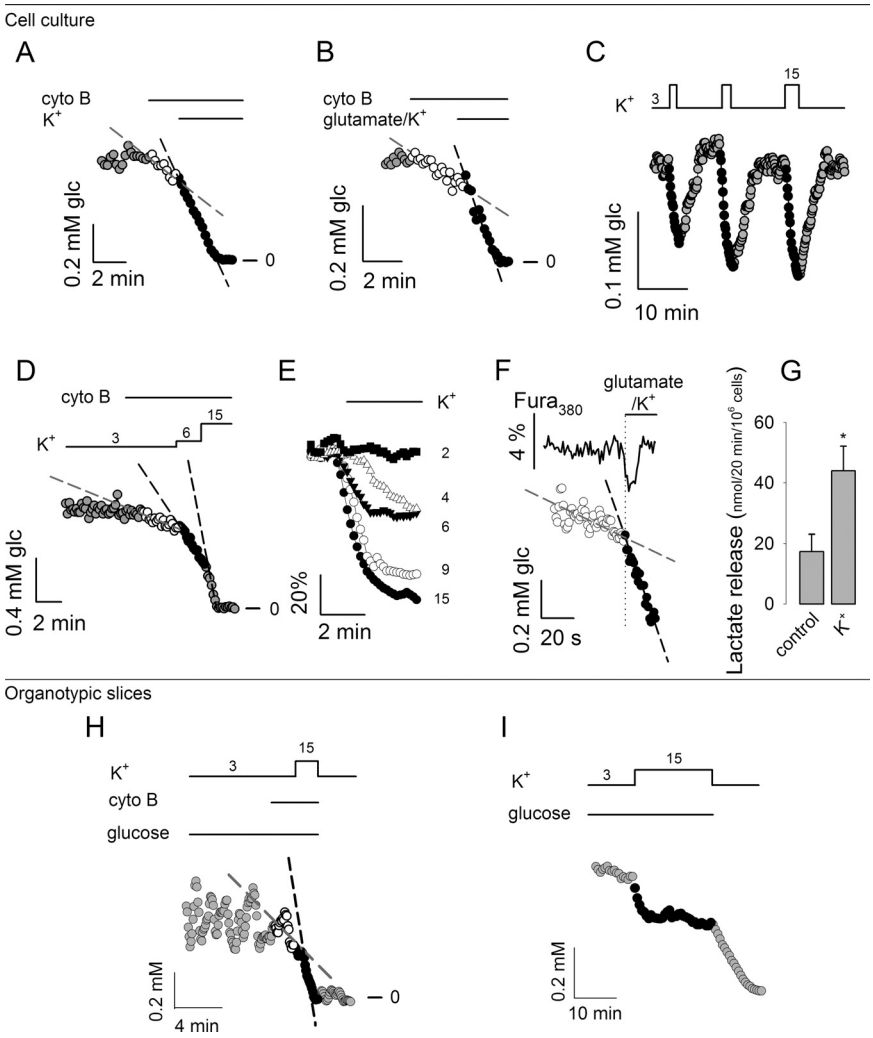

Figure 2. Acute stimulation of astrocytic glycolysis by K^+. ***A***, The basal rate of glycolysis in a single astrocyte was first estimated over 2 min by applying 20 μM cytochalasin B, followed by addition of 12 mM K^+. Dashed lines represent the rates before and after addition of K^+. ***B***, A similar experiment testing the effect of simultaneous addition of 50 μM glutamate and 12 mM K^+. ***C***, Transient exposure to 15 mM K^+ brought about a fall in intracellular glucose, consistent with transient activation of glycolysis. Data are representative of 10 similar experiments. ***D***, Glycolytic rate was measured in the presence of increasing concentrations of K^+ (6 and 15 mM). ***E***, Subsequent exposures of a cell to increasing K^+ concentrations (2, 4, 6, 9, and 15 mM) caused stronger perturbations in glucose concentration. ***F***, Intracellular glucose was measured every 2 s in cells with Fura 2, allowing nonquantitative reciprocal imaging of intracellular Ca^{2+} at 380 nm excitation (continuous line). The onset of the Fura 2 deflection (dotted vertical line) marked the arrival of 50 μM glutamate/15 mM K^+ to the cell. The intercept between linear regressions before and after the Fura 2 deflection (dashed lines) was computed as the onset of metabolic activation. The difference between the Fura 2 deflection and the intercept was computed as the stimulation delay, which for this specific cell was zero. ***G***, Lactate release by pure astrocytic cultures was measured over a period of 20 min in 3 mM K^+ (control) or 15 mM K^+ ($n = 3$ experiments paired with Fig. 1*F*). ***H***, The rate of glucose metabolism in a single astrocyte in an organotypic hippocampal slice was measured with 20 μM cytochalasin B before and after exposure to 15 mM K^+. Similar results were obtained in other eight experiments. ***I***, Effect of 15 mM K^+ on the concentration of glucose in an astrocyte, representative of five similar experiments.

while switching from a control level (3 mM) to an elevated 15 mM K^+. This maneuver strongly stimulated astrocytic glycolysis by 371 ± 134% (Fig. 2*A*) ($n = 15$ cells in four experiments, $p < 0.05$), a similar degree of stimulation to that achieved when K^+ is applied together with glutamate (Fig. 2*B*). In the absence of a glucose transport blocker, K^+ caused a quick drop in the steady-state concentration of glucose (Fig. 2*C*), showing that the stimulation of hexokinase is not evenly matched by glucose transporter stimulation. Control experiments confirmed that K^+ does not modulate GLUT1 in the short term (supplemental Fig. S4, available at www.jneurosci.org as supplemental material); thus, the rapid fall in cytosolic glucose provides a cytochalasin B-free demonstration of the stimulatory effect of K^+ on glycolysis. After K^+ removal, glucose concentration bounced back immediately, evidence of a fast "off" mechanism (Fig. 2*C*). A modest rise in K^+ from 3 to 6 mM was also effective, with stimulation of 200–300% (Fig. 2*D*). Mathematical modeling that will be described elsewhere showed that in the absence of changes in glucose transport kinetics, the initial rate of glucose decrease is directly proportional to the degree of activation, allowing the consecutive testing of multiple K^+ concentrations on the same cell. This type of experiment showed that glycolysis can be stimulated by small increases in extracellular K^+ (Fig. 2*E*). In the presence of 4 mM, i.e., an increase of just 1 mM over basal level, the stimulation seen was 30 ± 5% of the stimulation observed at 15 mM K^+ ($n = 17$ cells in three experiments). To pinpoint the speed of glycolytic activation, astrocytes were exposed to K^+ together with glutamate, which triggers a Ca^{2+} transient that marks the arrival of the agonists at the cell surface. Figure 2*F* shows a cell in which the calcium transient is simultaneous with the activation of glycolysis. In all 30 cells studied in this way (from seven experiments), the activation developed within 15 s, and in 19 cells, the activation was detected within 4 s. Control confocal microscopy in calcein-loaded cells revealed K^+-induced cell swelling, but at a negligible rate compared to the rate of glucose decrease, ruling out glucose dilution as a significant artifact (supplemental Fig. S2, available at www.jneurosci.org as supplemental material). The response of glycolysis to K^+ was robust, being detected in almost every astrocyte tested, regardless of culture conditions, from 3 to 21 d in culture; from 30% to 100% confluency and independent of the presence of neurons (data not shown). Supplemental Figure S3 (available at www.jneurosci.org as supplemental material) shows that short-term effects of glutamate and K^+ on glucose metabolism can also be detected at near physiological temperature. The activation of glycolysis by K^+ was accompanied by a marked increase in lactate release (Fig. 2*G*). To demonstrate the phenomenon in brain tissue, the glucose nanosensor was expressed in organotypical hippocampal slices by means of an adenoviral vector, which transduces specifically in astrocytes (Bittner et al., 2010). In slices, K^+ was also capable of activating glycolysis and reducing intracellular glucose (Fig. 2*H*,*I*).

Na^+/K^+ ATPase pump involvement in the stimulation of glycolysis by glutamate and K^+

The experiment in Figure 3*A* shows that the long-term stimulatory effect of glutamate on astrocytic glycolysis was reversibly inhibited by the Na^+/K^+ ATPase blocker ouabain, which regis-

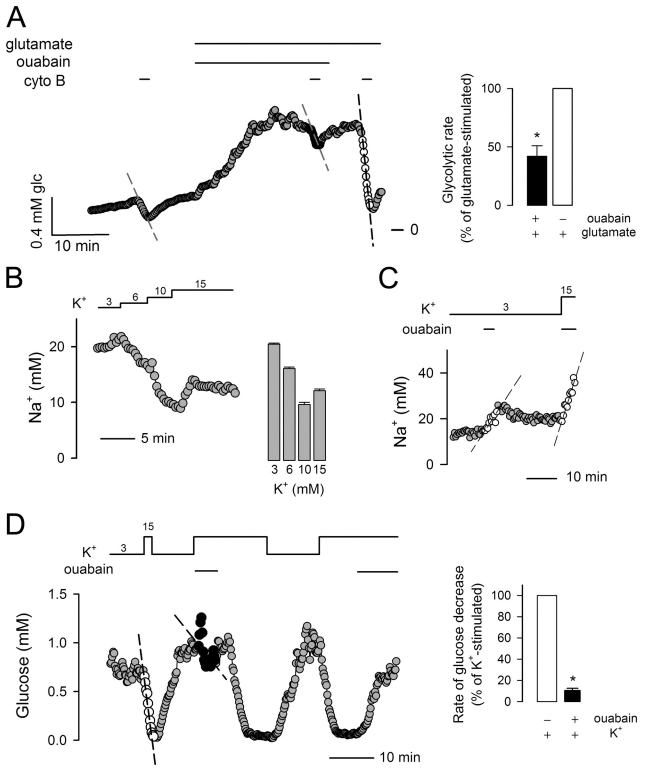

Figure 3. Involvement of the Na^+/K^+ ATPase in the modulation of astrocytic glycolysis by glutamate and K^+. ***A***, After measurement of a control glycolytic rate with 20 μM cytochalasin B (1.4 μM/s), an astrocyte was exposed to 50 μM glutamate in the presence of 100 μM ouabain. A second rate measurement was performed after 20 min exposure to this medium (1.43 μM/s), and a third measurement was carried 5 min after ouabain washout, in the continuous presence of glutamate (7.3 μM/s). The bar graph shows normalized values for glutamate-stimulated rates in the absence and presence of ouabain ($n = 57$ cells in six experiments). ***B***, Effect of extracellular K^+ on intracellular Na^+ in a single astrocyte. The bar graph shows average values after 4 min for $n = 95$ cells in five experiments. ***C***, Na^+ permeability was measured in the presence of 1 mM ouabain before and during exposure to 15 mM K^+. Na^+ permeability was increased by 93 ± 31% ($n = 60$ cells in five experiments). ***D***, Effect of 1 mM ouabain on the activation of glycolysis by 15 mM K^+. The graphs summarize the effects of ouabain on the initial rate of glucose decrease ($n = 25$ cells in four experiments).

ters with previous deoxyglucose data. As reported earlier (Rose and Ransom, 1996), exposure of astrocytes to high K^+ led to a decrease in astrocytic Na^+ concentration (Fig. 3*B*). This decrease occurred despite a rise in Na^+ permeability, estimated by blocking Na^+ extrusion with ouabain, at a concentration of 1 mM to prevent competition by K^+ (Fig. 3*C*). A Na^+ decrease together with higher Na^+ permeability provides a demonstration of Na^+/K^+ ATPase pump activation by K^+. Having shown that the pump is activated by K^+, we observed that the effect of K^+ on glucose concentration was strongly and reversibly inhibited by ouabain (Fig. 3*D*). Finally, the basal rate of glycolysis was inhibited by 85 ± 4% after a 5 min preincubation with 100 μM ouabain ($n = 47$ cells in five experiments). These results show that the Na^+/K^+ ATPase pump is a nodal point for basal control of glycolysis, for its long-term modulation by glutamate as well as for its short-term modulation by K^+.

Discussion

The main observation in this article is that K^+, a molecule released by postsynaptic terminals during excitatory neurotransmission, is capable of strong and reversible stimulation of astrocytic glycolysis that evolves on the order of seconds. Glutamate, a presynaptic signal, induced a delayed, long-term stimulation of astrocytic glycolysis, which persisted long after removal of the agonist. These results suggest that the lactate surge observed in brain tissue during synaptic activity originates—at least partially—in astrocytes, and therefore support a role for these cells in neurovascular coupling (Gordon et al., 2008). Whereas the speed and strength of the glycolytic activation by K^+ in astrocytes are compatible with the existence of a fast astrocyte-to-neuron lactate shuttle (Pellerin and Magistretti, 1994), it remains to be determined how neuronal glycolysis reacts to K^+ (and glutamate) in the short term. Neuronal K^+ is also released in the vicinity of glial cells at nonglutamatergic synapses, at the neuromuscular junction, and by axons during the action potential, suggesting a general mechanism of neurovascular/metabolic coupling.

Excitatory synaptic activity releases glutamate into the synaptic cleft and a few milliseconds later K^+ leaks out of the postsynaptic terminal, mirroring the large inward Na^+ current carried by ionotropic glutamate receptors (Attwell and Laughlin, 2001). Microelectrodes inserted in brain tissue and peripheral nerve have shown that K^+ concentration can reach up to 12 mM under maximum stimulation (Kofuji and Newman, 2004), but given the discordance between the tip of the electrodes (micrometers) and the size of the extracellular space (20 nm), this figure may be an underestimate (Fröhlich et al., 2008). Nevertheless, we observed that K^+-stimulated glycolysis was already detectable at 4 mM K^+, and reached 200–300% at 6 mM, close to the concentrations present in brain interstitium under physiological conditions (Heinemann et al., 1990; Fröhlich et al., 2008). On the other hand, a limitation of this study is that we used bulk changes of K^+, which are not necessarily equivalent to the local release of K^+ in a spatially constrained pattern. It has already been proposed that K^+ stimulates astrocytic glycolysis (Brookes and Yarowsky, 1985), but several studies designed to detect changes using 2-deoxyglucose uptake over 20 or more minutes found little or no stimulation, even at 50 mM K^+ (Peng et al., 1994; Takahashi et al., 1995; Sokoloff et al., 1996).

The short-term insensitivity of glycolysis to glutamate was unexpected, but with the benefit of hindsight, it seems compatible with the dynamics of Na^+ handling by these cells. Nonstimulated astrocytes maintain a high resting Na^+ level of ~15–20 mM, which is above the K_m of the Na^+/K^+ ATPase for Na^+ (Kimelberg et al., 1978; Rose and Ransom, 1996; Silver and Erecińska, 1997; Kelly et al., 2009). This means that under the basal conditions, the pump is already primed with Na^+, and that a further increase in intracellular Na^+ will not cause much stimulation. Moreover, due to the slow turnover of the Na^+-glutamate transporters, the rise in intracellular Na^+ elicited by glutamate is not instantaneous and in the first seconds amounts to only a few millimolar (Chatton et al., 2001; Chatton et al., 2003; Langer and Rose, 2009). For K^+, the scenario is more favorable. The arrival of K^+ is virtually instant and the astrocytic Na^+/K^+ ATPase has an unusually low affinity for extracellular K^+, with a K_m of ~5 mM (Rose and Ransom, 1996; Silver and Erecińska, 1997), which in combination with the low resting concentration in the brain interstitium (<3 mM) allows for potential stimulations of the Na^+/K^+ ATPase by >100%.

Over longer incubation periods, glutamate strongly stimulated glycolysis, a phenomenon dependent on an active sodium pump, confirming previous 2-deoxyglucose experiments and the purported role for glutamate in neurometabolic coupling (Pellerin and Magistretti, 1994; Takahashi et al., 1995; Pellerin et al., 2007; Magistretti, 2009). Remarkably, glycolysis remained stimulated long after glutamate withdrawal, a phenomenon that may

help to explain the local stimulation of glycolysis that persists *in vivo* up to 40 min after cessation of task performance (Madsen et al., 1995). Whereas glutamate did not stimulate glycolysis in the short term, it did cause an acute increase in intracellular glucose, which is consistent with GLUT1 stimulation, as shown with fluorescent glucose analogs (Loaiza et al., 2003; Porras et al., 2004; Barros et al., 2007; Porras et al., 2008; Chuquet et al., 2010). GLUT1 glucose transporters are preferentially located in astrocytic endfeet surrounding capillaries (Kacem et al., 1998), and in view of the severe intracellular glucose depletion caused by K^+ (e.g., Fig. 3*D*), acute GLUT1 activation by glutamate may be seen as a mechanism that prevents astrocytic hexokinase desaturation in conditions of high metabolic demand and that conditions gap junction-mediated exchange of glucose between neighboring regions (Rouach et al., 2000).

References

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21:1133–1145.

Barros LF, Deitmer JW (2010) Glucose and lactate supply to the synapse. Brain Res Rev 63:149–159.

Barros LF, Bittner CX, Loaiza A, Porras OH (2007) A quantitative overview of glucose dynamics in the gliovascular unit. Glia 55:1222–1237.

Bittner CX, Loaiza A, Ruminot I, Larenas V, Sotelo-Hitschfeld T, Gutiérrez R, Córdova A, Valdebenito R, Frommer WB, Barros LF (2010) High resolution measurement of the glycolytic rate. Front Neuroenergetics 2:26.

Brookes N, Yarowsky PJ (1985) Determinants of deoxyglucose uptake in cultured astrocytes: the role of the sodium pump. J Neurochem 44:473–479.

Caesar K, Hashemi P, Douhou A, Bonvento G, Boutelle MG, Walls AB, Lauritzen M (2008) Glutamate receptor-dependent increments in lactate, glucose and oxygen metabolism evoked in rat cerebellum in vivo. J Physiol 586:1337–1349.

Chatton JY, Shimamoto K, Magistretti PJ (2001) Effects of glial glutamate transporter inhibitors on intracellular Na+ in mouse astrocytes. Brain Res 893:46–52.

Chatton JY, Pellerin L, Magistretti PJ (2003) GABA uptake into astrocytes is not associated with significant metabolic cost: implications for brain imaging of inhibitory transmission. Proc Natl Acad Sci U S A 100:12456–12461.

Chuquet J, Quilichini P, Nimchinsky EA, Buzsáki G (2010) Predominant enhancement of glucose uptake in astrocytes versus neurons during activation of the somatosensory cortex. J Neurosci 30:15298–15303.

Deuschle K, Okumoto S, Fehr M, Looger LL, Kozhukh L, Frommer WB (2005) Construction and optimization of a family of genetically encoded metabolite sensors by semirational protein engineering. Protein Sci 14:2304–2314.

Fröhlich F, Bazhenov M, Iragui-Madoz V, Sejnowski TJ (2008) Potassium dynamics in the epileptic cortex: new insights on an old topic. Neuroscientist 14:422–433.

Gordon GR, Choi HB, Rungta RL, Ellis-Davies GC, MacVicar BA (2008) Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature 456:745–749.

Heinemann U, Schaible HG, Schmidt RF (1990) Changes in extracellular potassium concentration in cat spinal cord in response to innocuous and noxious stimulation of legs with healthy and inflamed knee joints. Exp Brain Res 79:283–292.

Hu Y, Wilson GS (1997a) A temporary local energy pool coupled to neuronal activity: fluctuations of extracellular lactate levels in rat brain monitored with rapid-response enzyme-based sensor. J Neurochem 69:1484–1490.

Hu Y, Wilson GS (1997b) Rapid changes in local extracellular rat brain glucose observed with an in vivo glucose sensor. J Neurochem 68:1745–1752.

Kacem K, Lacombe P, Seylaz J, Bonvento G (1998) Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a confocal microscopy study. Glia 23:1–10.

Kelly T, Kafitz KW, Roderigo C, Rose CR (2009) Ammonium-evoked alterations in intracellular sodium and pH reduce glial glutamate transport activity. Glia 57:921–934.

Kimelberg HK, Biddelcome S, Narumi S, Bourke RS (1978) ATPase and carbonic anhydrase activities of bulk-isolated neuron, glia and synaptosome fractions from rat brain. Brain Res 141:305–323.

Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. Neuroscience 129:1045–1056.

Langer J, Rose CR (2009) Synaptically induced sodium signals in hippocampal astrocytes in situ. J Physiol 587:5859–5877.

Loaiza A, Porras OH, Barros LF (2003) Glutamate triggers rapid glucose transport stimulation in astrocytes as evidenced by real-time confocal microscopy. J Neurosci 23:7337–7342.

Madsen PL, Hasselbalch SG, Hagemann LP, Olsen KS, Bülow J, Holm S, Wildschiødtz G, Paulson OB, Lassen NA (1995) Persistent resetting of the cerebral oxygen/glucose uptake ratio by brain activation: evidence obtained with the Kety-Schmidt technique. J Cereb Blood Flow Metab 15:485–491.

Magistretti PJ (2009) Role of glutamate in neuron-glia metabolic coupling. Am J Clin Nutr 90:875S–880S.

Mangia S, Garreffa G, Bianciardi M, Giove F, Di Salle F, Maraviglia B (2003) The aerobic brain: lactate decrease at the onset of neural activity. Neuroscience 118:7–10.

McNay EC, Fries TM, Gold PE (2000) Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. Proc Natl Acad Sci U S A 97:2881–2885.

Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A 91:10625–10629.

Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ (2007) Activity-dependent regulation of energy metabolism by astrocytes: an update. Glia 55:1251–1262.

Peng L, Zhang X, Hertz L (1994) High extracellular potassium concentrations stimulate oxidative metabolism in a glutamatergic neuronal culture and glycolysis in cultured astrocytes but have no stimulatory effect in a GABAergic neuronal culture. Brain Res 663:168–172.

Porras OH, Loaiza A, Barros LF (2004) Glutamate mediates acute glucose transport inhibition in hippocampal neurons. J Neurosci 24:9669–9673.

Porras OH, Ruminot I, Loaiza A, Barros LF (2008) $Na(^+)$-$Ca(^{2+})$ cosignaling in the stimulation of the glucose transporter GLUT1 in cultured astrocytes. Glia 56:59–68.

Prichard J, Rothman D, Novotny E, Petroff O, Kuwabara T, Avison M, Howseman A, Hanstock C, Shulman R (1991) Lactate rise detected by 1H NMR in human visual cortex during physiologic stimulation. Proc Natl Acad Sci U S A 88:5829–5831.

Rose CR, Ransom BR (1996) Intracellular sodium homeostasis in rat hippocampal astrocytes. J Physiol 491:291–305.

Rosenberg JC, Rush BF (1966) An enzymatic-spectrophotometric determination of pyruvic and lactic acid in blood. Methodologic aspects. Clin Chem 12:299–307.

Rouach N, Glowinski J, Giaume C (2000) Activity-dependent neuronal control of gap-junctional communication in astrocytes. J Cell Biol 149:1513–1526.

Silver IA, Erecińska M (1994) Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. J Neurosci 14:5068–5076.

Silver IA, Erecińska M (1997) Energetic demands of the Na+/K+ ATPase in mammalian astrocytes. Glia 21:35–45.

Sokoloff L, Takahashi S, Gotoh J, Driscoll BF, Law MJ (1996) Contribution of astroglia to functionally activated energy metabolism. Dev Neurosci 18:344–352.

Takahashi S, Driscoll BF, Law MJ, Sokoloff L (1995) Role of sodium and potassium ions in regulation of glucose metabolism in cultured astroglia. Proc Natl Acad Sci U S A 92:4616–4620.

Takanaga H, Chaudhuri B, Frommer WB (2008) GLUT1 and GLUT9 as major contributors to glucose influx in HepG2 cells identified by a high sensitivity intramolecular FRET glucose sensor. Biochim Biophys Acta 1778:1091–1099.