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Invited review

Astrocytic energetics during excitatory neurotransmission: What are contributions of glutamate oxidation and glycolysis?

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ABSTRACT

Astrocytic energetics of excitatory neurotransmission is controversial due to discrepant findings in different experimental systems in vitro and in vivo. The energy requirements of glutamate uptake are believed by some researchers to be satisfied by glycolysis coupled with shuttling of lactate to neurons for oxidation. However, astrocytes increase glycogenolysis and oxidative metabolism during sensory stimulation in vivo, indicating that other sources of energy are used by astrocytes during brain activation. Furthermore, glutamate uptake into cultured astrocytes stimulates glutamate oxidation and oxygen consumption, and glutamate maintains respiration as well as glucose. The neurotransmitter pool of glutamate is associated with the faster component of total glutamate turnover in vivo, and use of neurotransmitter glutamate to fuel its own uptake by oxidation-competent perisynaptic processes has two advantages, substrate is supplied concomitant with demand, and glutamate spares glucose for use by neurons and astrocytes. Some, but not all, perisynaptic processes of astrocytes in adult rodent brain contain mitochondria, and oxidation of only a small fraction of the neurotransmitter glutamate taken up into these structures would be sufficient to supply the ATP required for sodium extrusion and conversion of glutamate to glutamine. Glycolysis would, however, be required in perisynaptic processes lacking oxidative capacity. Three lines of evidence indicate that critical cornerstones of the astrocyte-to-neuron lactate shuttle model are not established and normal brain does not need lactate as supplemental fuel: (i) rapid onset of hemodynamic responses to activation delivers oxygen and glucose in excess of demand, (ii) total glucose utilization greatly exceeds glucose oxidation in awake rodents during activation, indicating that the lactate generated is released, not locally oxidized, and (iii) glutamate-induced glycolysis is not a robust phenotype of all astrocyte cultures. Various metabolic pathways, including glutamate oxidation and glycolysis with lactate release, contribute to cellular energy demands of excitatory neurotransmission.

1. Introduction

One of the hallmark characteristics of the brain is its regional, cellular, and subcellular heterogeneity, with compartmentation of function and metabolism. The glutamate–glutamine cycle is a classic example of astrocyte–neuron interactions that involve glycolytic, oxidative, biosynthetic, and neurotransmitter fluxes (Peng et al., 1993; Hertz et al., 1999; Hertz et al., 2000). In brief, the typical description glutamate–glutamine cycle portrays release of neurotransmitter glutamate from neurons, Na^+-dependent uptake of glutamate from the synaptic cleft by astrocytes and its conversion to glutamine, followed by glutamine release and uptake by neurons where glutamate is regenerated by the action of glutaminase and packaged into synaptic vesicles for release during neurotransmission. However, because the blood–brain barrier restricts glutamate uptake into brain from blood, the glutamate–glutamine cycle must be extended to include glutamate synthesis and degradation in brain. Anaplerosis is the de novo synthesis of glutamate from glucose in astrocytes. This process involves glycolysis and CO_2 fixation to generate the precursors (pyruvate and oxaloacetate) that condense to form citrate that is oxidized via the TCA cycle to form α-ketoglutarate, the precursor of glutamate and glutamine. Oxidative degradation of glutamate occurs mainly in astrocyte and involves entry of α-ketoglutarate into the TCA cycle,

Abbreviations: ANL, astrocyte–neuron–lactate; BOLD, blood oxygen level-dependent; CMR_{glc}, cerebral metabolic rate for glucose; CMR_{O2}, cerebral metabolic rate for oxygen; DG, 2-deoxy-D-glucose; MRS, magnetic resonance spectroscopy; TCA cycle, tricarboxylic acid cycle.

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exit of malate from the cycle and conversion of malate to pyruvate in the cytoplasm, followed by oxidative degradation of pyruvate in the TCA cycle. This process is called pyruvate re-cycling because the glutamate carbon skeleton was originally derived from two pyruvate molecules, and the 4-carbon backbone of the TCA cycle intermediates derived from α-ketoglutarate must exit the cycle for conversion to pyruvate to enable complete degradation of the molecule to CO_2. In spite of many studies of the pathways and fluxes that contribute to glutamate turnover and glutamatergic neurotransmission, the energetics of astrocytes has been a long-standing controversial issue because models describing astrocyte–neuron interactions and metabolite trafficking during excitatory neurotransmission predict that different pathways supply the ATP required by astrocytes and neurons. This review will briefly highlight studies of glutamate metabolism and evidence for glutamate oxidation as an astrocytic energy source, then contrast these findings with those of lactate shuttle models.

2. Glutamate is an important astrocytic energy source

2.1. Compartmentation of glutamate synthesis and degradation in astrocytes

Investigation of the in vivo labeling patterns of amino acids derived from various labeled precursors in the 1960–1970s led to the discovery that there is compartmentation of oxidative metabolism in brain, characterized by a large, 'energy' TCA cycle, and a smaller, 'synthetic' TCA cycle. Data obtained from many studies led to the conclusion that (i) the anaplerotic pathway for de novo synthesis of glutamate and glutamine from glucose resided in astrocytes and (ii) interconversion of labeled glutamate and glutamine reflects, in large part, excitatory neurotransmission (Balázs and Cremer, 1972; Berl et al., 1975). Assignment of the small, synthetic TCA cycle to astrocytes was confirmed by their high enrichment with the enzymes required for glutamine synthesis and de novo synthesis of glutamate from glucose, i.e., glutamine synthetase (Martinez-Hernandez et al., 1977) and pyruvate carboxylase (Yu et al., 1983; Shank et al., 1985), respectively. GABA turnover also involves these anaplerotic reactions and oxidative degradation in the astrocytic TCA cycle (Hertz, 1979; Schousboe et al., 1992; Schousboe and Waagepetersen, 2007), but these reactions are not included in the present discussion.

Many laboratories have demonstrated that exogenous glutamate had two major fates after its uptake into astrocytes, oxidation or conversion to glutamine, with the proportion metabolized by each pathway being concentration dependent. Uptake and metabolism of exogenous [^{14}C, ^{13}C, or ^{15}N]glutamate by brain slices and cultured astrocytes is associated with label incorporation into CO_2, aspartate, glutamine, and other compounds (Benjamin and Quastel, 1972, 1974; Schousboe et al., 1977; Yu et al., 1982; Waniewski and Martin, 1986; Yudkoff et al., 1986; Farinelli and Nicklas, 1992; Sonnewald et al., 1993). The higher the extracellular glutamate level the greater the fraction oxidized, with about half being oxidized at 0.5 mmol/L glutamate (McKenna et al., 1996). Because astrocytes have much greater glutamate oxidative rates than GABAergic neurons and the corresponding rates in glutamatergic neurons were negligible, glutamate degradation is predominantly astrocytic (Hertz et al., 1988; Waagepetersen et al., 2002). The conclusion that glutamate is an important energy substrate for astrocytes is strongly supported by (i) the glucose-sparing actions of extracellular glutamate in cultured astrocytes (Swanson et al., 1990; Yu et al., 1992; Peng et al., 2001; Qu et al., 2001) and in isolated, intact hippocampus from adult mice (Dunlop et al., 1984), (ii) robust stimulation of astrocytic respiration by glutamate (Eriksson et al., 1995), and (iii) similar rates of astrocytic oxygen consumption with either glucose or glutamate as sole substrate (Hertz and Hertz, 2003). Under steady state conditions, oxidation of glutamate and GABA approximates the anaplerotic rate, which is ~15% of the total pyruvate oxidation rate (Hertz, 2011; Rothman et al., 2011), and glutamate synthesis and degradation in astrocytes produces nearly as much ATP as direct oxidation of glucose (Hertz et al., 1999, 2007).

2.2. Glutamate oxidation can fuel glutamate uptake in astrocytes

Peng et al. (2001) tested the hypothesis that oxidation of exogenous glutamate provides the ATP required for Na^+ extrusion and demonstrated that (i) extracellular glutamate did not increase glucose utilization even though it inhibited glucose oxidation, (ii) treatment of astrocytes with D-aspartate, a transportable but non-metabolizable glutamate analog, did stimulate glucose utilization, and (iii) monensin, an ionophore that stimulates Na^+–K^+-ATPase activity, increased glucose utilization. These findings are consistent with studies in isolated, intact hippocampus from adult mice showing that extracellular glutamate was oxidized in greater amounts with increasing concentration, and glutamate reduced oxidation of glucose (Dunlop et al., 1984). Recently, the glutamate transporter was shown to form multi-enzyme complexes with glycolytic enzymes and mitochondria that facilitate oxidation of very low levels (8 μmol/L) of glutamate as it is transported into the astrocytes (Genda et al., 2011; Bauer et al., 2012).

Some, but not all, perisynaptic astrocytic processes in adult rodent brain contain mitochondria (Lovatt et al., 2007; Lavialle et al., 2011; Pardo et al., 2011) and these oxidation-competent filopodial structures are capable of metabolism of glutamate to support the energetics of its uptake. On the other hand, perisynaptic processes without mitochondria would depend on glycolysis. Because glutamate concentration in the synaptic cleft reaches millimolar levels (Bergles et al., 1999; Matsui et al., 2005) it is likely that a substantial fraction of the transmitter glutamate may be oxidized (McKenna et al., 1996) and provide ATP to help fuel glutamate uptake in oxidation-competent filopodia. Mitochondria are essential for both de novo glutamate synthesis from glucose and for its oxidative degradation, and it would, therefore, be of great interest to determine if mitochondria-containing perisynaptic filopodia preferentially surround synapses utilizing glutamate and GABA as neurotransmitters. The proportion and localization of perisynaptic filopodia endowed with mitochondria is a central issue in understanding astrocytic energetics of neurotransmission.

2.3. Does glutamate oxidation support dynamic mobility of astrocytic processes during neurotransmission?

Because astrocytic glycogenolysis and oxidative metabolism, not just glycolysis, rise during brain activation in vivo (Hertz et al., 2007; Dienel, 2012b), other unidentified energy-requiring processes may also be stimulated by excitatory neuronal signaling, not only the expense of Na^+ extrusion and glutamine synthesis. For example, filopodial and lamellipodial processes are specialized astrocytic structures that are enriched with specific proteins and are highly mobile; they spontaneously advance towards and retract from active synaptic terminals in brain slices (Hirrlinger et al., 2004; Reichenbach et al., 2010; Derouiche et al., 2012). Although the mechanisms and energetics of filopodial movements are poorly understood, glutamate induces formation of actin-containing filopodia in cultured astrocytes (Cornell-Bell et al., 1990), and actin is present in the fine peripheral astrocytic processes (Derouiche and Frotscher, 2001). These observations raise the question whether the ATP demands associated with actin dynamics (Chen et al., 2000; Bernstein and Bamburg, 2003; Carlier et al., 2003) contribute to the energetics of glutamatergic signaling and mobility of astrocytic processes in vivo. This issue underscores

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