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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.

Keywords

astrocyte; brain activation; glucose; glutamate; lactate; neuron

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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 of the 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 CO₂ 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 astrocytes and involves entry of α -ketoglutarate into the TCA cycle, 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 CO₂. 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 [¹⁴C, ¹³C, or ¹⁵N]glutamate by brain slices and cultured astrocytes is associated

with label incorporation into CO₂, 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; Hertz et al., 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; Lavielle 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 the importance of better knowledge of filopodial mitochondrial localization and their association with mobility.

2.4. Differential turnover of glutamate pools in brain in vivo

Berl and Frigyesi (1969) labeled brain amino acid pools in cat brain with [1-¹⁴C]acetate, and measured clearance of label from glutamate, glutamine, aspartate and GABA. They observed similar biphasic decay curves for all four amino acids, with fast components having half-lives of 11–18 min and slow components with half lives of 2.5–4h. The similar decay rates were interpreted to imply a close association for the overall metabolism of the four amino acids via the TCA cycle (Berl and Frigyesi, 1969). These results are in agreement with an earlier study in cats showing that one pool of glutamate had a half life of minutes, the other hours (Berl et al., 1962), and with subsequent studies in rat brain in vivo and in brain slices that identified pools of glutamate with very different sizes and turnover rates (Badar-Goffer et al., 1992; Shank et al., 1993; Kauppinen et al., 1994; Lukkarinen et al., 1997). The rapidly-turning over components of the TCA cycle-derived amino acid pools may be associated with neurotransmission, whereas the long half-lived components may perhaps contribute to glutamate and glutamine dilutions observed in magnetic resonance spectroscopic studies. The fractional enrichment of glutamate and glutamine C4 never reaches the theoretical maximum of half that of [1-¹³C- or 6-¹³C]glucose, and dilution of these pools is ascribed to lactate and glutamine exchange, respectively (Rothman et al., 2011). Lactate exchange would reduce brain lactate specific activity due to efflux of labeled lactate to blood coupled with influx of unlabeled lactate to maintain the steady state mass of lactate in brain. However, with relatively short (5–30 min) labeling assays, the lactate specific activity was close to theoretical maxim in awake rat brain during rest, sensory stimulation, and recovery from stimulation and during spreading depression (Dienel and Cruz, 2009), and lack of label dilution is in good agreement with findings in awake mice (Van den Berg et al., 1969) and in brain slices (Badar-Goffer et al., 1992; Kauppinen et al., 1994). These data contrast the finding of lactate dilution at the end of 300 min infusion assays in anesthetized rats (Duarte et al., 2011). The anatomical sites of rapid amino acid turnover and actual causes of glutamate and glutamine dilutions remain to be directly established, but degradation of the short half-lived fraction of the transmitter-related amino acids may be linked to astrocytic energetics of neurotransmission.

2.5. Selective inhibition of the astrocytic TCA cycle

Fluoroacetate and fluorocitrate selectively block the astrocytic TCA cycle at the aconitase step (Fonnum et al., 1997), and these gliotoxins are useful tools for analysis of astrocytic metabolism and energetics. For example, fluoroacetate and fluorocitrate (1 mmol/L in slices or 2 mg/kg, i.p. in vivo) strongly inhibited incorporation of label from [¹⁴C]glutamate, [¹⁴C]aspartate, [¹⁴C]acetate, and [¹⁴C]GABA into glutamine in brain slices and mouse brain in vivo (Clarke et al., 1970). Because these drugs did not inhibit purified glutamine synthetase and the slices were incubated in high glucose, the authors suggested that the ATP

required for glutamine synthesis from [^{14}C]glutamate is probably provided by the astrocytic TCA cycle, consistent with conclusions drawn from metabolism of exogenous glutamate discussed above, i.e., glutamate oxidation may help pay for its uptake.

Treatment of mice with a higher dose of fluoroacetate (100 mg/kg, i.p.) blocked labeling of glutamine by [1,2- ^{13}C]acetate, reduced labeling of glutamine C4 from [1- ^{13}C]glucose, and increased the C3/C4 ratio (a measure of TCA cycling) in glutamine compared with glutamate (Hassel et al., 1997). These findings were explained by inhibition of the astrocytic TCA cycle, exchange-mediated labeling of [^{13}C]glutamate from [1- ^{13}C]glucose in neurons, and neuronal release of [^{13}C]glutamate, followed by its astrocytic uptake and conversion to [^{13}C]glutamine. The high cycling ratio of labeled glutamine was interpreted in terms of association of the [^{13}C]glutamate with a (neuronal) TCA cycle that turned over faster than the overall brain TCA cycle, in agreement with faster turnover of transmitter glutamate compared with the metabolic pool of glutamate (see (Hassel et al., 1997) and references cited therein). Faster turnover of the neurotransmitter glutamate pool supports the conclusion that oxidation of some glutamate taken up by astrocytes provides energy to fuel its uptake in vivo.

2.6. Glutamate oxidation via a truncated TCA cycle

Incomplete astrocytic oxidation of glutamate was revealed by incorporation of label from exogenous glutamate into aspartate (Benjamin and Quastel, 1972, 1974; Schousboe et al., 1977; Yu et al., 1982; Sonnewald et al., 1993), i.e., there is sequential conversion of the 5-carbon glutamate to α -ketoglutarate, oxaloacetate, and aspartate, with preservation of the 4-carbon skeleton. Qu et al. (2001) extended these findings by showing that addition of 0.5 mmol/L glutamate to the incubation medium of cerebellar astrocytes reduced the amount of [U- ^{13}C]glucose consumed, decreased the flux through the pyruvate carboxylase step, and increased the amount of aspartate. Unlabeled glutamate also increased synthesis of labeled glutamate derived from the first and second turns of the TCA cycle. Thus, glutamate substituted for glucose as an energy substrate, it spared glucose use by the anaplerotic pathway, it increased synthesis of glutamate and aspartate, and it revealed compartmentation of intracellular oxaloacetate pools in astrocytes (Qu et al., 2001). Conversion of glutamate to oxaloacetate increases the capacity of the TCA cycle (i.e., the amount of TCA cycle intermediates) without requiring pyruvate carboxylase, and shunting of glutamate through the TCA cycle with regeneration of glutamate produces substantial quantities of ATP.

2.7. Astrocytic energetics and glutamate oxidation

Depolarization-evoked release of neuronal K^+ to extracellular fluid is followed by synaptic vesicle fusion and release of glutamate from excitatory neurons into the synaptic cleft. Increases in extracellular [K^+] within the range of 3–12 mmol/L stimulate glycogenolysis in brain slices in a concentration-dependent manner (Hof et al., 1988), and K^+ uptake into cultured astrocytes mediated by both Na^+, K^+ -ATPase and $\text{Na}^+, \text{K}^+, \text{Cl}^-$ cotransporter1 (NKCC1) is blocked by inhibition of glycogen phosphorylase even in the presence of glucose (Xu et al., 2012). K^+ transport is fueled by glycogen, whereas glutamate uptake is not (Magistretti et al., 1981). Astrocytic K^+ uptake also causes intracellular pH and glutamine content to increase (Brookes and Turner, 1993, 1994), and alkalinization will stimulate glycolysis due to the high pH sensitivity of phosphofructokinase (Trivedi and Danforth, 1966; Halperin et al., 1969), the major rate-limiting enzyme of the glycolytic pathway (Passonneau and Lowry, 1964). Small increases in extracellular [K^+] in hippocampal slice preparations also stimulate glycolysis-dependent protein synthesis which does not occur when pyruvate replaces glucose and it is not related to differences in ATP concentration (Lipton and Robacker, 1983). Thus, both K^+ and glutamate are cleared from

extracellular fluid by uptake into astrocytes and they cause metabolic activation of different pathways in astrocytes.

Glutamate taken up into an astrocyte can be either directly converted to glutamine or it can enter into the TCA cycle. For simplicity, the energetic balance sheet illustrated in Fig. 1 is based on uptake of 2 glutamate molecules, one entering each of these pathways. The carbon skeleton of glutamate entering the TCA cycle can also have two fates, shunting through the TCA cycle (i.e., entry, oxidation, and regeneration) or complete degradation. Entry of one glutamate into the truncated TCA cycle generates 7.5 ATP when it is converted to oxaloacetate (this is lower than theoretical ATP yield is due to proton leakage, see (Hertz et al., 2007)), thereby providing an excess of energy compared with the 3 ATP needed to synthesize glutamine from the second glutamate and extrude the Na^+ taken up with the 2 glutamate molecules (Fig. 1). Regeneration of glutamate from this oxaloacetate requires oxidation of one pyruvate without the need for pyruvate carboxylase activity, and passage of the molecule through the rest of the TCA cycle generates an additional 5 ATP (Fig. 1) and completes the process of shunting of glutamate through the TCA cycle. The net ATP yield when two glutamate are converted to two glutamine (one directly and one indirectly via shunting through the TCA cycle) is 9.5 ATP, with consumption of 0.5 molecules of glucose (Fig. 1). When glutamate is completely oxidized via the pyruvate re-cycling pathway (Cerdan et al., 1990), malate exits from the TCA cycle (arrow from TCA cycle to pyruvate, Fig. 1) and is converted to pyruvate in the cytoplasm, followed by pyruvate oxidation, for a yield of 20 ATP per glutamate molecule (Fig. 1; for details, see (Hertz et al., 2007)). Due to the large amount of ATP produced by glutamate metabolism, oxidation of only ~10–20% (depending on shunting or complete oxidation) of one incoming neurotransmitter glutamate molecule would match the energy demands of 2 ATP for Na^+ extrusion and conversion to glutamine. Furthermore, glutamate oxidation is glucose-sparing, as is glycogenolysis (Dinuzzo et al., 2012), thereby freeing up endogenous glucose for use by nearby neurons, for astrocytic glycogen turnover, or other astrocytic activities. Increasing the flux through the glutamate shunt or complete glutamate degradation when glutamate levels are high can generate surplus of ATP for use by astrocytic activities that are upregulated during strong excitatory neurotransmission, such as dynamic movements of perisynaptic processes.

2.8. Compartmentation of glutamate-glutamine turnover arising from astrocytic gap junctional communication

After uptake into astrocytes from the synaptic cleft or generation within activated astrocytic processes, K^+ , Na^+ , NADH, NADPH, glucose, lactate, glutamate, glutamine, and other compounds (see discussion in (Gandhi et al., 2009b) and (Dienel, 2012b)) can diffuse via gap junctions to other regions of the same astrocyte and to other astrocytes (Fig. 1). Gap junctional trafficking can spread the workload of astrocytic activation within the large syncytium and raises the possibility that glutamate oxidation and glutamate biosynthesis may occur in different regions of a single astrocyte or in different astrocytes (Fig. 1). Thus, glutamate oxidation may take place in mitochondria-containing perisynaptic processes and de novo synthesis taking place in more distant processes of the same astrocyte or in different astrocytes. K^+ stimulates pyruvate carboxylase (Kaufman and Driscoll, 1992) and increases anaplerotic flux, which rises with brain activity (Öz et al., 2004). Depending on the extent of activation of CO_2 fixation and glutamate biosynthesis by K^+ , the concentrations of glutamate and glutamine may increase in other regions of the cell and compensate for net oxidation of glutamate in perisynaptic processes. Diffusion of metabolites and compounds related to neurotransmitter cycling among coupled astrocytes increases the complexity of modeling and measuring interactions between immediately-adjacent processes of astrocytes and neurons.

A net change in total tissue glutamate level need not, however, arise solely from its turnover; it could also be due to interconversion of amino acids via transaminase reactions. For example, total glutamate concentration increases and aspartate level falls during sensory stimulation of awake rats (Dienel et al., 2002) and humans (Mangia et al., 2007; Lin et al., 2012). Mangia et al. (2007) attributed the concentration changes to increased malate-aspartate shuttle activity, assuming that the mitochondrial glutamate-aspartate antiporter is rate limiting. The malate-aspartate shuttle transfers reducing equivalents from cytoplasmic NADH to mitochondrial NADH and it is required for the formation of pyruvate as an oxidative substrate. When this redox shuttle becomes limiting cytoplasmic NAD⁺ is regenerated by the action of lactate dehydrogenase, and lactate will be produced in increased amounts. Determination of the capacity and regulation of the malate-aspartate shuttle is very important because it can help explain the rise in lactate production during brain activation. The malate-aspartate shuttle is stimulated by low cytoplasmic calcium levels and inhibited by greater influx of calcium into mitochondria, indicating that neuronal calcium dynamics during brain activation may be involved in regulation of neuronal lactate production (Contreras et al., 2007; Satrustegui et al., 2007; Bak et al., 2009; Contreras and Satrustegui, 2009; Bak et al., 2012).

2.9. Changes in glutamate metabolism may contribute to the CNS pathophysiology of diabetes

Elucidation of the role of glutamate oxidation in energetics of brain activation is not only essential for understanding neuron-astrocyte interactions during normal brain function, it is also critical for evaluation of the pathophysiology of complex diseases that affect the eye and brain. For example, retinopathy is common in diabetic subjects who can also develop progressive cognitive and sensory-processing deficits (Kodl and Seaquist, 2008; Reijmer et al., 2010). The underlying causes of these acquired abnormalities remain to be established but they may, in part, involve glutamate turnover. In retina of diabetic rats, glutamate levels are elevated and conversion of glutamate to glutamine is reduced (Lieth et al., 1998) due to (i) impaired glutamate oxidation, probably arising from inhibition of glutamate transamination by branched chain amino acids and (ii) decreased activity and content of glutamine synthetase, suggesting that abnormal glutamate metabolism in retinal Müller cells may contribute to retinopathy via glutamate excitotoxicity (Lieth et al., 2000; Gowda et al., 2011). The glutamate transporter GLAST is also dysfunctional in retinal Müller cells, presumably due to oxidative damage (Li and Puro, 2002), and glutamate uptake is increased in glial plasmalemmal vesicles isolated from diabetic brain regions, whereas glutamate transporter levels are not altered in various brain structures (Coleman et al., 2004; Coleman et al., 2010). Magnetic resonance spectroscopic (MRS) studies have also identified changes in metabolic fluxes in brain of diabetic rats. Incorporation of label from [1-¹³C]glucose into glutamate, glutamine, and GABA is reduced, whereas incorporation of ¹³C from [1,2-¹³C₂]acetate into these compounds increased (Garcia-Espinosa et al., 2003). Regional abnormalities in glycogen and amino acid homeostasis and reduced glutamate-glutamine cycling are also evident in diabetic rat brain (Sickmann et al., 2010; Sickmann et al., 2012). Diabetes and exposure to amyloid-beta, which is sometimes elevated in experimental diabetes, also impair astrocytic gap junctional communication with a time course that lags onset of oxidative stress without involvement of endoplasmic reticulum stress (Cruz et al., 2010; Gandhi et al., 2010; Ball et al., 2011; Lind et al., 2013). Thus, diabetes causes metabolic disturbances in neuronal and astrocytic pathways related to energy metabolism, glucose storage, and excitatory and inhibitory neurotransmission.

In addition to abnormal metabolism during diabetes and chronic hyperglycemia, recurrent bouts of acute, insulin-induced hypoglycemia disrupt the metabolic and functional integrity of brain cells in diabetic subjects (Amaral, 2012). Oxidation of endogenous metabolites is a

compensatory mechanism for an inadequate supply of glucose, and insulin-induced hypoglycemia depletes brain levels of glycolytic and TCA cycle intermediates, glutamate, glutamine, GABA, alanine, and phospholipids, and increases levels of aspartate and ammonia (Siesjö, 1978). These findings are consistent with the faster decline in the rate of glucose utilization compared with oxygen consumption after onset of hypoglycemia and the slower recovery of oxygen utilization rate after glucose levels are restored because glucose is used for energy and also for de novo synthesis of the compounds consumed during the hypoglycemic episode (Ghajar et al., 1982). Thus, both hyperglycemia and hypoglycemia have a high impact on many pathways involved in glutamatergic and GABAergic neurotransmission in diabetes.

2.10. Summary

Glutamate synthesis and degradation are critical functions of astrocytes required for neurotransmission. Exogenous glutamate can be completely degraded or shunted through the TCA cycle with regeneration of glutamate. The high ATP yield of glutamate oxidation, the glucose-sparing effects of glutamate, and lack of stimulation of glucose utilization by extracellular glutamate in many studies support the conclusion that astrocytic energetics are supported, at least in part, by oxidation of neurotransmitter glutamate. The advantage of glutamate as an energy source is that it is supplied to activated perisynaptic domains of astrocytes at the site where fuel is needed simultaneously with the onset of increased ATP demand. Glutamate oxidation can buffer glucose use in astrocytes and make more glucose available for neurons. Diabetes and other disease states that affect glutamate-glutamine turnover can disrupt brain function.

2.11. Outstanding questions for future work

Critical issues include localization of mitochondria in perisynaptic filopodia that surround glutamatergic synapses, energetic contributions to astrocytic functions during brain activation of glutamate oxidation compared with glycolysis, glycogenolysis, and glucose oxidation, filopodial mobility related to excitatory synaptic activity, compartmentation of glutamate-glutamine turnover within and among astrocytes, roles of the malate-aspartate shuttle in lactate formation, and involvement of glutamate metabolism pathways in the pathophysiology of diabetes.

3. Astrocytic energetics, glycolysis, and lactate shuttling

3.1. Models for energetics of excitatory neurotransmission

Oxidation of glutamate by astrocytes has been firmly established by work carried out during the past four decades in many laboratories. However, glutamate oxidation and its ATP yield have not yet been explicitly incorporated into models for astrocytic energetics and glutamate-glutamine cycling based on tissue culture experiments, in vivo ¹³C-MRS metabolic studies, and lactate-loading assays (i.e., experiments in which the lactate concentration greatly exceeds normal levels observed during brain activation, i.e., about 2 μmol/g tissue, by either putting large amounts of lactate into the incubation medium or infusing large amounts of lactate into subjects to raise blood and brain lactate levels) (Magistretti et al., 1999; Hyder et al., 2006; Rothman et al., 2011; Pellerin and Magistretti, 2012). Instead, strong emphasis has been placed on ATP derived from astrocytic glycolysis accompanied by transfer of lactate from astrocytes to neurons as a major oxidative fuel (Jolivet et al., 2010; Pellerin and Magistretti, 2012). The notion of lactate trafficking has diverted attention from experimental examination of important issues related to oxidative metabolism in astrocytes (see section 2, above) and of neuronal glucose utilization, and strengths and limitations of evidence for metabolic models need to be re-evaluated.

3.2. Astrocyte-to-neuron lactate (ANL) transfer-oxidation model

Based on their observation that treatment of cultured astrocytes with glutamate stimulated glycolysis and release of lactate, Pellerin and Magistretti (1994) proposed a model for coupling of glucose utilization with neuronal activity. The model *assigns* the 2 ATP generated by glutamate-evoked glycolysis to satisfy the astrocytic energetic demands of glutamate-glutamine cycling, i.e., one to fuel Na^+, K^+ -ATPase to extrude the Na^+ taken up along with glutamate and one to convert glutamate to glutamine. In addition, the lactate released to the culture medium was stated to be taken up and oxidized by nearby neurons *in vivo*, serving as a major fuel during excitatory neurotransmission. This model mandates glycolytic glucose consumption in perisynaptic astrocytic processes and lactate oxidation in nearby neurons, in sharp contrast with astrocytic glutamate oxidation to fuel Na^+ extrusion and glutamine synthesis. The astrocytic energy balance arising from uptake of 2 glutamate and their conversion to glutamine consumes 2 glucose and produces 4 ATP, with release of 4 lactate (glycolytic compartment, Fig. 1).

In recent reviews, a small number of selected studies were cited in support of the ANL transport-oxidation model (Jolivet et al., 2010; Pellerin and Magistretti, 2012). However, studies in many laboratories during the past 40 years that were not cited in the above reviews clearly demonstrate that cultured neurons and synaptosomes isolated from adult brain are capable of substantially increasing glucose uptake, glycolysis, and glucose oxidation (Dienel, 2012a). Furthermore, critical aspects of ANL transport, including the cellular origin of lactate produced during activation and the direction and magnitude of lactate shuttling have not been directly established in brain of normal awake subjects. An alternative model, the redox shuttle proposed by Cerdan and colleagues, provides a different mechanism to have high rates of glycolysis in astrocytes without net transfer of lactate to neurons; in this model astrocyte-derived lactate is oxidized by neurons to generate NADH that is oxidized by the neurons, and the resulting pyruvate is released to extracellular fluid where it can cycle back to astrocytes for oxidation (Cerdan et al., 2006). Lactate production during activation is generally assumed to be astrocytic, but this remains to be proven *in vivo*; it could be astrocytic, neuronal, or both.

3.3. Neuron-to-astrocyte lactate transfer

Predicted transport and pathway flux rates and directions depend on model assumptions, and a model that uses different assumptions and accounts for different kinetics of the neuronal and astrocytic glucose transporters predicts that neurons metabolize most of the glucose consumed during brain activation and that lactate is generated in neurons and transferred to astrocytes (Mangia et al., 2011). Experimental evidence that considerable lactate production during activation may be neuronal (Ueda and Ikemoto, 2007; Caesar et al., 2008; Contreras and Satrustegui, 2009; Ivannikov et al., 2010; Bak et al., 2012) and that neurons and astrocytes can oxidize both glucose and lactate (Zielke et al., 2007, 2009) has led to serious challenges of the validity of the ANL transport-oxidation model, a synopsis of which is presented below.

3.4. Glutamate-stimulated glycolysis is not a robust phenotype of astrocyte cultures

Glutamate-stimulated glucose utilization and lactate release occurs in astrocyte preparations of the Pellerin-Magistretti group (Pellerin and Magistretti, 1994; Debernardi et al., 1999; Chatton et al., 2003) and Takahashi et al. (1995). However, more laboratories have reported that glutamate treatment caused either no change or reduced glucose consumption (Dunlop et al., 1984; Swanson et al., 1990; Hertz et al., 1998; Peng et al., 2001; Qu et al., 2001; Liao and Chen, 2003; Dienel and Cruz, 2004) and no change or diminished lactate release (Dunlop et al., 1984; Gramsbergen et al., 2003; Liao and Chen, 2003; Dienel and Cruz, 2004). The basis for these phenotypic differences has never been established, but it probably

lies within the culturing procedures (Hertz et al., 1998). The fact that some astrocyte preparations exhibit glutamate-induced glycolysis whereas others do not demonstrates that the cornerstone of the ANL shuttle is not established.

3.5. Astrocytes upregulate oxidative pathways fluxes during brain activation

Assignment of glycolytic ATP to fuel sodium extrusion and glutamine synthesis is reasonable if no other energy-providing fluxes are increased. However, in vivo studies in awake subjects under activating conditions have shown that astrocytes upregulate oxidative metabolism (Dienel et al., 2001; Dienel et al., 2003; Cruz et al., 2005; Dienel et al., 2007b; Wyss et al., 2009; Wyss et al., 2011) and glycogenolysis (Swanson et al., 1992; Madsen et al., 1999; Cruz and Dienel, 2002; Dienel et al., 2007a). Thus, astrocytic ATP is generated by metabolism of endogenous (glutamate and glycogen) and exogenous (glucose and acetate) substrates, and the source(s) of ATP actually used to extrude Na^+ and synthesize glutamine remain to be directly identified. By 1994 when the ANL transport-oxidation model was first proposed, there was already a large literature demonstrating oxidation of exogenous glutamate by astrocytes, and studies since 1994 support the conclusion that glutamate oxidation can help fuel its uptake, as proposed by Peng et al. (2001).

3.6. Assays of the oxidative pathways greatly underestimate total glucose utilization

Astrocytic lactate production coupled with lactate oxidation in nearby neurons requires proportionate increases in total glucose utilization and oxidative metabolism of glucose and oxygen consumption. However, many studies carried out during the past 30 years reported that the magnitude of upregulation of the rates of the oxidative pathways assayed with [1- or 6- ^{14}C]glucose is ~50% or less of the rate of total glucose utilization assayed in parallel with [^{14}C]deoxyglucose (DG, which measures the hexokinase reaction and total glucose utilization) in awake rodents during normal and pathophysiological states (Dienel, 2012b). These findings indicate that substantial quantities of products of [^{14}C]glucose metabolism are not retained in activated tissue, even during brief 5–10 min assays. Any release of labeled lactate from activated tissue causes a proportionate underestimation of CMR_{glc} when assayed with [^{14}C]glucose. Similar findings would be anticipated for MRS studies of activation using [^{13}C]glucose.

3.7. Lactate release model: lactate release from activated brain is fast and facilitated by astrocytes

Direct assays of lactate release from brain during spreading cortical depression showed that labeled lactate was detected in cerebral venous blood within 2 min after pulse labeling with [6- ^{14}C]glucose and by 4–8 min, an approximate steady state was attained in which efflux of both labeled and unlabeled lactate accounted for ~20% of the labeled or unlabeled glucose entering the brain (Cruz et al., 1999). Because this accounted for only about half of the magnitude of underestimation of CMR_{glc} with labeled glucose our laboratory undertook a series of in vivo studies to establish the routes for release of ^{14}C -labeled metabolites during activation. Major findings reviewed in (Dienel, 2012b) include the following: (i) significant label is lost from [1- ^{14}C]glucose via the pentose phosphate shunt pathway (e.g., 25% of glucose oxidized in the inferior colliculus during acoustic stimulation; calculated from data of (Cruz et al., 2007) and illustrated in Fig. 6C,c-1 of (Dienel, 2012b)), (ii) most locally-generated lactate is not locally oxidized (Ball et al., 2010), (iii) label dispersal within activated tissue is reduced by inhibition of lactate transporters and gap junctions (Cruz et al., 2007), (iv) astrocytes have a 2–4-fold higher rate and capacity for uptake of lactate from extracellular fluid and for shuttling among gap junction-coupled astrocytes compared with neuronal lactate uptake and lactate shuttling to neurons (i.e., astrocytes are poised to take up, disperse, and discharge lactate over a wide range of lactate levels, ranging from 2–40 mmol/L) (Gandhi et al., 2009a), and (v) label derived from microinfused ^{14}C -labeled-lactate or

glucose is recovered in meninges, indicating that labeled metabolites are also released via lymphatic drainage systems in addition to blood (Ball et al., 2010) and that assays of arteriovenous differences underestimate metabolite release from brain. Furthermore, labeled metabolites of [6-¹⁴C]glucose are retained in greater quantities if the polarized localization of aquaporin 4 at astrocytic endfeet is disrupted, consistent with lactate release from endfeet in association with endfoot water movement (Cruz et al., 2013). Continuous lactate release into the perivascular-lymphatic drainage system may, in fact, be important for widespread stimulation of blood flow (Laptook et al., 1988; Hein et al., 2006; Yamanishi et al., 2006; Gordon et al., 2008). Together, the above studies demonstrate that most lactate generated from blood-borne glucose within brain is quickly released, not locally oxidized. These in vivo findings in normal awake rats strongly support the predominant lactate release model (Gandhi et al., 2009a; Dienel, 2012b) and demonstrate that the second cornerstone of the ANL transport-oxidation model, local oxidation of lactate within the brain as major neuronal fuel, is not established.

3.8. Astrocytic gap junctional communication reduces the focal energetic burden of glutamate uptake

Pictorial representations of astrocyte-neuron interactions related to the ANL transport-oxidation model (Pellerin and Magistretti, 2012) portray a tight linkage between glutamate cycling, Na⁺ extrusion, and glutamine synthesis within immediately-adjacent neuronal and astrocytic structures, with the implication that these cellular elements form a 'closed' temporal-spatial domain. However, it is well established that astrocytes are highly coupled to each other, and extracellular glutamate and K⁺ increase gap junctional communication (Enkvist and McCarthy, 1994; De Pina-Benabou et al., 2001), thereby enhancing trans-astrocytic fluxes of Na⁺ (Langer et al., 2012), glucose, lactate, NADH, and NADPH (Gandhi et al., 2009b), and probably glutamate and glutamine, during excitatory neurotransmission (Fig. 1). The astrocytic syncytium that can be comprised of as many as ten thousand coupled cells (Ball et al., 2007) is an 'open system', and the energetic burden of glutamate uptake, Na⁺ extrusion, and glutamine synthesis is, therefore, not restricted to perisynaptic processes. Glutamate, K⁺, and Na⁺ can be dispersed among coupled cells to minimize their accumulation at active sites, and the workload can be shared.

3.9. Discordant contributions of lactate to total oxidation in vitro and in vivo

Based on results of tissue culture and lactate loading studies, lactate is claimed to be the 'preferred' neuronal substrate compared with glucose (Jolivet et al., 2010; Pellerin and Magistretti, 2012). However, lactate oxidation by cultured neurons in studies by the Pellerin-Magistretti group greatly exceeds that in normal adult human brain, particularly in 'lactate-loading' assays. At levels of lactate achieved in brain during activation (<2–3 mmol/L), lactate contributes a small fraction (<8%) to total oxidation in human brain, sharply contrasting the 45–75% in neurons cultured from embryonic rats (Fig 2). Lactate metabolism in these cultured neurons is not equivalent to that in adult brain, even at low lactate levels. Lactate is a supplemental brain fuel that spares glucose when its blood concentrations are high (Fig. 2), but the physiology of lactate utilization during lactate loading and muscular work is not the same as in sedentary subjects with low plasma and brain lactate levels (Dienel, 2012a, b). Lactate transport is passive and follows concentration gradients. Lactate influx into cells with a proton can cause intracellular acidification and depletion of NAD⁺, thereby inhibiting glycolysis by biochemical regulatory mechanisms expected for a glucose-sparing alternative substrate.

3.10. Glycogenolysis rates that sustain neuronal function in the absence of glucose are very low

Astrocytic glycogen maintains neuronal function during severe hypoglycemia or aglycemia, and some investigators (Wender et al., 2000; Brown et al., 2005; Brown et al., 2012) claim that these findings provide proof of principle that lactate shuttling is important for *normal* neuronal function. This notion was evaluated by calculating overall rates of glycogenolysis during glucose deprivation as the difference in glycogen content (expressed as μmol glucosyl units/g) at the onset and endpoint of the experimental interval divided by experimental duration. These values are compared to in vivo CMR_{glc} and $\text{CMR}_{\text{glycogen}}$ in normal or ischemic tissue (Fig. 3A). CMR_{glc} varies regionally (Sokoloff et al., 1977) (Fig. 3A, gray bars), and it is much lower in brain slices due to deafferentation and postischemic recovery (gray hatched bar, hippocampus). Glycogenolysis rate in resting rodent cerebral cortex is $0.01 \mu\text{mol/g/min}$ (Watanabe and Passonneau, 1973), or about $\sim 1\%$ of cerebral cortical CMR_{glc} (Fig. 3A). During forebrain ischemia, glucose utilization increases by as much as 6-fold (diagonal-lined bars), and glycogenolysis rises as much as 200-fold, reaching $2 \mu\text{mol/g/min}$ (vertical-lined bars); ischemic parietal cortex and hippocampus also have large increases in CMR_{glc} and $\text{CMR}_{\text{glycogen}}$. These utilization rates during ischemia were calculated from substrate disappearance during the initial 30–60 seconds of ischemia when endogenous compounds were consumed at high rates.

In sharp contrast to responses to ischemia, glycogenolysis rates that preserve brain or nerve function during severe hypoglycemia or aglycemia are extremely low, ranging from about 0.01 to $0.06 \mu\text{mol/g/min}$ (black bars, Fig. 3A), corresponding to about <1 – 10% of resting CMR_{glc} in the respective structures. Glycogen use rates in isolated optic or sciatic nerve given electrical stimulation to evoke *maximal* compound action potentials in the absence of glucose (Wender et al., 2000; Brown et al., 2012) are much lower than $\text{CMR}_{\text{glycogen}}$ during ischemia in situ and they are also considerably less than CMR_{glc} in normal or ischemic nerve (Fig. 3A). This is also true for preservation of evoked potentials in hippocampal slices, even when pre-stimulation glycogen levels were elevated by supplementation of the pre-incubation medium with pyruvate to increase glycogen level over the control value (Shetty et al., 2012). K^+ -evoked glycogenolysis is linked to lactate shuttling and preservation of synaptic function (Choi et al., 2012), but here, too, the rate is very low (Fig. 3A). These severe glucose-deprivation paradigms are not likely to reflect the actual magnitude of glycogenolysis to functional energetics when glucose is available, and the contributions of neuronal metabolism of glucose to these functions remains to be established. Interpretation of glucose deprivation studies in terms of normal metabolism must be made with special care because survival of the cell or organism now becomes an issue. As an extreme example, cutting the arm off of a right-handed person will increase use of the left arm to carry out normal right-arm activities even though the left arm was normally rarely used for these tasks.

The specific roles of glycogen during brain activation are not well understood, but the magnitude of its turnover appears to be quite high. For example, when glycogen phosphorylase is inhibited during sensory stimulation of awake rats there are large, region-specific compensatory increases in utilization of blood-borne glucose. The net increase in CMR_{glc} during blockade of glycogenolysis in the most-activated structures (sensory cortex and layer 4 of sensory and parietal cortex) is about 0.4 – $0.5 \mu\text{mol/g/min}$ (Dienel et al., 2007a), which is more than 7–50 times greater than $\text{CMR}_{\text{glycogen}}$ in the above experiments in the absence of glucose (Fig 3A). Presumably, the compensatory rise in glucose utilization reflects glycogen turnover and glycogen consumption. In a separate study, we found that less than 1% of the $[6\text{-}^{14}\text{C}]$ glucose-derived label recovered in metabolites was in glycogen during rest, sensory stimulation, and recovery from stimulation (Dienel et al., 2002). This suggests that glucose label that passes through glycogen to the glycolytic pathway is

probably released from brain, since most of the activation-evoked increase in metabolite labeling by [^{14}C]glucose is not retained in brain by trapping in the large amino acid pools derived from the oxidative pathway (Dienel, 2012b). Thus, little, if any, glycogen-derived label is shuttled to neurons, in agreement with the very low glycogenolysis rates that preserve brain function in the absence of glucose.

The supplemental energy provided by glycogenolysis to help maintain EEG, synaptic potentials, and axonal conduction in the absence of glucose is functionally very important, particularly for diabetic patients. However, its low magnitude suggests that either these process may not require much ATP or that other endogenous substrates (e.g., amino acids) are also oxidized to provide most of the ATP required to maintain overall function. When interpreting the role of glycogen in sustaining neuronal function it is important to consider the following points. (i) Severe hypoglycemia and aglycemia do not occur in normal brain because compensatory mechanisms maintain plasma glucose levels and glucose delivery to brain, even during prolonged starvation (Owen et al., 1967). Glucose depletion does occur in diabetic patients who receive too much insulin, but this is abnormal. Nevertheless, the fact that glycogenolysis prolongs EEG (Fig. 3A) and reduces neuronal death during severe hypoglycemia (Suh et al., 2007) is very important for diabetic patients. (ii) ATP levels are maintained near-normal during graded hypoglycemia (Fig. 3B) due to oxidation of available endogenous substrates, including amino acids and TCA cycle intermediates (see section 2.9 and (Siesjö, 1978)), until a critical point at which energy failure abruptly ensues (Fig. 3B). Thus, even a very small energy source can prolong brain functions by helping maintain ATP production just above the point of collapse. (iii) The cells that use most of the glycogen-derived energy are not established. Use of glycogen by astrocytes to support K^+ homeostasis (Hof et al., 1988; Xu et al., 2012) would help maintain neuronal function, as would substrate oxidation by glia surrounding axons (Hargittai and Lieberman, 1991). Small fluxes can maintain function and prevent energy failure without making major contributions to energetics under normal conditions.

3.11. Summary

Two essential components of the ANL transfer system, glutamate-induced glycolysis and local oxidation of a substantial fraction of the lactate generated within activated tissue, are not sufficiently documented *in vivo* to validate the foundation of this model. Lactate may be produced by neurons, astrocytes, or both cell types. Energetics of astrocyte-neuron and astrocyte-astrocyte interactions are much more complex than portrayed by the model. Strong *in vivo* evidence supports rapid, substantial release of lactate from activated brain, suggesting that maintenance of high glycolytic flux is far more important than lactate shuttling coupled with its oxidation. These findings and the physiological responses to brain activation described in section 4 support the conclusion that lactate release predominates during brain activation and that normal brain does not need lactate as a supplemental fuel.

3.12. Outstanding questions for future work

Key issues related to lactate production and release during brain activation include identification of the processes that preferentially upregulate glycolysis, the cellular basis of lactate production, determination of the magnitude, direction, and time courses of lactate fluxes, identification of roles and metabolic fate of glycogen, and metabolic contributions of the astrocytic syncytium to local synaptic activity.

4. Glucose and oxygen supply exceed demand during brain activation

4.1. Fast onset of hemodynamic responses and surplus oxygen delivery

If glucose delivery to brain via arterial blood were limiting for brain metabolism during activation, it would be critical for brain cells to consume all available fuel. However, regulation of cerebral blood flow is described as a 'feed-forward' process because signals derived from neuronal activity also stimulate vasodilation and blood flow (Attwell et al., 2010). Measures of hemodynamic responses include vascular volume, blood flow rate, and a positive blood oxygen level-dependent (BOLD) signal that reflects increased oxygen content of venous blood, i.e., oxygen delivery exceeds its utilization (Ogawa et al., 1990; Ogawa, 2012). The onset of the hemodynamic response in anesthetized subjects is very fast, occurring within 0.75s in most studies (Fig. 4A). Fuel delivery to brain is not limited by a slow response of blood flow to activation.

4.2. Glucose delivery exceeds demand by a wide margin over a large range of glucose levels

Glucose influx into brain tissue exceeds its phosphorylation by ~60% over a 5-fold range of CMR_{glc} when plasma and brain glucose levels range from 4.9–12.6 $\mu\text{mol/mL}$ and 1.8–4.8 $\mu\text{mol/g}$, respectively (Fig. 4B). Because the K_m of hexokinase for glucose is ~0.05 $\mu\text{mol/mL}$ (Wilson, 2003), the enzyme is saturated and can operate at maximal velocity until brain glucose levels fall below ~0.5 $\mu\text{mol/g}$ (note: for simplicity, the water content (~80%) of brain tissue is ignored, and small concentration differences arise when converting in vitro concentrations to those per gram tissue). Rat brain glucose level is ~20% of that in arterial plasma and ranges from ~2–3 $\mu\text{mol/g}$ (Dienel et al., 1991). If CMR_{glc} abruptly doubles and rises from 1 to 2 $\mu\text{mol/g/min}$, then 1 $\mu\text{mol/g}$ would be consumed in one min. This leaves 1–2 $\mu\text{mol/g}$ in the brain precursor pool of unmetabolized glucose, even without the continuous supply of glucose from blood at a rate that matches the resting CMR_{glc} . Rapid blood flow upregulation and excess delivery of oxygen and glucose indicate that normal brain does not 'need' lactate as a supplemental fuel. Some extracellular lactate is probably consumed at stimulus onset, but analysis of available data indicates that this lactate makes a minor contribution to CMR_{glc} (Dienel, 2012a).

4.3. Stimulus-induced increases in glycolysis are highest in awake subjects

Anesthesia is required to avoid movement artifacts during some types (e.g., positron emission tomographic and MRS) of metabolic assays. However, stimulatory effects of activation are not equivalent in awake and anesthetized subjects, and pathways of fuel use may differ in the two conditions. Parallel studies in which glucose utilization was assayed during rest and activation using [^{14}C]DG or labeled glucose reveal that the resting basal rates are higher in awake subjects and the stimulus-induced rise in CMR_{glc} is generally much greater in awake compared with anesthetized subjects (Fig. 5). Furthermore, when assayed with [^{14}C]DG, the increases usually exceeded those determined with [^{13}C - or ^{14}C]glucose, consistent with disproportionate upregulation of glycolysis compared with glucose oxidation, particularly in awake subjects (Fig. 5). These findings suggest that suppression of brain functions by anesthesia simultaneously blunts the magnitude of cellular activities that activate the glycolytic pathway.

4.4. Increase in CMR_{O_2} sets an upper limit for the ANL transport-oxidation model

If all of the lactate produced in brain were locally oxidized, the rates of respiration or TCA cycle would rise in parallel with total glucose utilization. However, this is not the case because the percent rise in CMR_{O_2} during brain activation is relatively small, generally ~20% (Fig. 6). The increase in CMR_{O_2} establishes the upper limit for any lactate trafficking

linked to its local oxidation because if shuttled lactate were the *sole respiratory substrate* for neurons, then all oxygen consumption could be ascribed to lactate oxidation. However, neurons and astrocytes each phosphorylate ~50% of the glucose under resting conditions (Nehlig et al., 2004). If neurons directly oxidize the pyruvate generated from glucose they phosphorylate during activation (i.e., accounting for half of the rise in CMR_{O2}, or 10%) and astrocytes account for 20–30% of total oxidation (Hertz, 2011; Rothman et al., 2011), the *maximal* contribution of lactate shuttling to total oxidation is, on average, <10%. This fraction does not constitute a major fuel.

4.5. Summary

The conclusion that glucose is the major fuel for normal brain during activation of relatively sedentary subjects is strongly supported by the rapid increase in blood flow, glucose influx in excess of demand, disproportionate increases in glycolysis compared with oxidative metabolism, a small rise in CMR_{O2}, lack of substantial lactate accumulation in brain, and rapid, continuous lactate release that can contribute to upregulation of blood flow. Lactate is a supplemental fuel when blood levels rise during muscular activity and lactate infusions and during pathophysiological conditions. Use of lactate as a supplemental fuel varies with the physiology of the subject. Use of alternative substrates under special conditions does not mean that these compounds are normally major fuels.

4.6. Outstanding questions for future work

Major issues are how glucose and lactate are distributed within the brain during rest, activation, and intense physical activity, identification of processes dependent on glycolysis and those that cause increased production, elucidation of processes and pathways that are down regulated by anesthesia, functions fulfilled by oxidative metabolism and glycolysis, and the subcellular basis for glycolytic and oxidative metabolism in neurons and astrocytes.

5. Concluding comments

5.1. Glutamate vs. glycolysis to fuel astrocytic filopodial activities

Glutamate oxidation provides a simple mechanism to satisfy the temporal-spatial energy demands of mitochondria-containing perisynaptic astrocytes during excitatory neurotransmission. Exogenous glutamate is taken up and oxidized by cultured astrocytes immediately, and turnover of the neurotransmitter glutamate pool in vivo is faster than that of the metabolic pool. Glutamate can either be shunted through the TCA cycle and regenerated by consumption of 0.5 glucose, or it can be completely oxidized by the pyruvate re-cycling pathway. Oxidation of <20% of the transmitter glutamate taken up by either pathway is sufficient to satisfy the energy demands of its uptake, and oxidation of a larger fraction of the glutamate can support increased ATP demand for other activities during strong excitatory neurotransmission. Glutamate oxidation is glucose-sparing, freeing up glucose for use by neurons and by other processes in astrocytes, including glycogen turnover, and it does not rule out glycolytic upregulation in astrocytes and neurons. The conclusion that glutamate helps fuel the energetics of its uptake is consistent with results of many in vitro and in vivo studies, but more work is required to establish the quantitative contributions of neurotransmitter oxidation to astrocytic energetics in vivo. Labeling of neurotransmitter glutamate C4 in neurons by C1- or C6-labeled glucose followed by glutamate shunting through the astrocytic TCA cycle will cause formation of C2,4 and C2,3 isotopomers of glutamate and glutamine, and in vivo studies in the presence and absence of fluoroacetate may be useful to evaluate contributions of glutamate shunting to formation of these isotopomers.

5.2. Refine models for metabolic activation and astrocyte-neuron interactions

The two cornerstones of the ANL transport-oxidation model, i.e., glutamate-induced glycolysis and substantial local neuronal oxidation of lactate during brain activation, have never been validated in vivo. Normal brain does not need lactate as a supplemental fuel because compensatory responses to brain activation increase oxygen and glucose delivery within seconds, and fuel supplies exceed demand by a wide margin. Glycolysis is preferentially upregulated by unidentified cells during brain activation, and the fractional change is much lower in anesthetized subjects, suggesting that suppression of brain function by anesthesia also reduces demand for glycolysis. Fast lactate washout from activated tissue supplied with excess glucose implies that maintenance of elevated glycolytic rates is more important than local lactate utilization. Maximal contributions of lactate to total oxidation are estimated to be <10%, consistent with its being a minor fuel in human brain at low levels of lactate during exercise and lactate flooding that correspond to lactate levels during brain activation of sedentary subjects (less than about 2 mmol/L). Astrocytic glycogen helps maintain brain function during severe glucose depletion, but the rates that preserve function are very low, and the cells that use the glycogen-derived energy remain to be identified in most studies.

5.3. 'Raise the bar' for critical analysis and interpretation of translational research

Lactate trafficking coupled with substantial neuronal oxidation has been widely accepted in some scientific circles, but it is intensely debated in others because it has been perpetuated with little direct in vivo evidence and without critical evaluation within the broad scope of the brain energy metabolism literature over the past 30–40 years. It is not sufficient to provide supporting correlative evidence from a few selected studies, from modulation of monocarboxylic acid transporter levels, from the use of high lactate loads, or from assays carried out in the absence of glucose. Three issues must be addressed to generate a more accurate metabolic model of astrocyte-neuron interactions associated with excitatory neurotransmission, (i) identification of the processes and cells that generate lactate during activation in vivo, (ii) quantification of the direction, magnitude, and rate of lactate shuttling coupled with the rate of local lactate oxidation in vivo, and (iii) incorporation of substantial lactate release from activated brain in vivo. Although astrocytic glutamate oxidation is well established, the filopodia surrounding glutamatergic synapses that contain mitochondria in vivo need to be identified and quantified and the contribution of glutamate oxidation must be evaluated. Glutamate oxidation is an efficient, economical process that can help satisfy astrocytic energetics of excitatory neurotransmission. Advantages of glutamate oxidation include the following: fuel is supplied with commensurate with demand, no metabolite trafficking is required, critical fuel is not subject to washout, and glucose is spared for use by neurons and astrocytes for different functions. In addition, glutamate oxidation enables increased glycolysis with continuous lactate release to perivascular fluid, facilitating stimulation of blood flow to activated tissue. These important issues have been neglected by current models of excitatory neurotransmission, and new experimental approaches must be developed to advance the field.

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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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Highlights

- Glutamate oxidation by astrocytes can fuel glutamate uptake and spare glucose
- Glutamate-evoked glycolysis and neuronal lactate oxidation in vivo are not proven
- Glucose and oxygen supply exceed demand during brain activation]
- Very low rates of glycogenolysis can support neuronal functions
- Models for astrocyte-neuron interactions are incomplete

Figure 1. Energetics of astrocytic glutamate uptake

K^+ released from neurons is taken up by astrocytes and fueled by glycogenolysis. Glutamate (Glu) uptake into perisynaptic processes of astrocytes that contain mitochondria has two fates, conversion to glutamine (Gln) or oxidation. When partially oxidized, the glutamate is converted to α -ketoglutarate (α KG), then oxaloacetate (OAA). This OAA can be converted to aspartate (not shown) and it can serve to increase the catalytic capacity of the TCA cycle without increased pyruvate (Pyr) carboxylase (PC) activity. Glu can be regenerated after condensation of OAA with acetyl CoA (AcCoA) and passage through the TCA cycle. Alternatively, Glu can be completely oxidized by exit of malate from the TCA cycle (blue arrow from TCA cycle to Pyr), its conversion to pyruvate in the cytoplasm, and reentry of pyruvate into the TCA cycle (See text). Energy derived from glutamate oxidation is obtained from its shunting through the TCA cycle and by complete oxidative degradation. Because electrolytes, metabolites, and signaling compounds can diffuse within the astrocytic syncytium via gap junctions, local K^+ concentrations may increase in regions of the astrocyte distant from the perivascular zone or in gap junction-coupled astrocytes, stimulate pyruvate carboxylase and increase de novo glutamate synthesis. Compartmentation of glutamate oxidation in perisynaptic regions and glutamate synthesis in more distant structures is denoted by the red and green borders, respectively. A glycolytic compartment is included for structures that do not contain mitochondria.

Figure 2. Lactate oxidation in cultured neurons greatly exceeds that in adult human brain
Lactate oxidation (CMR_{lac}) is expressed as percent of the TCA cycle rate (V_{TCA}). Lactate oxidation in cultured neurons was assayed in the presence of glucose, either 5.5 or 1.1 mmol/L, and different levels of lactate in the medium. Lactate oxidation in human brain was assayed when blood and brain lactate levels were increased by moderate exercise (van Hall et al., 2009) or lactate infusion (Boumezbeur et al., 2010).

A. Rates of glucose and glycogen utilization in normal, ischemic, and aglycemic conditions

B. Energy levels during severe hypoglycemia

Figure 3. Low rates of glycogen utilization preserve neuronal function during glucose depletion (A) Regional rates of glucose utilization (CMR_{glc}) in normal awake resting rat brain are from (Sokoloff et al., 1977) and CMR_{glc} for hippocampal slices is from (Newman et al., 1996). Pairs of values for CMR_{glc} and $\text{CMR}_{\text{glycogen}}$ during ischemia in situ were determined in cerebral cortex (Lowry et al., 1964), parietal cortex (Gatfield et al., 1966), and hippocampus (Gatfield et al., 1966); data from these references are plotted by denoting the respective brain structure on the abscissa for the CMR_{glc} values and the respective literature reference for the $\text{CMR}_{\text{glycogen}}$ values. Glycogenolysis rate in cerebral cortex during severe insulin-induced hypoglycemia was calculated from the change in glycogen concentration during the interval in which cortical EEG was maintained (Suh et al., 2007). Glycogenolysis was also calculated from net changes in glycogen concentration in aglycemic hippocampal slices (i) during the interval in which field excitatory postsynaptic potentials fell to 50% of control for samples with initial glycogen levels that were generated by pre-incubation of the slices with glucose or with glucose plus pyruvate (+Pyr) that raised the initial glycogen level above that with glucose and gave a higher glycogenolysis rate (Shetty et al., 2012), and (ii) during the interval with preservation of synaptic function (Choi et al., 2012); the author thanks Drs. Choi and Macvicar for providing the information needed to convert the percent changes to glycogen amount for these calculations. CMR_{glc} and $\text{CMR}_{\text{glycogen}}$ were assayed in sciatic nerve during ischemia (Stewart et al., 1965), and CMR_{glc} in the intact optic nerve is from Table 3 in (Dienel, 2012b). Glycogenolysis rates in isolated optic and sciatic nerves perfused without glucose were based on net concentration changes from onset of electrical stimulation until maximally-evoked compound action potentials fell by 50% (Wender et al., 2000; Brown et al., 2012). (B) Phosphocreatine (PCr) and ATP levels are maintained at normal levels during progressive insulin-induced hypoglycemia until a critical point is

reached. PCr levels fall prior to the decline of ATP concentration, with energy failure occurring when both compounds are depleted. Plotted from data of (Lewis et al., 1974).

A. Hemodynamic response time to stimulation

B. Glucose delivery exceeds demand over wide range of metabolic rates and brain and plasma glucose levels

Figure 4. Fuel delivery to activated brain is quickly upregulated and exceeds demand. (A) Hemodynamic responses include blood volume, blood flow, and blood oxygen level-dependent (BOLD) signal. Data obtained in studies in anesthetized subjects are plotted from studies summarized in Table 1 of (Masamoto and Kanno, 2012). Note the onset time is <0.75 s in most studies. **(B)** Comparison of paired glucose delivery and phosphorylation rates determined in the same tissue sample. Samples were derived from rats assayed under different experimental conditions designed to vary blood and brain glucose levels and brain glucose utilization rates over a wide range. Data are from (Cremer et al., 1983; Hargreaves et al., 1986). The regression line is $y = 1.59x + 0.21$ ($r^2 = 0.77$). Modified from Fig. 3 of (Dienel, 2012b) with permission of the author.

Figure 5. Upregulation of glucose utilization in awake rats exceeds that in anesthetized rats
 Data are plotted from studies summarized in Table 4 of (Dienel, 2012b). Values are pairs of rates obtained in the same brain structure during rest and activation, and they represent different stimuli (sensory, chemical, or electrical) to different brain regions. Values that fall above the line of identity (solid line) indicate upregulation of CMR_{glc} during activation compared with rest. Individual points cannot be directly compared with each other because the responses vary with brain region and stimulus paradigm. Nevertheless, increases in CMR_{glc} in awake subjects are greater and have a wider range compared with anesthetized subjects that are outlined by the dashed rectangle.

Figure 6. Increase in CMRO₂ during brain activation

Values are means and SD of tabulated results of CMRO₂ in two reviews (n=17 studies (Shulman et al., 2001); n = 15 studies (Giove et al., 2003)) or mean values (with SD for responses to different visual stimulus rates) from representative studies in human or rat brain (Madsen et al., 1995; Madsen et al., 1999; Feng et al., 2003; Zhu et al., 2009; Lin et al., 2010).