The Astrocyte: Powerhouse and Recycling Center

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Brain metabolism is characterized by fuel monodependence, high-energy expenditure, autonomy from the rest of body, local recycling, and marked division of labor between cell types. Although neurons spend most of the brain's energy on signaling, astrocytes bear the brunt of the metabolic load, controlling the composition of the interstitial fluid, supplying neurons with energy substrates and precursors for biosynthesis, and recycling neurotransmitters, oxidized scavengers, and other waste products. Outstanding questions in this field are the role of oligodendrocytes, the metabolic behavior of the different subtypes of astrocytes during development and disease, and the emerging notion that metabolism may participate directly in information processing.

The energy requirements of the central nervous system (CNS) are very high compared with those of other organs. Although the brain accounts for merely 2% of body weight, it receives ~15% of cardiac output, and uses 20% of the oxygen and 25% of the glucose of the total body turnover (Magistretti 2008). A special microarchitecture has evolved to support this extreme need, in which glial cells play a central role (Fig. 1). The microvasculature consists of a complex and dense network of highly interconnected blood vessels (Weber et al. 2008; Blinder et al. 2013) to ensure adequate delivery of oxygen and glucose. Although oxygen diffuses freely into the parenchyma, glucose and other hydrophilic energy substrates are translocated across membranes via specific transporter proteins (Fig. 1). The astrocyte is a polarized cell. One set of astrocytic processes ensheaths the vasculature and a second set reaches toward the syn-

apse, the site of highest energy demand (Harris et al. 2012). Much of the ATP produced in the brain is spent by neurons on the recovery of ion gradients challenged by postsynaptic potentials, with a smaller investment in action potentials and neurotransmitter recycling (Harris et al. 2012). Astrocytes consume considerable energy for their own needs and the cycling of metabolically relevant substances for neurons. These metabolic processes in astrocytes and neurons are the basis for brain mapping using functional magnetic resonance imaging and positron emission tomography (PET)—methods that capture local metabolism either directly or indirectly via hemodynamic changes (Magistretti 2008; Belanger et al. 2011a).

Levels of energy consumption in the whole brain are almost constant. However, individual neurons can increase their consumption dramatically. For example, a striatal neuron may

Editors: Ben A. Barres, Marc R. Freeman, and Beth Stevens
Additional Perspectives on Glia available at www.cshperspectives.org

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Cite this article as *Cold Spring Harb Perspect Biol* 2015;7:a020396

Figure 1. Overview of astrocytic metabolism. (*Inset*) Astrocytes (green) reside between blood vessels (red) and neurons (yellow). Neurons and oligodendrocytes (blue) are not in direct contact with vessels. Astrocytes form metabolic domains and are coupled by gap junctions, through which they exchange metabolites and ions. **Energy:** The main energy substrate of the brain is glucose (glc), which crosses the endothelium and enters astrocytes via the glucose transporter GLUT1. Glucose is converted by glycolysis into pyruvate (pyr), which is oxidized to CO₂ by the mitochondrial Krebs cycle, producing NADH and then ATP. Pyruvate also generates lactate (lac), which is shuttled via monocarboxylate transporters MCT4 and MCT2 to be used as an energy substrate by neurons. Astrocytes store energy in the form of glycogen. (*Legend continues on following page.*)

jump from electric silence to a firing rate of 40 Hz within milliseconds, demanding an estimated 30-fold increase in fuel supply (Attwell and Laughlin 2001). The large dynamic range of energy metabolism at the single-cell level is unique to nervous tissue. It could be argued that a general principle of design is the dampening of this dynamic range to make it sustainable in terms of fuel and oxygen delivery. One dampening strategy is “sparse coding,” whereby information is encoded by only a few neurons at any given point in time (Kording et al. 2003; Olshausen and Field 2004). Astrocytes also help to reduce the metabolic impact of neuronal signaling by diluting the individual demands of synapses in space and time.

Astrocytes are the metabolic workhorses of the brain. In addition to catering for themselves, astrocytes subsidize neurons, oligodendrocytes, and possibly microglia by providing energy and building blocks for biosynthesis. Astrocytes energize *trans*-cellular cycles, inactivate toxic waste, and express complex biochemical pathways, including synthesis, assembly, and degradation of enzymes and ancillary proteins, all of which demand energy and information. Moreover, metabolites are water soluble, requiring osmotically obliged water and, therefore,

space. Relieved from metabolic duties, neurons can allocate more of their resources to information processing.

MORPHOLOGY OF ASTROCYTES AND THEIR ROLE AS INTERFACE CELLS

For understanding metabolism, neurons, glial cells, and the cerebral vasculature have to be considered a tightly coupled functional unit. Astrocytes are responsible for the cohesion of this unit (Fig. 1). They are spongiform cells with a central cell body and a dense radial arrangement of prolongations parceling the neuropil into largely nonoverlapping domains. It is estimated that each astrocyte enwraps about four neuronal somata, one or two capillaries, and 10^5 synapses (Bushong et al. 2002; Halassa et al. 2007; Oberheim et al. 2008). Astrocytes cover virtually the entire basal lamina of the cerebral vasculature (Mathiisen et al. 2010) with delicate processes termed “perivascular endfeet” (Reichenbach 1989). They also extend peripheral processes (Derouiche and Frotscher 2001) that make close contact with neurons at somata, dendrites, and axons. The term “tripartite synapse” (Araque et al. 1999) comes from the fact that most of the synapses, pre- and postsynapse, are touched

Figure 1. (Continued) A fast mechanism of neuronal energization is redox cycling, whereby astrocytic lactate is exchanged for neuronal pyruvate, with the net transfer of one energy-rich NADH per cycle. Neurons capture glucose via the glucose transporter GLUT3 to be metabolized via glycolysis and the pentose phosphate pathway (PPP) to promote antioxidation. Neurotransmission: Glutamate and γ -aminobutyric acid (GABA) are captured by astrocytic excitatory amino acid transporters (EAAT) and GAT, respectively, converted into glutamine and shuttled back to neurons for recycling via multiple astrocytic and neuronal transporters (SN1, SAT/ATA, and others). Biosynthesis: Glucose diverted through the astrocytic PPP generates NADPH and precursors for the synthesis of nucleotides and amino acids. Pyruvate is carboxylated into the Krebs cycle intermediate oxaloacetate (OAA), which is a precursor of multiple biosynthetic pathways in astrocytes and, through shuttling of glutamine, is also the main precursor for neuronal biosynthesis. Waste recycling: Reactive oxygen species (ROS) in neurons are scavenged by ascorbate (AA) with the production of dehydroascorbic acid (DHA), which diffuses through the glucose transporters toward astrocytes to be recycled into AA, and is returned to neurons via anion channels and the SVCT2. Glutathione (GSH) reacts with ROS to generate glutathione disulfide (GSSG), or with xenobiotics to generate conjugated glutathione (GS-X), both of which are discarded via multidrug resistance proteins (MDR). GSH synthesized and released by astrocytes is cleaved in the interstice to cysteine, which controls the neuronal synthesis of GSH. Ammonia (NH_3) released by the deamidation of glutamine into glutamate leaves neurons by an unknown pathway and is captured as ammonium (NH_4^+) by K^+ channels in astrocytes, where it is recycled by glutamine synthase. Some nitrogen is returned to astrocytes in the form of alanine and other amino acids (not shown). K^+ released by neurons during synaptic activity enters astrocytes via K^+ channels and the Na^+/K^+ ATPase. Methylglyoxal is a side product of glycolysis, which is detoxified mostly in astrocytes by the glyoxalase system.

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by astrocytic processes (Ventura and Harris 1999). The synapse–astrocyte interface has attracted much attention in relation to neurotransmission (Halassa and Haydon 2010), but it is also of paramount importance for metabolism. The astrocyte removes the neurotransmitter glutamate from the synapse through high-affinity surface transporters, triggering various intracellular metabolic events, which are described elsewhere in this article. The spatial relationship between astrocytes and synapses is complex (Bernardinelli et al. 2014a). Coverage of individual synapses by astrocytic processes varies across brain regions, with this being 100% in cerebellum (Grosche et al. 1999) and only 29% in neocortex (Spacek 1985). The degree of coverage is dynamic and dependent on synaptic activity (Bernardinelli et al. 2014b) and modulates local levels of glutamate (Oliet et al. 2001), as well as its cotransmitter D-serine (Panatier et al. 2006). Conceivably, coverage may also modulate the metabolic exchange between astrocytes and neurons.

Many textbooks and reviews have reported that the cerebral vasculature, particularly in the neocortex, is constructed according to functional neuronal units, such as whisker barrels or cortical columns, in general. However, this notion is not supported by recent quantitative analyses of the cerebral cortical vasculature (Tsai et al. 2009; Keller et al. 2011; Hirsch et al. 2012; Blinder et al. 2013). Astrocytic networks, however, do seem to be organized according to neuronal functional units. Astrocytes are coupled by gap junctions, through which metabolites are exchanged (Giaume et al. 2010). This metabolic coupling is confined to individual barrels, suggesting that astrocytes, rather than the vascular network, are responsible for microscopic distribution of energy substrates (Houades et al. 2008; Giaume et al. 2010; Roux et al. 2011). The astrocytic response to various forms of CNS insults includes a significant remodeling of gap junctional communication between astrocytes (Li et al. 1998; Oberheim et al. 2008; Giaume et al. 2010). Around the large pial and diving vessels, astrocytes define the Virchow–Robin space. This perivascular space is a clearing route for interstitial solutes, a role played by

the lymphatic system for the rest of the body (Iliff et al. 2012, 2013; Iliff and Nedergaard 2013; Xie et al. 2013). This so-called glymphatic system consists of a para-arterial influx route, a paravenous clearance route, and a *trans*-parenchymal pathway facilitated by the astrocytic aquaporin-4 water channel (Iliff and Nedergaard 2013). Because of the dominance of diffusion over short distances, the glymphatic system is likely to play a minor role in local metabolism. However, it may prove relevant for convective transfer between distant regions of the brain, particularly for large protein-bound metabolites, such as cholesterol.

METABOLIC PATHWAYS AND COMPARTMENTS

The blood–brain barrier provides protection against circulating toxins (Obermeier et al. 2013), behavioral stability in the face of starvation and disease, and the possibility of metabolic specialization, for example, the co-option of the amino acid glutamate for the purposes of neurotransmission. A significant trade-off is that complex and energetically expensive chemical reactions need to be performed “in-house.” Neurons are deficient in several metabolic pathways, which are correspondingly stronger in astrocytes, including the production of building blocks for biosynthesis (Yu et al. 1983; Herrero-Mendez et al. 2009), antioxidation (Schmidt and Dringen 2012), and waste disposal (Bak et al. 2006; Belanger et al. 2011b). There are no metabolic reactions in astrocytes that are not also present in other cell types of the body. The uniqueness of astrocytic metabolism stems from its intimate and heavily biased relationship with the super specialized neuron, in a context of relative insulation from the circulation.

Metabolic processes, occurring within neurons and astrocytes, are distributed between membrane compartments and are the work of enzymes and transporters. Whereas enzymes transform molecules, transporters move them between compartments. As the control of flux is distributed among multiple nodes of the metabolic network, specific enzymes or transporters are no longer considered to be rate limiting.

Mitochondria, the endoplasmic reticulum, and other membrane-bound organelles host specific reactions, integrated with the rest of the network through exchange with the cytosol. The nucleus is well connected to the cytosol, behaving as a metabolic buffer. The high-diffusion coefficient and high prevailing concentration of most metabolites, together with the slow rate of enzymes, indicates that the cytosol should be a well-mixed compartment (Barros and Martinez 2007; Martinez et al. 2010), a prediction confirmed for glucose in cultured astrocytes (Kreft et al. 2013). However, a recent study of astrocytes *in situ* revealed that diffusion in endfeet is 20 times slower than in the rest of the cell (Nuriya et al. 2013), which may determine metabolic compartmentation within the cytosol of single astrocytes, particularly for low-concentration molecules like AMP, NADH, and some glycolytic intermediates.

Energy Uptake, Storage, and Delivery

The adult mammalian brain is energized by the oxidation of glucose (Siesjö 1978). This fuel monodependence has been explained by the inability of albumin-bound fatty acids to cross the blood–brain barrier and the fact that ATP production requires less oxygen from glucose than from fat. Minor substrates, like lactate and ketone bodies, may become significant if their blood levels are elevated, as occurs in strenuous exercise or when fasting (Dalsgaard et al. 2004; Yellen 2008; Rasmussen et al. 2011). Glucose enters the brain via the endothelial transporter GLUT1 and is then captured by astrocytic endfeet, which are also rich in GLUT1 (Kacem et al. 1998). The activity of the astrocytic GLUT1 is acutely stimulated by glutamate (Loaiza et al. 2003; Porrás et al. 2008; Chuquet et al. 2010), a mechanism coupling synaptic activity to fuel supply. The paracellular route is seemingly minor, as endfeet wrap 99.7% of the capillary surface (Mathiisen et al. 2010), and even water requires astrocytic channels to enter the parenchyma (Haj-Yasein et al. 2011). However, the barrier function of endfeet decreases under pathological conditions and may conceivably be a physiologically regulated variable (Nuriya

et al. 2013). Unlike muscle cells and adipocytes, astrocytes maintain a substantial pool of glucose (Bittner et al. 2010, 2011; Prebil et al. 2011; Ruminot et al. 2011). Glucose may leave astrocytes at the perisynaptic processes via GLUT1, which by virtue of its relatively low affinity for glucose is similarly equipped to facilitate influx and efflux. Neurons, on the other hand, express GLUT3, a higher affinity transporter that performs better in the influx mode (Barros and Deitmer 2010).

Astrocytes consume more glucose than neurons in cultured cells and tissue slices (Bouzier-Sore et al. 2003, 2006; Barros et al. 2009b; Jakoby et al. 2013), although mitochondrial oxidative phosphorylation, the ATP harvesting phase of metabolism, is somewhat weaker in astrocytes (Hyder et al. 2006), a deficit explained at both transcriptional (Lovatt et al. 2007; Cahoy et al. 2008) and posttranslational levels (Herrero-Mendez et al. 2009; Halim et al. 2010). This mismatch produces a chronic surplus of pyruvate, which is exported as lactate, an efficient oxidative fuel for neurons *in vitro* and *in vivo* (Schurr et al. 1988; Bouzier-Sore et al. 2006; Wyss et al. 2011). In neurons, glycolysis is blocked at the level of phosphofructokinase (PFK) by tonic proteosomal degradation of PFK2 (Herrero-Mendez et al. 2009; Fernandez-Fernandez et al. 2012). A vectorial flux is thus generated, termed the astrocyte–neuron lactate shuttle (ANLS) (Pellerin and Magistretti 1994). Glycolysis in astrocytes is acutely modulated by two parallel readouts of neuronal activity: glutamate (Pellerin and Magistretti 1994) and K^+ (Bittner et al. 2011). The effect of K^+ develops within seconds, is quickly reversible, and is mediated by the Na^+ /bicarbonate cotransporter NBCe1 (Ruminot et al. 2011). Stimulation by glutamate develops within minutes, persists long after removal, and is mediated by Na^+ /glutamate cotransporters (Pellerin and Magistretti 1994; Voutsinos-Porche et al. 2003; Bittner et al. 2011). Both mechanisms require an active Na^+ / K^+ ATPase pump (Pellerin and Magistretti 1994; Bittner et al. 2011). Glutamate may further promote lactate production by inhibiting astrocytic respiration (Azarias et al. 2011). The driving forces for the ANLS are the gradients in

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pyruvate concentration and NADH/NAD⁺ ratio between astrocytes and neurons. The combination of low-affinity isoforms of lactate dehydrogenase (LDH5) and the monocarboxylate transporter (MCT4) in astrocytes, and high-affinity isoforms LDH1 and MCT2 in neurons act as a rectifier, facilitating a one-way flux from astrocytes to neurons (Fig. 1) (Aubert et al. 2005; Barros and Deitmer 2010). A nonexclusive, fast mechanism of neuronal fueling is lactate/pyruvate exchange, which transfers one reducing equivalent per cycle from astrocytes to neurons (Cerdan et al. 2006; Hung et al. 2011). It is not clear, at this stage, to what extent neurons are energized by the ANLS, redox cycling, or glucose. Also unclear is the relative contribution from each of the three fueling mechanisms in neuronal subtypes, across brain regions, and in response to neural activity.

Astrocytes store glycogen, the sole energy reserve of brain tissue. The brain does not express significant levels of glucose-6-phosphatase, so it cannot transform glycogen into glucose with high efficiency. However, glycogen can be transferred to neurons as lactate (Dringen et al. 1993). Glycogen breakdown is induced during hypoglycemia and ischemia (Brown and Ransom 2007; Oz et al. 2007, 2009; Matsui et al. 2012) and also under physiological conditions. In studies of optic nerve in vitro, firing was equally sustained by lactate or glucose (Brown et al. 2003) and impaired by pharmacological inhibition of glycogen phosphorylase, the enzyme that degrades glycogen (Tekkok et al. 2005). In vivo, glycogen phosphorylase inhibition caused amnesia (Gibbs et al. 2006; Newman et al. 2011; Suzuki et al. 2011), which was reverted by lactate (Newman et al. 2011; Suzuki et al. 2011). Moreover, lactate rescued the amnesic effect of knocking down the astrocytic lactate transporter MCT4, but not that of the neuronal lactate transporter MCT2, highlighting the transfer of lactate from astrocytes to neurons (Suzuki et al. 2011). Several neuronal signals are capable of mobilizing glycogen, including noradrenaline, adenosine, and vasoactive intestinal peptide (VIP), although glutamate proved ineffective (Sorg and Magistretti 1991). A link between local synaptic activity and glycogen mo-

bilization was suggested recently, based on the observation that glycogen may be mobilized by elevated extracellular K⁺ via the bicarbonate-sensitive soluble adenylyl cyclase (Choi et al. 2012). Intriguingly, a sizable fraction of the glucose captured by astrocytes may be converted to glycogen before being metabolized to pyruvate, a phenomenon termed the “glycogen shunt” (Shulman et al. 2001; Walls et al. 2009).

Energization of Intercellular Cycles

Astrocytic intervention in neuronal metabolism also occurs via a prominent participation in the recycling of neurotransmitters and other molecules. Instead of being recaptured directly by neurons, most of the glutamate released during synaptic activity is diverted to astrocytes via high-affinity excitatory amino acid transporters (EAATs), converted to glutamine, and then shuttled back by the concerted work of multiple glutamine transporters (Deitmer et al. 2003; Bak et al. 2006). Because of the additional steps, the cycle becomes more expensive, but the burden is on astrocytes, allowing neurons to reduce their direct ATP investment in glutamate recycling by 60%.³ Further, neurons are spared the logistics of expressing the exceedingly high density of transporters required for fast glutamate removal (Rusakov and Kullmann 1998). γ -aminobutyric acid (GABA), dopamine, and noradrenalin are other neurotransmitters that are released by active neurons and recycled via astrocytes.

Supply of Building Blocks

Krebs cycle intermediates are the starting material for the synthesis of several classes of structural molecules, including fatty acids, sterols, amino acids, and porphyrins. Astrocytes replenish their own pool of Krebs intermediates, a process termed anaplerosis, by carboxylating

³The glutamate transporters import 3 Na⁺ and 1 H⁺, requiring the use of 1.33 ATP molecules. The neuronal glutamine transporter SNAT imports 1 Na⁺, requiring 0.33 ATP molecules. These expenditures add to the 1 H⁺ (0.33 ATPs) used by the vesicular glutamate transporter.

glycolytic pyruvate to oxaloacetate (OAA). Neurons do not express pyruvate carboxylase and, for anaplerosis, they rely on glutamine imported from astrocytes, which is converted to glutamate and then into the Krebs intermediate α -ketoglutarate (Yu et al. 1983; Schousboe et al. 1997). Thus, the building blocks for neuronal biosynthesis come from astrocytes. In addition, the glutamate/glutamine cycle is leaky, as some glutamate gets oxidized. Again, the losses of glutamate and glutamine are compensated by astrocytic anaplerosis. An estimated 33%–50% of all pyruvates entering mitochondria in astrocytes serve anaplerosis instead of producing energy (Hertz and Dienel 2002; Hyder et al. 2006). The export of molecules from astrocytes to neurons may also occur at higher levels. For instance, based on the differential expression of enzymes involved in the synthesis, export, and degradation of cholesterol, it has been suggested that astrocytes are net producers of cholesterol and neurons are net consumers (Pfrieger and Ungerer 2011). The brain pool of cholesterol, a major constituent of neuronal membranes, is completely isolated from the rest of the body. Oligodendrocytes have received less attention and it is not clear whether they are capable of anaplerosis or whether they rely on astrocytes (Amaral et al. 2013). The synthesis of myelin does seem to involve astrocytes as it is stimulated by lactate (Rinholm et al. 2011) and also by *N*-acetylaspartate (NAA), a highly abundant neuronal metabolite that is synthesized from glutamine imported from astrocytes (Amaral et al. 2013). Little is known about the metabolism of microglia and their likely metabolic exchange with astrocytes and/or neurons. Capable of proliferation and strong nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, microglia are presumed to metabolize glucose at high rates. However, their hexose transporter is GLUT5, which, in humans, does not transport glucose but fructose (Augustin 2010), a sugar, which is almost absent in blood and cerebrospinal fluid. On activation, microglia augment their expression of MCT1 and MCT2 (Moreira et al. 2009), but it is not known whether they are lactate importers or exporters.

Waste Recycling

The most abundant waste products of neuronal activity are CO_2 , reactive oxygen species (ROS), ammonia (NH_3), and K^+ . Although CO_2 diffuses across astrocytes into the blood and ROS are scavenged in situ, NH_3 and K^+ are temporarily retained by astrocytes and then returned to neurons. NH_3 is a gas that is stoichiometrically released during the glutamate and GABA cycles and when glutamine is used for anaplerosis. In the neuronal cytosol, NH_3 captures a proton and becomes ammonium (NH_4^+), equilibrating at a ratio of 100:1 ($\text{NH}_4^+:\text{NH}_3$). Lacking glutamine synthase, neurons cannot process the excess of nitrogen, which returns to astrocytes as NH_4^+ , NH_3 , or in the form of amino acids (Fig. 1) (Bak et al. 2006; Cooper 2012; Rothman et al. 2012). NH_4^+ enters astrocytes through K^+ channels and transporters (Nagaraja and Brookes 1998; Kelly and Rose 2010) to be returned to neurons as glutamine. This recycling and detoxifying mechanism, which works efficiently at the normal micromolar levels of NH_4^+ , becomes overloaded during hyperammonemia. Competition between NH_4^+ and K^+ for channel-mediated uptake increases interstitial K^+ , leading to NKCC1-mediated neuronal disinhibition and neurotoxicity (Rangroo Thrane et al. 2013). During chronic hyperammonemia, astrocytes adapt to the excess NH_4^+ by accumulating glutamine, which is released back into the bloodstream. However, high glutamine levels eventually damage the mitochondria and cause astrocytic swelling, leading to Alzheimer type II astrocytosis, the histochemical signature of hepatic encephalopathy (Albrecht and Norenberg 2006; Brusilow et al. 2010).

Other important waste products of brain cells derive from glutathione (GSH) and ascorbate (AA). GSH participates in the detoxification of ROS, endogenous toxins, and xenobiotics. Most of the oxidized glutathione (SGGS) is reduced in situ, but a minor fraction is lost via multidrug resistance (MDR) proteins, which also mediate the export of conjugated glutathione (GS-X). These losses are replenished by de novo synthesis of GSH, which, in neurons, is limited by cysteine supplied by astrocytes

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(Fig. 1) (Schmidt and Dringen 2012). AA (vitamin C) is a cofactor for many enzymatic reactions and is the most abundant free-radical scavenger in neurons. After oxidation by ROS, AA is reduced locally by reactions consuming NAD(P)H, but some ends up as dehydroascorbic acid (DHA), a toxic compound that diffuses to astrocytes via glucose transporters to be recycled into AA by GSH or enzymatic reduction. The loop is completed in an activity-dependent manner by the export of AA via swelling-activated ion channels, followed by accumulation in neurons via the Na⁺-dependent AA cotransporter SVCT2 (Fig. 1) (Siushansian et al. 1996; Harrison and May 2009). Metabolism also generates toxins that are not free radicals, for example, methylglyoxal, an inevitable side product of glycolysis that promotes the formation of advanced glycation end (AGE) products and slowly progressing cell degeneration. As expected from their high rate of glycolysis, astrocytes express a robust glyoxalase system (Fig. 1) that protects both themselves and neurons against methylglyoxal toxicity (Belanger et al. 2011b).

MEASURING METABOLISM, TOWARD SINGLE-CELL READOUTS

New discoveries are often associated with methodological advances. It is remarkable to see how new techniques for visualizing structures or processes have influenced the way neuroscientists design experiments and formulate questions. This, too, is true for the astrocytic involvement in brain metabolism, and it is very likely that imaging techniques will become the forefront method in this field.

Studies of brain metabolism span a wide range, from the behavior of single enzymes using molecular dynamics to the long-term variation of brain glucose consumption in aging individuals. Glial involvement was first studied in vitro, which enables optimal control of experimental variables. Neuron and astrocyte cultures, in combination with radiolabeled substrate analogs, have been instrumental for the identification of molecular mechanisms. Strong small-scale interactions and mechanisms are relatively insensitive to large-scale context,

thus the success of the reductionist in vitro approach. However, in cultured conditions, some specific boundary conditions are lost. For example, although the biophysical properties of a neuronal ion channel are insensitive to blood flow, hemodynamics may only be understood within the context of the intact tissue. To understand the concerted processes of brain energy metabolism involving neurons, glial cells, and blood vessels, in vivo experiments are essential.

Kety and Schmidt (1948) introduced one of the most influential early techniques to quantify global cerebral blood flow and metabolic rate in vivo. The method measures arteriovenous differences in the concentration of a tracer and is based on the brain absorbing an inert diffusible gas from arterial blood in a manner that only depends on perfusion. Sokoloff et al. (1977) took the next big step, developing the deoxyglucose method for measuring the local metabolic rate of glucose with autoradiography. A noninvasive variation of this technique soon became applicable to human subjects with the introduction of PET (Raichle et al. 1978). Resolution at cell level is now possible using cell-specific labeled compounds, such as acetate, which is unambiguously taken up by astrocytes (Waniewski and Martin 1998). Apart from chromatographic separation of metabolites from lysates and, subsequently, scintillation counting, [¹⁴C]-labeled acetate has frequently been used together with autoradiography to map astrocytic metabolism (Muir et al. 1986; Dienel et al. 2001). On the basis of this method, an activity-dependent regulation of oxidative metabolism in astrocytes has been shown (Cruz et al. 2005), which was later replicated using the PET-tracer [1-¹¹C]-acetate in rat and human (Wyss et al. 2009). Acetate can also be labeled with the stable isotope [¹³C] for nuclear magnetic resonance (NMR) spectroscopy. In contrast to radiotracer-based methods, [¹³C]-NMR spectroscopy presents a substantial advance with respect to the identification of metabolic products. With the appropriate kinetic models, the observed dynamics of the [¹³C]-label in metabolic pathways permits quantitative determination of flux rates. Although individual fluxes in neurons and as-

trocytes require assumptions with [^{13}C]-glucose, separation with the astrocyte-specific [^{13}C]-acetate is more direct. For example, using [2- ^{13}C]-acetate, flux through the astrocytic Krebs cycle was found to be 0.14 ± 0.06 mmol per gram per minute, accounting for $\sim 15\%$ total cerebral oxidative flux (Lebon et al. 2002). This flux is two times higher than that measured using [^{13}C]-glucose, reflecting glucose entry into neurons and astrocytes and mixing of labels between the cell types because of glutamate/glutamine cycling. Apart from a low spatial and temporal resolution, low sensitivity is another serious disadvantage of NMR spectroscopy. Consequently, a high concentration of label is required, which alters metabolic fluxes. This difficulty may, in part, be overcome with dynamic nuclear polarization (Mayer et al. 2009; Park et al. 2013).

All of the above-mentioned methods fail to resolve single cells, which is a severe drawback for the investigation of heterogeneous tissues, like the brain. Fluorescence optical imaging, confocal, as well as two-photon microscopy, achieve subcellular resolution and have made important contributions to glial research. Metabolism may also be investigated at cellular resolution with fluorescent glucose analogs, such as 6-NBDG and 2-NBDG, which probe glucose transport and consumption (Kim et al. 2012). Although these large molecules are transported and metabolized much more slowly than glucose, they still serve as tracers for studies in vitro and in vivo (Loaiza et al. 2003; Rouach et al. 2008; Barros et al. 2009a; Chuquet et al. 2010).

Genetically encoded fluorescent sensors specific for energy metabolites, glucose, glutamate, ATP, NADH, lactate, and pyruvate have been successfully used in brain cells and brain slices (reviewed in San Martín et al. 2014). Providing high spatiotemporal resolution, these sensors report cell-specific metabolite levels, absolute concentrations, and even metabolic fluxes, and are instrumental for the discovery of new phenomena and their molecular underpinnings. One example is the NBCe1-dependent stimulation of astrocytic glycolysis within seconds of an increase in extracellular K^+ , that is, within the time frame of the activity-dependent

lactate surge detected in brain tissue (Barros 2013). Sensors continue to be developed and optimized in terms of dynamic range and signal-to-noise ratio, which, together with new protein expression strategies and imaging techniques, are permitting the subcellular, subsecond characterization of brain metabolism.

CONCLUDING REMARKS

We have outlined the central role of astrocytes in brain metabolism. To conclude, we would like to highlight four areas related to metabolic processes and astrocytes, which are not dealt with in the review. All four areas are currently in the spotlight and we believe that their significance will increase.

Diversity

Various types of neurons have been identified and it is well accepted that a “standard neuron” does not exist. Indeed, attempts to establish a unifying systematic taxonomy of neurons have been made (for a recent classification scheme of GABAergic interneurons, see DeFelipe et al. 2013). Different types of astrocytes can also be distinguished, according to morphology, electrophysiological properties, and expression of specific proteins (Kimelberg 2009), so it seems likely that different metabolic phenotypes may also exist. The metabolic machinery of astrocytes is likely to differ in its relationship with the vasculature, in gap junctional communication with other astrocytes and oligodendrocytes, between brain areas, within species, and during development and in pathophysiology. The advent of single-cell resolution metabolic analysis will certainly shed more light on the metabolic diversity of astrocytes.

The Oligodendrocyte: The Other Astrocyte of Energy Metabolism?

Oligodendrocytes are responsible for the myelination of axons in the CNS, which forms the basis of efficient transmission of action potentials over long distances. In this context, oligo-

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dendrocytes have long been known to support axonal functioning. Recent research now indicates that oligodendrocytes, much like astrocytes, also play a pivotal role in metabolism, particularly with respect to energizing axons (Saab et al. 2013). More specifically, inactivation of oxidative metabolism in oligodendrocytes through conditional knockout of the *Cox10* gene failed to have a detrimental effect on cell functioning even in the long term. This strongly suggests that oligodendrocytes rely on aerobic glycolysis (Funfschilling et al. 2012). Moreover, oligodendrocytes were also shown to release lactate, which may fuel axons. An experimentally disrupted expression of MCT1, normally abundantly expressed in oligodendrocytes but also reduced in patients suffering from amyotrophic lateral sclerosis, leads to axonal degeneration (Lee et al. 2012). This negative effect is likely caused by the inability of oligodendrocytes to transport lactate and coincides with the hypothesis that oligodendrocyte-derived lactate is channeled through the myelin sheath and taken up by axons as an oxidative energy substrate. Oligodendrocytes show relatively low expression of glycolytic enzymes, but they communicate with astrocytes through gap junctions that are essential for myelin maintenance, suggesting substantial transfer of lactate and other metabolites between the two cell types (Tress et al. 2012).

Metabolism and Signaling

Mainstream neuroscience regards metabolism as a low-level domain, that is, an important but ultimately contingent platform that becomes interesting for information processing only when it goes wrong. However, there may be a more horizontal relationship between metabolism and signaling. Whereas brain tissue is energetically expensive, the process of encephalization occurred in a context of chronic food shortage, which implies that energy metabolism is a source of constraint for the evolutionary design of the brain (Aiello and Wheeler 1995; Navarrete et al. 2011). Moreover, metabolism and signaling share critical hubs, the most prominent example being glutamate, the main

excitatory neurotransmitter, and also a pivotal metabolic intermediate.

Metabolism and signaling could have been kept separate, as exemplified by serotonin, acetylcholine, and noradrenalin neurotransmission, but they were not. Why? Another link between signaling and metabolism is adenosine, a degradation product of purine nucleotides but also a major neuromodulator involved in the coordination of synaptic networks, neurovascular coupling, and the physiology of sleep (Pascual et al. 2005; Blutstein and Haydon 2013). Further, cross talk is provided by lactate, which diffuses beyond the active zone and modifies the activity of neurons and astrocytes in neighboring regions acting via the NADH/NAD⁺ ratio, ion channels, and the G protein-coupled lactate receptor HCA1. Involved in a hierarchy of processes from neurovascular coupling to memory formation, lactate is both fuel and intercellular messenger (Suzuki et al. 2011; Barros 2013; Bozzo et al. 2013; Lauritzen et al. 2013; Tang et al. 2014). One defining characteristic of metabolic signals, like adenosine and lactate, is that they are local, conveying information to neighboring neuronal units and possibly helping to distribute resources between adjacent areas of the brain.

The “Selfish” Astrocyte

Outsourcing of metabolic processes makes neurons more efficient signaling machines, but also renders them vulnerable to potential deficits in astrocytic function. In response to metabolic or oxidative stress, astrocytes become “reactive” or “gliotic,” terms that describe a phenotype characterized by cytoskeletal rearrangements, hypertrophy, loss of structural complexity, changes in metabolism, and secretion of inflammatory cytokines. Gliosis is an adaptive response, instrumental to the effective containment of acute injury and wound repair, but it may become harmful if astrocytes neglect their supportive role toward neurons, as is suspected to occur in chronic conditions, such as Alzheimer’s disease. The metabolism of reactive astrocytes is not well understood, particularly in

vivo, and may prove to be a relevant target for therapeutic intervention (Allaman et al. 2011; Steele and Robinson 2012).

ACKNOWLEDGMENTS

We are grateful to our colleagues for their continuing support and discussions. We thank Dr. Karen Everett for critical reading of the manuscript. This work is partly supported by Fondecyt Grant 1130095 and the Swiss National Science Foundation. The Centro de Estudios Científicos (CECs) is funded by the Chilean Government through the Centers of Excellence Basal Financing Program of La Comisión Nacional de Investigación Científica y Tecnológica (CONICYT).

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