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Review Article

Brain lactate metabolism: the discoveries and the controversies

Gerald A Dienel

Department of Neurology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

Potential roles for lactate in the energetics of brain activation have changed radically during the past three decades, shifting from waste product to supplemental fuel and signaling molecule. Current models for lactate transport and metabolism involving cellular responses to excitatory neurotransmission are highly debated, owing, in part, to discordant results obtained in different experimental systems and conditions. Major conclusions drawn from tabular data summarizing results obtained in many laboratories are as follows: Glutamate-stimulated glycolysis is not an inherent property of all astrocyte cultures. Synaptosomes from the adult brain and many preparations of cultured neurons have high capacities to increase glucose transport, glycolysis, and glucose-supported respiration, and pathway rates are stimulated by glutamate and compounds that enhance metabolic demand. Lactate accumulation in activated tissue is a minor fraction of glucose metabolized and does not reflect pathway fluxes. Brain activation in subjects with low plasma lactate causes outward, brain-to-blood lactate gradients, and lactate is quickly released in substantial amounts. Lactate utilization by the adult brain increases during lactate infusions and strenuous exercise that markedly increase blood lactate levels. Lactate can be an 'opportunistic', glucose-sparing substrate when present in high amounts, but most evidence supports glucose as the major fuel for normal, activated brain.
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Glucose is the major fuel for the brain, and its metabolism by different pathways has important functions related to energetics, neurotransmission, oxidation–reduction (redox) reactions, and biosynthesis of essential brain components (Figure 1). For many decades, lactate production in the brain was viewed as a consequence of inadequate oxygen delivery, disruption of oxidative metabolism, or mismatch between glycolytic and oxidative rates (Siesjö, 1978), but more recently, the conceptual role of lactate metabolism and function in the normal brain have undergone major changes, shifting from developmental fuel and glycolytic waste product to include its use as a supplemental fuel and signaling molecule. Starting in the 1970s to 1980s studies carried out in different laboratories with diverse experimental interests related to brain function brought attention to upregulation of glycolysis, lactate production, lactate release into the blood, the possibility of lactate shuttling among cell types within the brain, lactate fueling adult brain during exercise, and roles of lactate in the regulation of blood flow; some of these topics are controversial and highly debated. The experimental paradigm and physiologic status of subjects are critical for interpretation of data, and this review first presents a brief historical overview of studies related to brain lactate transport and metabolism, then compares sets of data to provide a perspective and context within which the consistency of similar experiments and their *in vivo* relevance can be compared and assessed. Space and reference limitations prevent citation of many important studies, and selected initial reports and reviews for specific topics are cited.

Correspondence: Dr GA Dienel, University of Arkansas for Medical Sciences, Department of Neurology, Slot 830, 4301 West Markham Street, Shorey Building, Room 715, Little Rock, AR 72205, USA.
E-mail: gadienel@uams.edu
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Brief Thematic and Historical Perspective of Brain Lactate Metabolism and Trafficking

Compartmentation of Lactate Metabolism in the Brain

During the 1960 to 1970s, *in vivo* studies of precursors of brain amino acids revealed compartmentation

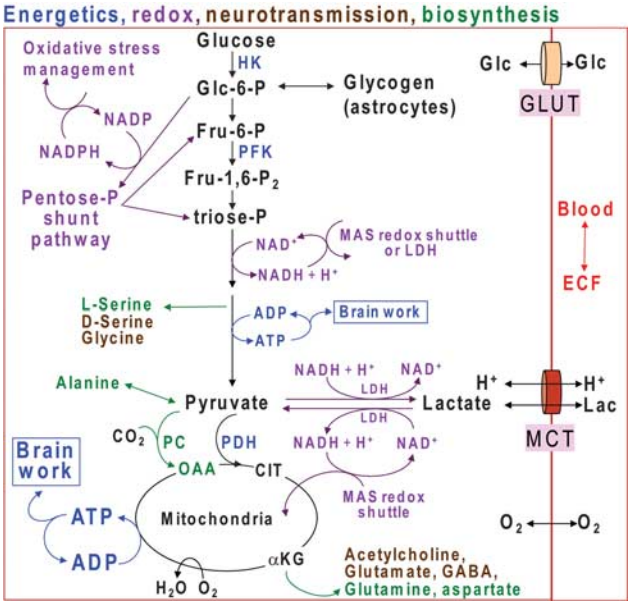

Figure 1 Multifunctional roles of glucose metabolism. Color coding denotes different functional roles of pathways of glucose metabolism. Glucose (Glc) and lactate (Lac) plus H^+ are transported into brain cells from blood or extracellular fluid (ECF) by equilibrative transporters, GLUTs and MCTs (monocarboxylic acid transporters), respectively, whereas oxygen diffuses into brain cells. Energetics (blue) involves ATP production by the glycolytic (glucose to pyruvate; Fru = fructose) and oxidative (pyruvate to CO_2 + H_2O) pathways. Glycolytic rate is modulated by regulation of hexokinase (HK), phosphofructokinase (PFK), and other enzymes. Glucose is stored as glycogen, mainly in astrocytes. Pyruvate enters the oxidative pathway of the mitochondrial tricarboxylic acid cycle, with formation of 3 CO_2 and regeneration of oxaloacetate (OAA). Neurotransmitters and neuromodulators (brown) are synthesized through the glycolytic and oxidative pathways. Other biosynthetic pathways produce amino acids (green) and sugars (not shown) used to synthesize complex carbohydrates for glycoproteins and glycolipids. Net synthesis of a 'new' four- or five-carbon compound (aspartate, glutamate, glutamine, GABA) requires pyruvate carboxylase (PC), which is only located in astrocytes. CO_2 fixation by PC converts pyruvate to OAA. OAA is transaminated to form aspartate or it condenses with acetyl CoA derived from a second pyruvate molecule by the action of pyruvate dehydrogenase (PDH) to form citrate (CIT). Decarboxylation of this 'new' six-carbon compound forms α-ketoglutarate (αKG) that can be converted to a new molecule of glutamate, glutamine, and GABA. These compounds can also incorporate label from labeled glucose in neurons and astrocytes by means of reversible exchange reactions, but their net synthesis requires the astrocytic PC reaction. Acetylcholine is also derived from glucose through citrate in neurons. Entry of glucose-6-P into the pentose phosphate shunt pathway (purple) results in oxidative decarboxylation of carbon one of glucose and generates NADPH, which is used to detoxify reactive species that can cause oxidative stress. The nonoxidative branch of pentose shunt produces fructose-6-phosphate (Fru-6-P) and triose-P that reenter the glycolytic pathway; nucleotide precursors are also generated by the pentose shunt pathway. NAD^+ is required for glycolysis, and it is regenerated from NADH by the malate–aspartate shuttle (MAS) or lactate dehydrogenase (LDH) (purple). The MAS redox shuttle transfers reducing equivalents from the cytosol to the mitochondria and is required to generate pyruvate for oxidation by the tricarboxylic acid cycle; it is also required for oxidative metabolism of lactate. Regeneration of NAD^+ by LDH removes pyruvate from the oxidative pathway.

of metabolism in the brain, with identification of different precursors that preferentially labeled the large (neuronal) glutamate pool and the small (astrocytic) glutamate pool that is the precursor for glutamine; pool labeling assignments were based on the ratio of the specific activity of purified glutamate to that of glutamine (reviewed in the volume edited by Balázs and Cremer (1972)). Early studies have shown that glucose (Cremer, 1964) and lactate labeled the large glutamate pool, whereas butyrate and acetate labeled the small pool (O'Neal and Koeppe, 1966). However, lactate is oxidized by cultured cortical and cerebellar neurons and astrocytes (Dienel and Hertz (2001) and references cited therein) and by both neurons and astrocytes *in vivo* (Zielke *et al*, 2007; Zielke *et al*, 2009).

Rapid Lactate Release from the Brain into the Blood

Hawkins *et al* (1973) showed that an ammonia injection increases the rate of cerebral glucose utilization (CMR_{glc}) and oxygen consumption (CMR_{O2}) in the rat brain and increases lactate release to blood from 3.5% (as glucose equivalents) of glucose uptake at rest to 15% after ammonia. The brain lactate level was less than that in blood, suggesting sites with locally high lactate levels from which lactate diffused into blood. In humans, positron emission tomographic imaging studies using [^{11}C]glucose detected release of ^{11}C-acidic metabolites into blood within 4 minutes (Blomqvist *et al*, 1990). During spreading cortical depression, release of ^{14}C-lactate was detectable within 2 minutes after pulse labeling of the rat brain with [6-^{14}C]glucose; maximal lactate efflux equaled 20% of glucose uptake, and [^{14}C]lactate accounted for nearly all of the ^{14}C discharged into the blood (Cruz *et al*, 1999). In humans given stressful mental testing, lactate release corresponded to 7% of glucose uptake (Madsen *et al*, 1995). The above studies show that the resting brain also releases small amounts of lactate (~3% to 7% of glucose uptake), and that lactate efflux quickly increases by 3- to 4-fold during activation. A recent positron emission tomographic study in a resting young adult human brain revealed regional heterogeneity in the mismatch between local rates of glucose and oxygen utilization (Vaishnavi *et al*, 2010), suggesting that lactate release from various brain structures probably differs under basal conditions.

Lactate Release from Brain Cells

Lactate is released in larger quantities from 'resting' cultured astrocytes than neurons, but both cell types produce lactate under various conditions (Walz and Mukerji, 1988). Dringen *et al* (1993) discovered that lactate, not glucose, is released from cultured astrocytes during glycogenolysis, and suggested that lactate may function as fuel for neighboring cells. These and related *in vitro* studies underlie the

widely held notion that astrocytes may be the major source of brain lactate, but the cellular origin and cellular metabolic fate of lactate *in vivo* remain to be experimentally established.

Underestimation of Metabolic Activation with Labeled Glucose and Lactate Release from the Brain

Functional metabolic brain imaging studies in conscious rats (Collins *et al*, 1987; Ackermann and Lear, 1989; Adachi *et al*, 1995; Cruz *et al*, 2007) and humans (Blomqvist *et al*, 1990) found that the magnitude of increased CMR_{glc} evoked by sensory stimulation, seizures, spreading depression, and voluntary finger tapping was greatly underestimated (by approximately $\geqslant$50%) with labeled glucose compared with labeled deoxyglucose, suggesting upregulation of glycolysis and rapid lactate release (Collins *et al*, 1987; Lear and Ackermann, 1989; Lear, 1990). Studies that our laboratory designed to understand the neurobiology underlying the above discrepant results obtained with glucose and deoxyglucose showed that brain lactate is quickly labeled by blood glucose, lactate is readily diffusible, and rapid lactate efflux to the blood causes loss of labeled products from the brain (Adachi *et al*, 1995; Cruz *et al*, 1999; Dienel and Cruz, 2009). Focal label retention in activated structures is enhanced by blockade of lactate transporters and astrocytic gap junctions (Cruz *et al*, 2007), and astrocytes have a much higher rate and capacity for lactate uptake from extracellular fluid and for dispersion within the astrocytic syncytium compared with lactate shuttling from astrocytes to neurons (Gandhi *et al*, 2009). Most lactate derived from glucose microinfused into interstitial fluid is not locally oxidized, and extracellular metabolites are released through perivascular flow into the lymphatic drainage systems (Ball *et al*, 2010). Taken together, these findings indicate that increased glycolysis during activation is associated with substantial loss of lactate from the brain through vascular and perivascular drainage systems within 5 minutes in normal subjects with low blood lactate levels ($\sim$0.5 to 1 mmol/L) and modest ($\sim$2-fold) or large ($>$3- to 8-fold) increases in brain lactate level.

'Uncoupling' of Cerebral Blood Flow, Oxygen Consumption (CMR_{O2}), and CMR_{glc} During Sensory and Mental Stimulation

In the resting brain, nearly all of the glucose metabolized is oxidized, and many, but not all, studies report that the resting CMR_{O2}/CMR_{glc} ratio is close to the theoretical maximum of 6.0 (i.e., 6 O_2 are required to oxidize 1 glucose). However, during activation, disproportionately larger increases in cerebral blood flow (CBF) and CMR_{glc} compared with CMR_{O2} were reported by Fox and Raichle (1986) and Fox *et al* (1988), and confirmed in humans (Madsen *et al*, 1995) and rats (Madsen *et al*, 1999). The CMR_{O2}/CMR_{glc} ratio falls in most, but not all, activation studies by a variable magnitude, showing that nonoxidative metabolism usually increases much more than oxidative metabolism, which can be either unchanged or increased somewhat ($\sim$10% to 25%), depending on the paradigm and brain structures involved (Dienel and Cruz (2008) and cited references). The basis for this phenomenon (sometimes called aerobic glycolysis) remains to be elucidated, and it contrasts the brain's capacity to increase CMR_{O2} by 2- to 3-fold during seizures and maintain the increase for 2 hours (Meldrum and Nilsson, 1976; Borgström *et al*, 1976). The activation-induced CMR_{O2}–CMR_{glc} mismatch is consistent with increased glycolysis without local oxidation of the lactate equivalents generated.

Lactate and Neuronal Function in Brain Slices

Levels of lactate transporters at the blood–brain barrier and enzymes that metabolize ketone bodies decrease drastically after weaning (Cremer, 1982; Vannucci and Simpson, 2003), and blood lactate and ketones are not major fuels for the adult brain unless their concentrations increase markedly. However, during hypoxia/ischemia, glucose/glycogen-derived lactate accumulates in brain tissue. The notion that lactate may 'jump start' neuronal recovery after restoration of blood flow and oxygen delivery was proposed after the discovery that lactate supported electrically evoked action potentials in brain slices (Schurr *et al*, 1988; Schurr, 2006). However, other investigators previously found that lactate and other alternative substrates cannot substitute for glucose, and evoked action potentials fail even though ATP levels are maintained (see Figure 4 and related text in Dienel and Hertz (2005)). The ability of lactate to support evoked action potentials depends on the speed of slice preparation and other technical issues that are not fully understood (Okada and Lipton, 2007). Moreover, lactate cannot prevent anoxic depolarization in slices from P12 and P28 rats when glycolysis is completely inhibited (Allen *et al* (2005) and discussion therein). These findings indicate that lactate oxidation can support cellular functions or contribute to brain energetics under specific experimental conditions. However, glycolytic metabolism of glucose satisfies critical functions (Figure 1) that cannot be fulfilled by lactate or mitochondrially generated ATP, and maintenance of specific brain function requires glucose, not lactate, under many experimental conditions.

Extracellular Lactate Levels Increase During Activating and Pathophysiologic Conditions

Microdialysis (Korf and de Boer, 1990) and microelectrode (Hu and Wilson, 1997*a*,) technology enabled monitoring of extracellular glucose and

lactate levels. Many investigators have reported ~2-fold increases in extracellular lactate levels during various behaviors or stresses, and these findings are often used to support the idea that glycolytic flux increases. However, lactate concentration changes must be interpreted with caution (Veech, 1991) because metabolite concentration is the net result of input to and output from a pool, and it does not report flux through the pool.

Lactate and Excitatory Neurotransmission

In 1994, Pellerin and Magistretti (1994) reported that glutamate stimulated CMR_{glc} and lactate release in cultured astrocytes, and proposed that glutamate uptake stimulates astrocytic glycolysis and the lactate serves as fuel for nearby neurons. This concept, the astrocyte–neuron lactate shuttle hypothesis, posits that (1) the two ATP required by astrocytes to dispose of the Na^+ taken up with glutamate and to convert glutamate to glutamine are satisfied by glycolysis and (2) there is a predominant cellular compartmentation of glycolytic and oxidative metabolism in astrocytes and neurons, respectively, during excitatory neurotransmission, with lactate shuttling to neurons and neuronal oxidation of lactate as major fuel (Hyder *et al*, 2006; Pellerin *et al*, 2007; Pellerin, 2008; Magistretti, 2009; Jolivet *et al*, 2010).

Cerdán *et al* (2006) proposed a different mechanism and role for astrocyte–neuron lactate trafficking, i.e., redox shuttling in which reducing equivalents are hypothesized to be transferred from astrocytes to neurons. In this model, lactate release from astrocytes and its uptake and oxidation to pyruvate in neurons transfers NADH to neurons. However, the pyruvate is not retained and oxidized in the neurons. Instead, pyruvate is released, taken up by astrocytes, and reduced to lactate to regenerate NAD^+ in the astrocyte. This mechanism could thereby support glycolytic metabolism in astrocytes by means of a transcellular redox shuttle cycle instead of the intracellular, malate–aspartate shuttle (MAS) that transfers reducing equivalents from cytoplasmic NADH to the mitochondria for oxidation and ATP generation (Figure 1).

Discordant metabolic effects of glutamate on cultured astrocytes, complex biochemical and cellular responses to activation, oxidation of lactate by both neurons and astrocytes *in vitro* and *in vivo*, and rapid, substantial lactate release from the brain during *in vivo* activation have been cited as evidence against the brain's use of lactate as a major fuel during normal adult brain activation under physiologic conditions (Hertz *et al*, 1998, 2004, 2007; Chih *et al*, 2001; Chih and Roberts, 2003; Dienel and Cruz, 2003, 2004, 2006, 2008; Dienel and Hertz, 2001, 2005; Mangia *et al*, 2009*a*; Zielke *et al*, 2009). In addition, major metabolic responses to activation of the cerebellum *in vivo* are linked to postsynaptic events, with no detectable effect of blockade of astrocytic glutamate uptake on evoked metabolic activity. The AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid) receptor blockade, not astrocytic glutamate transport inhibition, eliminates stimulus-induced increases in extracellular lactate level, CMR_{glc}, CMR_{O2}, and CBF in the cerebellum *in vivo* (Caesar *et al*, 2008), separating metabolic activation from glutamate transport.

A contrasting transport-metabolism model that emphasizes concentrations and kinetic properties of cellular glucose and lactate transporters predicts that neurons take up most glucose during activation and release lactate to astrocytes, i.e., a neuron-to-astrocyte lactate shuttle (Simpson *et al*, 2007; Mangia *et al*, 2009*b*, 2011; DiNuzzo *et al*, 2010*a*,). A mechanism that may explain, in part, increased neuronal lactate production during activation comes from *in vitro* studies of regulation of mitochondrial metabolism by calcium (Bak *et al*, 2009; Contreras and Satrústegui, 2009). In brief, extramitochondrial Ca^{2+} binds to the aspartate–glutamate carrier (aralar) that is predominant in neurons and a component of the MAS. The MAS transfers reducing equivalents from cytoplasmic NADH to the mitochondria and regenerates NAD^+ to maintain glycolytic flux and produce pyruvate for oxidative metabolism (Figure 1). Small $[Ca^{2+}]$ signals stimulate MAS activity, whereas large $[Ca^{2+}]$ signals arising from Ca^{2+} entry into the mitochondria via the Ca^{2+} uniporter activate pyruvate, α-ketoglutarate, and isocitrate dehydrogenases and increase tricarboxylic acid (TCA) cycle flux (Pardo *et al* (2006) and cited references). However, Ca^{2+} activation of the MAS and TCA cycle are competitive, with preferential retention of α-ketoglutarate in the TCA cycle, thereby limiting its role in the MAS; low MAS activity would cause lactate production to increase in activated neurons (Bak *et al*, 2009; Contreras and Satrústegui, 2009) and it would impair neuronal lactate oxidation (Figure 1).

To sum up, the role of lactate during activation has been a difficult, controversial topic owing, in part, to technical difficulties associated with comprehensive, quantitative *in vivo* assays of metabolism and metabolite trafficking and to temporal-spatial limitations of current methodology. Brain lactate metabolism is complex and *in vivo* studies are required to establish its role during brain activation.

Lactate is Fuel for the Human Brain when Exercise Increases Blood Lactate Levels

During strenuous physical work, human plasma lactate increases from ~0.5 to 1 mmol/L to 20 to 30 mmol/L, and whole-brain studies of metabolic activity during exercise reveal progressive increases in brain lactate uptake and metabolism as work load and plasma lactate levels increase (Ide *et al*, 1999, 2000). Blood lactate is oxidized in the brain and more glucose is also consumed during exhaustive exercise,

but there is also a decline in the oxygen/(glucose + $\frac{1}{2}$ lactate) utilization ratio from ~6 to as low as 1.7, and there is a large, unexplained excess carbohydrate taken up into brain that is not accounted for by oxidative metabolism or tissue metabolite accumulation or release (Dalsgaard, 2006; Quistorff *et al*, 2008; van Hall *et al*, 2009).

Lactate can Stimulate Vasodilation

Gap junction-coupled astrocytes can avidly take up lactate from extracellular fluid and are poised to discharge it from their endfeet into perivascular fluid where pulsatile pressure can drive the lactate along the vasculature (Gandhi *et al* (2009); Ball *et al* (2007, 2010) and cited references). Several studies have reported that lactate increases vasodilation by different mechanisms (Hein *et al*, 2006; Yamanishi *et al*, 2006; Gordon *et al*, 2008), and continuous lactate release from the activated brain may serve a signaling function to increase blood flow and fuel delivery to the brain. As glucose delivery to the brain exceeds demand for glucose over a wide range of CMR_{glc} (Cremer *et al*, 1983; Hargreaves *et al*, 1986), lactate release and its use as a blood flow regulator need not be a 'waste' of fuel, because lactate can be used by peripheral tissues as fuel or as a gluconeogenic substrate.

Summary

Evidence for increased glycolysis and lactate release from the brain to the blood during brain activation in normal subjects with low plasma glucose levels during normal and pathophysiological conditions has accumulated since the 1970s. Strenuous exercise increases blood lactate levels and floods the brain with an alternative substrate that is oxidized in increased amounts. Flooding experiments in cultured cells and brain slices also show lactate oxidation and reduced glucose utilization, and these assays mimic strenuous exercise, not sedentary subjects. Lactate is generated and oxidized by neurons and astrocytes, but the magnitude and direction of cell-to-cell lactate shuttling coupled to its oxidation or release from the brain remains to be established *in vivo*. Continuous lactate release may serve an important CBF-regulatory function.

Aspects of Experimental Systems Relevant to Interpretation of Lactate as Brain Fuel

The lactate literature is very extensive and involves many different experimental systems. Experiments often focus on specific aspects of a more complex system, and comparative data interpretation requires a broad perspective, context, and attention to experimental details.

Properties and Physiology of the Experimental System

Assessment of all studies must take into account age, nutritional status, anesthesia, and physiologic state. Brain growth and metabolic and functional development have enormous spurts between 10 and 21 days, with slower increases thereafter (Baquer *et al*, 1975). Particular care must be taken when translating findings obtained in cells or tissue from prenatal, early postnatal, and weanling subjects to the adult brain owing to downregulation of specific transport and metabolic activities after weaning and to continued brain grown for weeks after weaning. Brain slices obtained from immature or adult brains have cell–cell interactions acquired through normal development, but they are damaged by preparative procedures and postmortem ischemia and have lower metabolic rates than *in vivo* owing to deafferentation. Slices have no blood flow and are dependent on diffusion of fuel and oxygen from the incubation medium. Cultured cells derived from embryonic and newborn animals have very low levels of metabolic enzymes when the tissue is harvested, and transport and metabolic capability may be geared to the early prenatal or postnatal and suckling stages of development, i.e., for use of lactate and ketone bodies more than glucose. Different cell types and brain regions mature at different ages, and neurons that survive tissue dissociation and multiply in culture are considered to be recently postmitotic neurons that have not developed a lot of processes. Cerebral cortical neuronal cultures obtained from ~15-day-old embryos are used as a model system for GABAergic neurons (culture conditions apparently select against glutamatergic neurons; Yu *et al* (1984)), and cerebellar granule neurons obtained from ~7-day-old postnatal rodents are used as a model system for glutamatergic neurons (Schousboe *et al* (1985); Hertz *et al* (1988) and cited references). Harvest age, culture duration, conditions, medium composition, and cellular development during culturing influence characteristics of cultures (Hertz *et al* (1998), Hertz (2004) and cited references), as well as any acquired pathophysiology during culturing (e.g., 15 to 30 mmol/L glucose causes diabetic complications; Gandhi *et al* (2010)). The capacity to use glucose or lactate by cultured astrocytes and neurons grown for <2 weeks *in vitro* need not be equivalent to the adult brain.

Lactate Concentration and Utilization Rates

Brain lactate concentration in normal, carefully handled resting subjects is ~0.2 to 1 μmol/g, and it approximately doubles during activation. The quantity of lactate that accumulates in the brain during an activation episode is <5% of the pyruvate formed from glucose (Dienel *et al*, 2007*a*). The lactate level in a normal resting brain is linearly related to that of pyruvate (Dienel and Cruz, 2008), and its increase

during activation probably reflects an increase in pyruvate concentration. The arterial plasma lactate level in resting subjects is often lower than that in the brain, and it increases with physical activity. However, even during exhaustive exercise, human brain lactate does not accumulate above ~1 mmol/L (Quistorff *et al*, 2008). Large increases in brain lactate level are abnormal (Siesjö, 1978), and metabolic assays using high, flooding doses of lactate (greater than ~3 mmol/L) mimic brain pathology or physically active subjects.

Fractional Contribution of Lactate to Overall Metabolism

Lactate is sometimes called a 'preferred substrate' compared with glucose. Within this context, the notion of 'equi-caloric' concentrations of glucose and lactate (1 glucose = 2 lactate) is sometimes used as a framework for testing relative concentrations of each substrate. However, this is a specious concept because glycolysis is highly regulated (by activation and inhibition) at many steps, whereas lactate dehydrogenase (LDH)-mediated formation of pyruvate from lactate is an equilibrative reaction (lactate + NAD^+ ↔ pyruvate + NADH + H^+) that is not governed by metabolic demand nor fine-tuned by intricate regulation. Lactate concentration is influenced by pyruvate level, pH, NADH/NAD ratio, and other reactions coupled to the NADH–NAD redox system (Veech, 1991). Lactate cannot fulfill many functions of glucose metabolism (Figure 1) and elevated concentrations of lactate reduce glucose utilization in a concentration-dependent manner in cultured astrocytes (Swanson and Benington, 1996; Rodrigues *et al*, 2009), cultured neurons (Bouzier-Sore *et al*, 2006), and brain *in vivo* (Wyss *et al*, 2011). This feature of lactate utilization is consistent with its use as an opportunistic, glucose-sparing substrate when available in blood in high amounts, as during intense exercise.

Transport of glucose and of lactate plus H^+ is equilibrative, and unidirectional uptake rates will increase with substrate concentration until the transporters are saturated. Brain glucose levels are typically ~20% to 25% that of arterial plasma, and once hexokinase is saturated (its K_m for glucose is ~0.05 mmol/L), further increases in glucose concentration do not increase glucose utilization rate in the rat brain *in vivo* (Orzi *et al*, 1988) or in isolated synaptosomes (Bradford *et al*, 1978) owing to feedback-regulatory mechanisms that coordinate CMR_{glc} with ATP demand and ADP availability as cosubstrate for reactions that produce ATP. In contrast, lactate-pyruvate interconversion is driven by concentration gradients. The higher the lactate level, the more pyruvate plus NADH + H^+ will be generated until inhibitory levels of pyruvate are reached or MAS activity to regenerate cytoplasmic NAD^+ becomes limiting (Figure 1). It must be noted that the actual metabolic situation in brain tissue is probably much more complex because of compartmentation of intracellular pyruvate/lactate pools and differential fates of pyruvate in different pools that are not considered in this simplified discussion (Cruz *et al*, 2001; Rodrigues *et al*, 2009). Utilization of pyruvate derived from either substrate by the oxidative TCA cycle pathways would then be governed by the same regulatory steps that modulate the rates of the pyruvate dehydrogenase reaction, TCA cycle, and oxidative phosphorylation.

Different monocarboxylic acid transporter (MCT) and LDH isoforms are present in neurons and astrocytes. These isoforms can influence the concentration dependence of the proportion of lactate taken up and metabolized by either cell type because of differences in their K_ms, V_{max}s, and LDH inhibition by pyruvate, but do not govern the direction of lactate flow (see discussions by Veech (1991), Chih *et al* (2001), Chih and Roberts (2003), Gandhi *et al* (2009), and Quistorff and Grunnet (2011*a*, *b*). Lactate transport and its oxidation to pyruvate generate intracellular H^+, and, depending on buffering capacity, reduced intracellular pH may inhibit phosphofructokinase and glycolytic rate. Depletion of NAD^+ by the LDH reaction will reduce its availability for glycolysis (Figure 1). Metabolites generated by lactate oxidation (citrate, ATP, and other TCA cycle compounds) can inhibit brain phosphofructokinase in a very complex manner that depends on the levels of many modulators of this enzyme and pH (Passonneau and Lowry, 1963; Lowry and Passonneau, 1966). Of interest is the lack of effect of glutamate (Passonneau and Lowry, 1963), 10 to 100 mmol/L glucose, 2 mmol/L creatine, 0.2 mmol/L pyruvate, 3 mmol/L lactate, 0.06 mmol/L acetyl CoA, and 0.3 mmol/L α-ketoglutarate on the activity of brain phosphofructokinase (Krzanowski and Matschinsky, 1969). In contrast, 5 to 10 mmol/L lactate inhibits skeletal muscle phosphofructokinase (Costa Leite *et al*, 2007), suggesting that there may be different regulatory mechanisms involving lactate in the muscle compared with the brain, as observed for TCA cycle intermediates that can modulate phosphofructokinase from the rat brain but not the rat heart (Passonneau and Lowry, 1963).

Many investigators have used high-lactate flooding experiments, and a critical issue that is not always addressed in competitive substrate assays is dilution of labeled pyruvate when labeled glucose or lactate is the tracer. This is important because pyruvate concentration is 10- to 13-fold lower than lactate owing to the LDH equilibrium constant. To interpret inhibition of metabolism of pyruvate derived from glucose compared with that derived from lactate, the specific activity or fractional enrichment of pyruvate (i.e., the ratio of the labeled to unlabeled pyruvate) must be determined and used to calculate the effects of different concentrations of lactate or glucose added to the assay. For example, if labeled glucose generates pyruvate with a specific activity of 1, and

addition of unlabeled lactate reduces pyruvate specific activity to 0.5 and the amount of glucose oxidized by 50%, then lactate had no effect on glucose oxidation. In other words, lactate only depressed pyruvate specific activity and, therefore, reduced the fraction of labeled pyruvate that entered the oxidative pathway. Increasing the level of unlabeled lactate will overwhelm labeling of pyruvate by glucose, whereas increasing glucose concentration will not have much effect on pyruvate labeled by lactate because of regulated metabolism of glucose.

Summary

High levels of extracellular lactate can 'flood the system' and provide a nonregulated source of pyruvate, thereby influencing glucose utilization. However, a 'preference' for lactate that arises from fine-tuned regulation glycolytic enzyme activities by many metabolites is not the same as preferring one of different candies of identical composition and caloric content. If brain-derived lactate was highly 'preferred' over blood-borne glucose as fuel, why would any lactate be released from the brain? Other factors must be involved in substrate utilization. The apparent simplicity of brain lactate metabolism and trafficking during brain activation *in vivo* is deceptive, and knowledge of pyruvate specific activity or fractional enrichment is necessary to interpret effects of lactate on glucose utilization. Unresolved issues include the cellular origin of lactate released into the extracellular fluid, flux through lactate pools, routes for dispersal and release of lactate, and the contribution of lactate oxidation to energetics of brain activation in neurons and astrocytes.

Unexplained Discordant Results Underlie Lactate-Related Controversies

When viewed in isolation, various studies may seem to support or oppose a model for brain lactate metabolism, but when evaluated within a broad context of different data sets related to the same issue, each set can 'speak for itself' and trends or anomalies are easily recognized.

Glutamate Transport-Evoked Glycolysis is not a Robust, Intrinsic Property of all Cultured Astrocytes

Increased CMR_{glc} and lactate production by cultured astrocytes exposed to glutamate in the culture medium is reproducibly observed in some laboratories but not in many others (Table 1). Responsive pure astrocyte cultures have different temporal responses to glutamate compared with astrocytes in mixed astrocyte–neuron cultures (Table 1). The basis for the presence or absence of a glycolytic response to glutamate is unknown (Hertz *et al*, 1998), but may be related to oxidative metabolism of glutamate, which stimulates astrocytic respiration and is oxidized in greater amounts with increasing extracellular level (Table 1). Use of ATP generated from glutamate oxidation to extrude sodium is consistent with the increase in CMR_{glc} evoked by nonmetabolizable D-aspartate (Table 1). In the cerebellum *in vivo*, glutamate transport blockade has no effect on metabolic activation and lactate increase, whereas these changes are eliminated by AMPA receptor inhibition (Caesar *et al*, 2008), ruling out astrocytic glutamate transport-induced glycolysis as a major factor governing blood flow-metabolism upregulation.

Brain Activation *In Vivo* Activates Glycogenolysis and Oxidative Metabolism in Astrocytes

Glycogen turnover is very slow under resting conditions, but astrocytes have significant resting oxidative activity, calculated to be ~15% to 38% of total oxidative metabolism of glucose (Hyder *et al*, 2006; Duarte *et al*, 2011; Hertz, 2011). The astrocytic filopodial processes that surround and interact with synaptic structures contain mitochondria (Lovatt *et al*, 2007; Pardo *et al*, 2011; Lavialle *et al*, 2011) and have the potential to oxidize glucose, glycogen, and glutamate during activation. If brain activation stimulated only glycolysis in astrocytes, it would be reasonable to assign the ATP derived from this pathway toward the energetics of glutamate uptake. However, this is not the case. *In vivo* studies have shown that glycogenolysis, TCA cycle flux, and pyruvate carboxylation (a biosynthetic pathway involving the TCA cycle that also generates NADH and ATP; see Figure 1 and Hertz *et al* (2007)) are all increased in astrocytes *in vivo* under activating conditions (Table 2).

In our studies of acoustic stimulation of conscious rats that assayed both glucose utilization by all cells and acetate oxidation by astrocytes in the inferior colliculus *in vivo*, CMR_{glc} increased by 0.49 μmol/g per min (from 0.71 to 1.20 μmol/g per min, or 69%) and acetate oxidation increased a minimal mean value of 0.02 μmol/g per min (from 0.126 to 0.146 μmol/g per min, or 16%) (see Table 5 in Cruz *et al* (2005)). Assuming 2 ATP produced by glycolysis and 32 ATP from the oxidative pathways (< 38 ATP owing to proton leak), the increase in glycolysis in all cells would produce $2 \times 0.49 = 0.98$ μmol ATP/g per min, and the increase in astrocytic oxidative metabolism would generate $32 \times 0.02 = 0.64$ μmol ATP/g per min. Thus, a minimal estimate of the contribution of increased astrocytic oxidative metabolism (assuming that changes in acetate oxidation reflect those of glucose) is 65% that of total glycolysis in all cells. If half of the glucose is metabolized in astrocytes, then increased oxidative metabolism produces a similar amount of ATP as the increase in glycolysis. If all glycolytic ATP were used to

Table 1 Discordant metabolic responses of cultured astrocytes to glutamate exposure

Preparation	*Glutamate concentration (μmol/L)*	*Glucose utilization*[a]	*Glucose uptake*[a]	*Lactate production*[a]	*O_2 utilization*[a]	*Metabolite oxidation (substrate)*[a]	*Reference*
Forebrain, cerebral cortical, or striatal astrocytes	10–500	+15 to +180		+40 to +55			Pellerin and Magistretti, 1994; Takahashi *et al*, 1995; Debernardi *et al*, 1999; Chatton *et al*, 2003
	10–1,000	+7 to −60	0 to −27	0 to −60		−25 to −75 (glucose)	Hertz *et al*, 1998; Swanson *et al*, 1990; Peng *et al*, 2001; Qu *et al*, 2001; Gramsbergen *et al*, 2003; Liao and Chen, 2003; Dienel and Cruz, 2004
	100				+55		Eriksson *et al*, 1995
	100 → 500					15% → 40% (glutamate)	McKenna *et al*, 1996
	200 20,000 (D-Aspartate, 500–1,000)	0[b] +18[b] +20					Prebil *et al*, 2011 Peng *et al*, 2001
Hippocampal astrocytes in mixed astrocyte–neuron cultures	500		2-NBDG, +110[c] 6-NBDG, +180[c]				Loaiza *et al*, 2003
	50, Acute 50, 20 minutes 5, 20 minutes (50, D-Aspartate)	−20[b] +275[b] +300[b] 0[b]		+130			Bittner *et al*, 2011

NBDG, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)2-deoxyglucose.

[a]Magnitude of response is expressed as approximate percentage change owing to treatment, 100[(treated−control)/control]. For details, see the original references and Tables 5 and 6 of Dienel and Cruz (2004). Except where noted, glucose utilization was assayed with deoxyglucose. Effects of D-aspartate, a nonmetabolizable substrate for the glutamate transporter, were also tested in some studies.

[b]Fluorescence resonance energy transfer (FRET) assays based on nanosensors that bind to intracellular glucose and report glucose concentration; glucose utilization is based on the change in glucose concentration, which does not reveal the pathway(s) that consume the glucose (glycolysis, pentose phosphate shunt pathway, glycogen synthesis, or sorbitol production if glucose levels are high).

[c]Uptake assays were 10 minutes and measured change in fluorescence with time. 6-NBDG reflects only transport, whereas 2-NBDG uptake can reflect both transport and phosphorylation because there was no washout of unmetabolized precursor.

Table 2 Stimulus-induced increased metabolism in astrocytes in the rat or human brain *in vivo*

Experimental condition and substrate fuel	*Percentage increase in pathway activity*		*References*
	Glycogenolysis (glycolysis)	*TCA cycle*	
Sensory stimulation-cerebral cortex, conscious rat			
Glycogen consumption (stimulation and recovery)	14–32		Cruz and Dienel, 2002
Release of label from glycogen	22		Dienel *et al*, 2007*a*
Anesthetized rat			
Acetate oxidation		14	Wyss *et al*, 2009
Metabolic washout of acetate-derived label		93	
Conscious human—washout of acetate label		62	Wyss *et al*, 2009
Acoustic stimulation-inferior colliculus, conscious rat			
Release of label from glycogen	12		Dienel *et al*, 2007*a*
Acetate oxidation		15–18	Cruz *et al*, 2005
Visual stimulation-conscious rat			
Release of label from glycogen, superior colliculus	13		Dienel *et al*, 2007*a*
Acetate oxidation superior colliculus		25–30	Dienel *et al*, 2007*b*
Acetate oxidation lateral geniculate		14–20	
Operant training, conscious rat			Dienel *et al*, 2003
Acetate oxidation in five brain regions		15–24	
Spreading cortical depression-cerebral cortex			
Conscious rat, acetate and butyrate oxidation		15–40	Dienel *et al*, 2001
Anesthetized rat, glycogen consumption	10–28		Krivanek, 1958
Pyruvate carboxylation, whole brain			
Pentobarbital-anesthetized to awake rat		~400	Öz *et al*, 2004

TCA, tricarboxylic acid.
Glycogen and pyruvate carboxylase are located predominantly in astrocytes, and acetate is preferentially oxidized by astrocytes. These markers can be used to reveal changes in pathway fluxes in astrocytes in the brain *in vivo*. The fate of glycogen-derived pyruvate *in vivo* is not known; hence, glycogenolysis is considered to reflect glycolysis. As (1) calculated TCA cycle rates determined with [^{13}C]acetate and [^{13}C]glucose are similar (Hyder *et al*, 2006) and (2) calculated [^{14}C]acetate oxidation is within the range of that estimated for astrocytic glucose oxidation (Cruz *et al*, 2005), it is likely that acetate oxidation rate reflects glucose oxidation rate in astrocytes. Pyruvate carboxylation is part of the anaplerotic pathway located in astrocytes (not neurons) for *de novo* synthesis of aspartate, glutamate, glutamine, and GABA from glucose (Figure 1). Increased anaplerotic flux generates ATP through NADH formed by pyruvate and isocitrate dehydrogenase reactions.

power Na^+-K^+-ATPase to extrude sodium taken up with glutamate into astrocytes, then other unidentified, upregulated energy-requiring processes consume at least half of the additional ATP generated by the astrocytes. Contributions of glycogenolysis and oxidative flux related to pyruvate carboxylase activity (Table 2) are not included and would increase the total ATP produced by astrocytes further. Although speculative and approximate, this calculation suggests that working astrocytes are not well understood, and that further experiments are required to evaluate the energetics of astrocytic activation *in vivo*.

Cultured Neurons and Presynaptic Endings from the Adult Brain Increase Glucose Utilization

Arguments used by Jolivet *et al* (2010) in support of the need of neurons for lactate as fuel during activation include inability of neurons to increase glucose transport (citing Porras *et al* (2004) and a few other studies) and glycolysis (citing Herrero-Mendez *et al* (2009)). The Bolaños–Almeida–Moncada group has carried out an elegant series of studies (reviewed by Bolaños and Almeida (2010)) designed to elucidate the basis for high sensitivity of cultured cerebral cortical neurons to respiratory inhibition by nitric oxide (NO) and neuronal inability to increase glycolysis when treated with NO (Table 3). In brief, they showed that the enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase isoform 3 (Pfkfb3) that makes a potent allosteric activator of 6-phosphofructo-1-kinase (i.e., PFK, see Figure 1) is constantly degraded in cultured cortical neurons but not in cultured cortical astrocytes. Their cortical neurons have a lower glycolytic rate than do astrocytes, and neurons divert glucose-6-phosphate into the pentose phosphate shunt pathway to produce NADPH for management of oxidative stress (Figure 1). The study by Herrero-Mendez *et al* (2009) extended these findings by showing upregulation of neuronal Pfkfb3 confers to neurons the ability to increase glycolysis and lactate production at the expense of glucose-6-P flux into the pentose shunt pathway (Table 3). Bolaños and Almeida (2010)

Table 3 Metabolic responses of cultured neurons derived from different brain regions to activating conditions

Brain region and harvest age[a]	*Treatment*	*Response magnitude*[b]	*Reference*
Cerebral cortex	(Cortical neurons, a model for GABAergic neurons[a])		
E14	5 → 50 mmol/L K^+	10% ↑ DG phosphorylation 20% ↑ [U-^{14}C]glucose to $^{14}CO_2$ (low rate) 20% ↓ [U-^{14}C]lactate to $^{14}CO_2$ 115% ↑ [2-^{14}C]pyruvate to $^{14}CO_2$ (very low rate)	Peng *et al*, 1994
E16–17	1.4 μmol/L nitric oxide	85% ↓ O_2 uptake, no change in lactate production	Almeida *et al*, 2001
	100 μmol/L Glutamate	No change in lactate production	Almeida *et al*, 2004
	Overexpression of 6-phospho-fructo-2-kinase/fructose-2, 6-bisphosphatase-3	550% ↑glycolysis, 190% ↑lactate level, 50% ↓pentose phosphate shunt pathway (PPP) flux (control neurons: PPP flux = 200% glycolytic flux)	Herrero-Mendez *et al*, 2009; Bolaños and Almeida, 2010
E17	Hypoxia, 3 days	200% ↑ glucose utilization 330% ↑ lactate concentration in medium	Malthankar-Phatak *et al*, 2008
E16–17	Hypoxia for 24 hours	200% ↑ lactate concentration in medium	Sher, 1990
E15	55 mmol/L K^+	50% ↑ cycling ratio for glutamate = increased TCA cycle activity	Waagepetersen *et al*, 2000
E18	33 μmol/L Glutamate	200–250% ↑ oxygen consumption with glucose substrate	Gleichmann *et al*, 2009
	7–32 μmol/L FCCP	200–250% ↑ oxygen consumption with glucose substrate	
E15	25 nmol Dinitrophenol	20% ↑ oxygen consumption with glucose substrate	Jameson *et al*, 1984
E17	2 μmol/L amyloid-β_{1-42} , 4 days	200% ↑ [1-^{14}C]glucose to $^{14}CO_2$; 155% ↑ [6-^{14}C]glucose to $^{14}CO_2$; 205% ↑ pentose phosphate shunt pathway	Soucek *et al*, 2003
Cerebellum	(Cerebellar granule neurons, a model for glutamatergic neurons[a])		
PN7	5 → 50 mmol/L K^+	75% ↑ DG phosphorylation 120% ↑ [U-^{14}C]glucose to $^{14}CO_2$ 20% ↑ [U-^{14}C]lactate to $^{14}CO_2$ 110% ↑ [2-^{14}C]pyruvate to $^{14}CO_2$	Peng *et al*, 1994
PN7	50 μmol/L Glutamate 500 μmol/L Glutamate 5.4 → 55 mmol/L K^+	30% ↑ DG phosphorylation 40% ↑ DG phosphorylation 75% ↑ lactate production rate; 75% ↑ [U-^{14}C]glucose to $^{14}CO_2$	Peng and Hertz, 2002
PN8	25 nmol Dinitrophenol	43% ↑ oxygen consumption with glucose substrate	Jameson *et al*, 1984
PN6–7	Hypoxia, 7h	100% ↑ lactate production	Sonnewald *et al*, 1994
PN8	100 μmol/L Glutamate	115% ↑ DG uptake plus phosphorylation (10 minute assay)	Minervini *et al*, 1997
	100 μmol/L NMDA	180% ↑ DG uptake plus phosphorylation (10 minute assay)	
	60 μmol/L Kainate	220% ↑ DG uptake plus phosphorylation (10 minute assay)	
	100 μmol/L Quisqualate	55% ↑ DG uptake plus phosphorylation (10 minute assay)	
PN5–7	Respiration assays in 25 mmol/L K^+		Jekabsons and Nicholls, 2004
	2 μmol/L FCCP	175% ↑ oxygen consumption with glucose substrate	
	250 μmol/L Glutamate + 25 μmol/L glycine	32–60% ↑ oxygen consumption with glucose substrate	
	300 μmol/L NMDA	33–36% ↑ oxygen consumption with glucose substrate	

Table 3 *Continued*

Brain region and harvest age[a]	*Treatment*	*Response magnitude*[b]	*Reference*
PN5–7	Respiration assays in 3.9 mmol/L K^+		Yadava and Nicholls, 2007
	3 μmol/L FCCP	250–325% ↑ oxygen consumption with glucose substrate	
	100 μmol/L Glutamate + 10 μmol/L glycine	250–325% ↑ oxygen consumption with glucose substrate	
PN7–8	DG assays in 3.9 mmol/L K^+		Ward *et al*, 2007
	100 μmol/L Glutamate + 10 μmol/L glycine for 10 minutes, then new medium with no glutamate + [^{3}H]DG for 20 minutes	50% ↑ DG phosphorylation (reflecting ↑ glucose utilization)	
Hippocampus			
Neurons in mixed Neuron–astrocyte Cultures (PN1–3)	500 μmol/L Glutamate or 20 μmol/L AMPA	75–80% ↓ 2- or 6-NBDG uptake—fast, reversible	Porras *et al*, 2004
	75 μmol/L veratridine	No change in 6-NBDG uptake for ~10 minutes, then 70% ↓	
	40 mmol/L KCl	No effect on 6-NBDG uptake	
Neurons (E18)	100 μmol/L Glutamate, 10 minutes	No effect on DG uptake	Patel and Brewer, 2003
	1 μmol/L FCCP + 10 mg/mL oligomycin	200% ↑ DG uptake at 5 minutes	
	100 μmol/L Glutamate + 1 μmol/L FCCP + 10 mg/mL oligomycin	135% ↑ DG uptake at 5 minutes	
Neurons (PN0)	Acute anoxia	40% ↑ DG uptake	Yu *et al*, 2008
	Acute anoxia after hypoxic preconditioning, 20 minutes/day for 6 days	90% ↑ DG uptake	

AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid; DG, deoxyglucose; NBDG, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)2-deoxyglucose; TCA, tricarboxylic acid.

[a]The age at which tissue was obtained for cultured cells is denoted by E = embryonic or PN = postnatal, followed by the age in days. Cerebral cortical neuronal cultures obtained from ~15-day-old embryos are used as a model system for GABAergic neurons, and cerebellar granule neurons obtained from ~7 day-old postnatal rodents are used as a model system for glutamatergic neurons (Yu *et al* (1984); Schousboe *et al* (1985); Hertz *et al* (1988) cited references). It must be noted that culture conditions and duration, composition of the culture medium, and cellular development during time in culture influence characteristics of the cultures (e.g., Hertz *et al* (1998); Hertz (2004) cited references).

[b]Magnitude of response is expressed as approximate percentage change owing to treatment, 100[(treated−control)/control)]. DG assays are typically used to measure hexokinase-dependent phosphorylation as a measure of glucose utilization. 2-NBDG is a fluorescent glucose analog that is transported and phosphorylated, whereas 6-NBDG is transported but not phosphorylated. Brief assays (< 5 minutes) with DG or 2-NBDG measure mainly transport (uptake), intermediate duration assays (5–10 minutes) can represent transport plus phosphorylation depending on washout of unmetabolized precursor from cells at the end of the assay, and longer assays with DG or 2-NBDG reflect mainly phosphorylation that can be overestimated somewhat if unmetabolized precursor is not completely washed out. 6-NBDG accumulation reflects transport until intracellular and extracellular levels equilibrate. See legend to Table 4 for more details related to sites of action of metabolic inhibitors.

stated that different regulatory mechanisms may operate in other preparations and brain regions.

In fact, many laboratories have shown that different types of cultured neurons can substantially upregulate glucose metabolism, whereas a few preparations have no or small responses. Many cerebral cortical neuron preparations (a model for GABAergic neurons) do respond to many treatments (e.g., depolarization, hypoxia, exposure to glutamate, treatment with uncouplers or amyloid-β) with quite large increases in glycolysis and glucose-supported respiration, indicating that glucose transport must increase in parallel (Table 3). Cultured cerebellar granule cell neurons (a model for glutamatergic neurons) also exhibit large metabolic responses to depolarization, uncouplers, hypoxia, glutamate, NMDA, and other conditions (Table 3). Cerebral glucose utilization in cultured hippocampal neurons increases during exposure to uncouplers and anoxia, and is not affected by glutamate (Table 3). Conversely, hippocampal neurons in mixed astrocyte–neuron cultures exhibit reduced NBDG uptake upon glutamate exposure, delayed inhibition of NBDG uptake after veratridine exposure, and no response to depolarization (Table 3). The inability of neuronal cultures to respond to activating conditions by increasing CMR_{glc}, glycolysis, pentose shunt flux, or respiration is an exception, not the rule (Table 3).

Synaptosomes embody the metabolic capabilities of nerve endings from the mature brain, although their capacity may be reduced by losses of soluble enzymes, ATP, Pi, and phosphocreatine during preparative procedures. Synaptosomes isolated from adult brain regions, including the hippocampus and cerebral cortex from different species, have high metabolic capacity and respond with large increases

in glucose-supported respiration to depolarization, uncouplers, anoxia, enhanced ion fluxes, and NO donors (Table 4). Inhibition of MAS with aminooxyacetate reduces uncoupler-evoked respiration (Table 4). Glycolysis and glucose-supported respiration in hippocampal and cortical synaptosomes are enhanced by K^+ and veratridine (Table 4), sharply contrasting the responses of hippocampal neurons in mixed cultures (Table 3). The magnitude of response to Na^+-stimulated glucose oxidation increases with developmental age, and is much higher in synaptosomes isolated from adult compared with the immature brain (Table 4).

To summarize, many preparations of cortical, cerebellar, and hippocampal neurons and synaptosomes upregulate various pathways of glucose metabolism under many different conditions. However, cultured neurons derived from different brain regions may not have the same metabolic capacities or responses to the same treatment. Synaptosomes are one structure of adult brain neurons that is readily isolated, and these nerve terminals can increase glycolysis and respiration by 5- to 10-fold *in vitro*. Glucose transport and glycolytic flux must increase in parallel with glucose-supported respiration. Citation of selected metabolic studies that support a point of view (Jolivet *et al*, 2010) does not provide an appropriate perspective of the field.

Neurons can Quickly Upregulate Glucose Transport Capacity During Activation

Glutamate inhibits NBDG transport into cultured neurons (Porras *et al*, 2004; Table 3) and stimulates glucose transport into cultured astrocytes (Loaiza *et al*, 2003; Table 1). These findings have been interpreted by Pierre *et al* (2009) as rerouting of glucose from neurons to astrocytes during glutamatergic neurotransmission, so neurons would depend on astrocyte-derived lactate as a fuel, in accordance with the astrocyte-neuron lactate shuttle hypothesis. However, these results sharply contrast those from other neuronal cultures that exhibit glutamate-induced increases in CMR_{glc} and 2- to 3-fold stimulation of glucose-supported respiration by glutamate in cultured cerebral cortical neurons and cerebellar granule neurons (Table 3). Moreover, nerve endings isolated from both immature and adult brains are capable of large increases in glycolysis and glucose-supported respiration (Table 4). Therefore, neuronal glucose transport must increase simultaneously with stimulation of its utilization.

Neuronal glucose transport capacity is enhanced within minutes by treatment of cultured neurons with glutamate, bicuculline, and a NO donor by increasing cell-surface expression of the neuronal glucose transporter (GLUT)3 throughout the neuronal processes and soma (Table 5). Upregulation of the GLUT3 protein level is slower than cell-surface translocation, and is stimulated *in vivo* by conditions that affect CMR_{glc} in the brain (Table 5). Glucose transport into neurons is critical for brain function, and in the adult rat brain, GLUT3 is localized in synaptic terminals, small neuronal processes, and postsynaptic structures, with significant intracellular localization (Leino *et al*, 1997). Glucose transporter-3 deficiency causes serious developmental abnormalities in mice (Table 5). Taken together, the rapid increases in glucose transport capacity by cultured hippocampal, cortical, and cerebellar neurons and synaptosomes plus increased glucose utilization show that neurons require and consume more glucose when activated.

Dendritic Spine Energetics: Is Monocarboxylic Acid Transporter-2 used for Lactate Release?

Neuronal MCT2 and AMPA receptor GluR2/3 are colocalized in postsynaptic densities of glutamatergic synapses between parallel fibers and Purkinje cells in the cerebellum (Bergersen *et al*, 2001, 2005), and these two proteins are translocated to the cell surface from intracellular stores in parallel under activating conditions (Pierre *et al*, 2009). Monocarboxylic acid transporter-2 localization and trafficking are claimed to facilitate uptake of astrocyte-derived lactate as oxidative fuel for these glutamatergic spines (Bergersen *et al*, 2005, 2007; Pierre *et al*, 2009). However, spines do not contain the mitochondria (Bergersen *et al*, 2001, 2002); hence, lactate, ADP, and phosphate must diffuse through the spine neck to the mitochondria in the dendritic shaft, followed by lactate oxidation and synthesis of ATP, then diffusion of ATP back to postsynaptic density for its utilization. This scenario does not include glucose transport and metabolism in spines, and to understand the energetics of dendritic structures more fully, it is important to know the relative levels of GLUT3 compared with MCT2 in presynaptic and postsynaptic structures and to evaluate glucose and lactate metabolism in these structures.

Most dendritic spines have very few mitochondria, in contrast to the shafts (Li *et al*, 2004; Bourne and Harris, 2008). Postsynaptic densities contain glycolytic enzymes that synthesize ATP (Wu *et al*, 1997), and GLUT3 is localized in synaptic endings and postsynaptic structures (Leino *et al*, 1997). Calcium clearance in activated cultured cerebellar granule neurons and in Purkinje cells in brain slices relies on glycolysis to power the plasma membrane Ca^{2+}-ATPase in the soma, dendrites, and spines, and inhibition of mitochondrial ATP generation does not affect operation of this pump (Ivannikov *et al*, 2010). These findings underscore the importance of glycolysis in neuronal dendritic spines and show that diffusion of ATP from the dendritic shaft into the spine cannot support calcium pumping at the plasma membrane of spines. Therefore, trafficking of MCT2 might be required to release lactate generated by glycolysis in the spine into extracellular fluid, so that

Table 4 Metabolic responses of synaptosomes to activating conditions

Substrate[a] *and treatment*[b]	*Response magnitude*[c]	*Tissue source and Reference*
Electrical stimulation	20–65% ↑respiration rate, 10–70% ↑ glycolysis	Seven brain regions from adult rat, sheep, or rabbit
Glucose + 50 mmol/L K^+	15% ↑ respiration rate, 30% ↑ glycolysis	Bradford, 1975
10 mmol/L glucose + 24 mmol/L KCl	137% ↑ respiration rate 85% ↑ [1-^{14}C]pyruvate decarboxylation	Cerebral cortex, adult rat Schaffer and Olson, 1980
10 mmol/L glucose + 40 mmol/L KCl 5 mmol/L pyruvate + 40 mmol/L KCl	69% ↑ respiration rate 63% ↑ respiration rate	Adult rat forebrain Erecińska *et al*, 1991
9.5 mmol/L [U-^{14}C]glucose + 72 mmol/L Na^+ + 100 μmol/L 2,4-dinitrophenol	180% ↑ glucose oxidation to $^{14}CO_2$ 370% ↑ glucose oxidation to $^{14}CO_2$	Whole brain from adult or immature rats Diamond and Fishman, 1973
Developmental age from 1 to 90 days	500%↑ Na^+-stimulated glucose oxidation to $^{14}CO_2$	
Developmental age from 10 to 20 days	200%↑ Na^+-stimulated glucose oxidation to $^{14}CO_2$	
1 mmol/L [U-^{14}C]glucose + 100 μmol/L veratridine	290% ↑ glucose oxidation to $^{14}CO_2$	Adult rat forebrain Harvey *et al*, 1982
10 mmol/L glucose + 100 μmol/L veratridine	300% ↑ respiration rate	Adult rat hippocampal mossy fiber synaptosomes Terrian *et al*, 1988
10 mmol/L glucose + 0.5 μmol/L FCCP 10 mmol/L glucose + 100 μmol/L veratridine, followed by 0.5 μmol/L FCCP	Immediate 500% ↑ respiration rate Immediate veratridine-induced 175% ↑ respiration, followed by an immediate additional 65% FCCP-evoked ↑ respiration	Cerebral cortex, 4–8-week-old guinea pig Scott and Nicholls, 1980
1.5 mmol/L glucose + [3,4-^{14}C]glucose + 100 μmol/L 2,4-dinitrophenol	120% ↑ glucose oxidation to $^{14}CO_2$	Adult rat forebrain Ksiezak and Gibson, 1981*a,b*
1.5 mmol/L glucose, severe hypoxia (O_2 tension 19 torr → <1 torr)	250% ↑ lactate production rate	
5 mmol/L glucose + 1 mmol/L cyanide or 6 μmol/L rotenone 5 mmol/L glucose + Nitrogen atmosphere (anoxia) 5 mmol/L glucose + A23187 (divalent cation ionophore)	Block respiration, 9-fold increase glycolysis Ten-fold increase in glycolysis Three-fold stimulation respiration, 5-fold stimulation glycolysis	Cerebral cortex, adult guinea pig Kauppinen and Nicholls, 1986*a*
10 mmol/L glucose + anoxia	335% ↑ lactate amount produced	Adult rat forebrain White *et al*, 1989
10 mmol/L glucose + 1 μmol/L FCCP 10 mmol/L glucose + 1 mmol/L arsenite (inhibit pyruvate oxidation)	900% ↑ glycolysis maintained for at least 30 minutes 500% ↑ respiration 35% inhibition respiration, 3-fold increase in glycolysis	Cerebral cortex, adult guinea pig Kauppinen and Nicholls, 1986*b*
5 mmol/L glucose + 45 mmol/L KCl 5 mmol/L glucose + 75 μmol/L veratridine 5 mmol/L glucose + 1 μmol/L Cl-CCP	55% ↑ glycolysis, 47% ↑pyruvate decarboxylation 250% ↑ glycolysis, 290% ↑pyruvate decarboxylation 650% ↑pyruvate decarboxylation	Cerebral cortex, 6–10-week-old guinea pig Kauppinen *et al*, 1989
10 mmol/L glucose + anoxia 10 mmol/L glucose + 10 μmol/L veratridine	2,100% ↑ lactate synthesis rate 160% ↑ respiration rate, 75% ↓ lactate synthesis rate	Adult rat cerebral cortex Gleitz *et al*, 1993
10 mmol/L glucose + 1 mmol/L nitroprusside or 100 μmol/L *S*-nitrocysteine 10 mmol/L glucose + 10 μmol/L rotenone	Aerobic conditions: 20–30% ↑ lactate synthesis rate; 70% ↑ lactate amount at 15 minutes 900% ↑ lactate synthesis rate	Adult rat forebrain Erecińska *et al*, 1995
10 mmol/L glucose + 40 μmol/L veratridine, 5 minutes 15 minutes 10 mmol/L glucose + 10 μmol/L monensin, 5 minutes	210% ↑ respiration, 515% ↑ lactate production 135% ↑ respiration, 620% ↑ lactate production 73% ↑ respiration, 1,100% ↑ lactate production	Adult rat forebrain Erecińska *et al*, 1996

Table 4 *Continued*

Substrate[a] *and treatment*[b]	*Response magnitude*[c]	*Tissue source and Reference*
15 minutes	70% ↑ respiration, 580% ↑ lactate production	
10 mmol/L glucose + 5 μmol/L nigericin, 5 minutes	58% ↑ respiration, 465% ↑ lactate production	
15 minutes	8% ↑ respiration, 200% ↑ lactate production	
10 mmol/L glucose + 100 (young rats) or 50 (old rats) μmol/L veratridine	120 or 110% ↑ respiration in synaptosomes from young or old rats, respectively	Whole brain from 3-month (young) or 24 (old)-month rats Joyce *et al*, 2003
10 mmol/L glucose + 240 nmol/L FCCP	150 or 88% ↑ respiration in synaptosomes from young or old rats, respectively	
15 mmol/L glucose + 4 μmol/L FCCP	300% ↑ respiration rate	Cerebral cortex, 17–20 day-old mouse Choi *et al*, 2009
10 mmol/L pyruvate + 4 μmol/L FCCP	230% ↑ respiration	
15 mmol/L glucose + 10 mmol/L pyruvate + 4 μmol/L FCCP	225% ↑ respiration rate	
15 mmol/L glucose + 2–5 μmol/L veratridine	100–135% ↑respiration, 200–300%, ↑extracellular acidification (i.e., ↑glycolysis with lactate production and release)	
15 mmol/L glucose + 10 mmol/L pyruvate + 2–5 μmol/L veratridine	210–165% ↑respiration, 180–250% ↑extracellular acidification	
15 mmol/L glucose + 10–100 μmol/L AOAA	No effect on basal respiration rate	
15 mmol/L glucose + 4 μmol/L FCCP + 10 μmol/L AOAA	15% inhibition of maximal FCCP-evoked rate	
+ 30 μmol/L AOAA	30% inhibition of maximal rate	
+ 100 μmol/L AOAA	45% inhibition of maximal rate	
15 mmol/L glucose + 10 mmol/L pyruvate + 0.05–1 mmol/L 4-aminopyridine	35% ↑respiration rate compared with without 4-aminopyridine	
15 mmol/L glucose + 10 mmol/L pyruvate + 4 μmol/L FCCP	Values normalized by number of bioenergetically competent synaptosomes: 447 or 452 ↑ respiration rate in dopamine transporter-enriched synaptosomes from the striatum or cortex, respectively 538 or 542 ↑ respiration rate in residual nondopaminergic synaptosomes from the striatum or cortex, respectively	Cerebral cortex or striatum from 3 to 4 month-old mice Choi *et al*, 2011

[a]Synaptosomes are heterogeneous populations of presynaptic nerve endings that contain mitochondria and are capable of glycolytic and oxidative metabolism of glucose and other substrates. Glycolysis generates ATP plus pyruvate when reducing equivalents are transferred from cytoplasmic NADH to the mitochondria by the malate–aspartate shuttle (MAS). NADH can also be oxidized to regenerate NAD^+ by production of lactate, which is sometimes used as a surrogate marker for glycolytic pathway flux. Oxidation of pyruvate through the tricarboxylic acid cycle generates NADH and $FADH_2$. The electron transport chain transfers electrons from NADH and $FADH_2$ to oxygen, along with extrusion of protons from the mitochondrial matrix. Proton reentry into the matrix through ATP synthetase drives ATP synthesis. In well-coupled mitochondria, respiration rate (oxygen consumption or uptake) is coupled to ATP synthesis, and 'respiratory control' is exerted by energy demand, i.e., ADP availability. Respiration in the absence of ATP synthesis is low and arises from proton leakage into the matrix.

[b]Synaptosomal energetics can be modulated by treatment with compounds that inhibit specific metabolic or transport reactions, abolish respiratory control, or increase energy demand. The MAS can be blocked by aminooxyacetate (AOAA), which inhibits pyridoxal-dependent enzymes (e.g., aminotransferases), thereby reducing activity of the malate–aspartate shuttle, preventing reoxidation of cytosolic NADH, and blocking the ability of mitochondria to use glycolytic pyruvate. 4-CIN (α-cyanocinnamate) inhibits monocarboxylic acid transporters and pyruvate transport into the mitochondria. Electron transport can be inhibited at different sites, complex I (NADH dehydrogenase complex) by rotenone; complex II (succinate dehydrogenase flavoprotein complex) by 2-nitropropionate, malonate, or methylmalonate; complex III (cytochrome bc_1 complex) by antimycin A; and complex IV (cytochrome a_1a_3 or cytochrome oxidase) by cyanide, azide, nitric oxide, carbon monoxide, or anoxia. Oligomycin inhibits proton reentry into the mitochondrial matrix through the ATP synthase and blocks ATP synthesis; the residual respiration reflects the proton leak into the matrix. FCCP (carbonylcyanide-p-trifluoromethoxy-phenylhydrazone), Cl-CCP (carbonylcyanide-m-chlorophenylhydrazone), and dinitrophenol are uncouplers or protonophores that allow proton reentry into the mitochondrial matrix and relieve respiratory control by diverting the proton current away from the ATP synthase and reducing the capacity to generate ATP. Uncouplers stimulate respiration, and FCCP treatment can reveal maximal respiratory capacity, i.e., the available capacity of nerve endings or cells for substrate delivery and electron transport to increase ATP synthesis in response to increased ATP demand. Ion movements can also be altered to increase ATP demand and stimulate metabolism. Increased extracellular K^+ levels are a consequence of neuronal activity, and depolarization of cells or nerve endings with high concentrations of KCl stimulates energy production. Veratridine prevents inactivation of voltage-activated sodium channels and causes intracellular Na^+ to increase, thereby increasing ATP use by Na^+-K^+-ATPase; veratridine also causes intracellular Ca^{2+} to increase, followed by glutamate release. 4-Aminopyridine (4-AP) is an inhibitor of A-type K^+ channels that causes synaptosomes to fire repetitive action potentials. NMDA, *N*-methyl-D-aspartate, is an agonist for a class of ionotropic glutamate receptors. Nigericin and monensin are ionophores that exchange H^+ for K^+ or Na^+, respectively, whereas A23187 exchanges a divalent cation (Ca^{2+} or Mg^{2+}) for $2H^+$. For more details regarding synaptosomal bioenergetics and responses to various treatments, see Nicholls (2003, 2009, 2010) and Erecińska *et al* (1996).

[c]Magnitude of response is expressed as approximate percentage change owing to treatment, 100[(treated−control)/control], or, when indicated (i.e., as '–fold' change), treated relative to control ratio.

Table 5 Neuronal glucose transporter GLUT3: treatment-evoked changes in cell surface expression and total protein level, and functional abnormalities in mice with GLUT3 deficiency

Preparation[a]	*Treatment*[a]	*Duration*	*Response Magnitude* [b]	*Reference*
Hippocampal neurons (E18)	100 μmol/L Glutamate + 1 μmol/L FCCP + 10 mg/mL oligomycin	5–20 minutes	60% ↑GLUT3 surface expression 130% ↑DG transport	Patel and Brewer, 2003
Cerebellar granule neurons (PN7)	100 μmol/L Glutamate + 10 μmol/L glycine	30–60 minutes	30–55% ↑GLUT3 surface expression	Weisová *et al*, 2009
Cerebral cortical neurons (E18)	50 μmol/L Bicuculline + 100 μmol/L 4-Aminopyridine (AP)	15 minutes 30 minutes	400% ↑GLUT3 surface expression 1,900% ↑GLUT3 surface expression	Ferreira *et al*, 2011
	50 μmol/L NOR3 (nitric oxide donor)	15 minutes	1,400% ↑GLUT3 surface expression	
Hippocampal neurons (E18)	50 μmol/L Bicuculline + 100 μmol/L 4-AP	15 minutes	300% ↑GLUT3 surface expression	
	50 μmol/L Bicuculline + 100 μmol/L 4-AP	15 minutes	80% ↑GLUT3 surface expression in dendrites	
	50 μmol/L NOR3 (nitric oxide donor)	5 minutes	700% ↑GLUT3 surface expression	
	50 μmol/L Bicuculline + 100 μmol/L 4-AP	15 minutes	60% ↑2-NBDG mainly phosphorylation	
Cerebellar granule neurons (PN8)	5→ 15 or 25 mmol/L KCl	8 days	150–200% ↑GLUT3 protein level 250–550% ↑DG transport	Maher and Simpson, 1994
	15 mmol/L KCl + 150 μmol/L NMDA		80% ↑GLUT3 protein level versus 15 mmol/L KCl 60% ↑DG transport versus 15 mmol/L KCl	
Adult rat	Repeated hypoglycemia	4 days	50% ↑GLUT3 protein level in forebrain	Lee *et al*, 2000
	Water deprivation	2–3 days	40–55% ↑GLUT3 protein level in neurohypophysis	Vannucci *et al*, 1994
	Streptozotocin diabetes	2 weeks	50% ↑GLUT3 protein level	
	Streptozotocin diabetes + 6 hours/day restraint stress	7 days	10–20% ↑GLUT3 protein level in regions of the hippocampus	Reagan *et al*, 1999
Heterozygous GLUT3-deficient mice	Tests were carried out with 7-day-old or 2–12-month-old mice		Developmental abnormalities leading to autism spectrum disorders including abnormal spatial learning, working memory, and cognitive flexibility. Animals also have EEG seizures and perturbed social behavior	Zhao *et al*, 2010

[a]The age at which tissue was obtained for cultured cells is denoted by E = embryonic or PN = postnatal, followed by the age in days. Bicuculline is a GABA receptor antagonist; see legends to Tables 3 and 4 for more details about actions of inhibitors.

[b]Magnitude of response is expressed as approximate percentage change owing to treatment, 100 [(treated−control)/control]. Deoxyglucose (DG) assays are typically used to measure glucose phosphorylation, but brief assays are also used to measure transport. 2-NBDG is a fluorescent glucose analog that is transported and phosphorylated; the 15 minutes assay in this study probably reflects mainly phosphorylation.

high glycolytic flux can be maintained within the spine at the site of the postsynaptic density. Avid lactate uptake by nearby astrocytes could then oxidize or disperse and discharge the lactate to more remote locations (Gandhi *et al*, 2009).

Net Transport of Lactate Across the Blood–Brain Barrier *In Vivo*

Arteriovenous differences are used to evaluate brain uptake and release of compounds, but limited access to venous drainage systems restricts these assays to the whole brain, cerebral cortex, and eye. Small amounts of lactate (~5% of glucose uptake) are released from resting brain, and during activation, lactate release increases to 15% to 22% of glucose influx (Tables 6A and 6B). Importantly, lactate release can occur even when global brain lactate levels are lower than blood (Table 6A), presumably owing to locally high brain lactate levels. Krebs (1972) noted that the eye is highly glycolytic, and lactate release from the eye exceeds that from the brain, ranging from ~20% to 100% of glucose uptake (Table 6C). Lactate is also released from the human brain during stressful cognitive testing (Table 6E). When blood lactate levels increase during sensory stimulation (Table 6D) or graded exercise (Table 6E), lactate enters the brain in progressively increasing amounts. Activation is associated with lactate

Table 6 Lactate release from the brain and eye during activation and lactate uptake during exercise

Tissue and experimental condition	*Glucose*	*Lactate*	*Lactate flux (%glucose influx)*[a]	*Reference*
A. Conscious rat brain, acute ammonia challenge[b]				Hawkins *et al*, 1973
Before ammonia injection				
Arterial (A) blood level (mmol/L)	5.10	2.64		
Cerebral venous (V) blood (mmol/L)	4.53	2.68		
A–V difference (mmol/L)	0.57	−0.04	−4%	
Brain level (μmol/g)	0.75	1.25		
4–5 minutes after ammonia injection				
Arterial blood level (mmol/L)	5.74	3.20		
Cerebral venous blood (mmol/L)	4.97	3.43[b]		
A–V difference (mmol/L)	0.77*	−0.23**[b]	−15%	
Brain level (μmol/g)	1.05**	2.39**[b]		
B. Conscious rat brain, spreading cortical depression[c]				Adachi *et al*, 1995;
Arterial blood level (mmol/L)	7.32	0.87		Cruz *et al*, 1999
A–V difference (mmol/L)	0.62	−0.27*	−22%	
Brain level (μmol/g)	1.2	8.5**		
C. Eye, anesthetized animals[d]				
Rabbit retina				
A–V difference (mmol/L)[e]	0.39	−0.37	−47%	Krebs, 1972
Rate of consumption or production (μmol/min)				Wang and Bill, 1997
Dark	0.204	−0.160	−39%	
Light 10 lux	0.197	−0.153	−39%	
Light 150 lux	0.206	−0.146	−35%	
Light	0.221	−0.212	−48%	
4 Hz flicker	0.258***	−0.242**	−47%	
Pig outer retina, A–V difference (mmol/L)				Wang *et al*, 1997*a*
Dark	0.304**	−0.372**	−61%	
Light	0.182	−0.160	−44%	
Pig inner retina, A–V difference (mmol/L)				Wang *et al*, 1997*b*
Dark	0.731*	−0.296	−20%	
Light	0.625	−0.324	−26%	
Cat outer retina, use or release rate (μmol/min)				Wang *et al*, 1997*c*
Dark	0.236**	−0.409**	−87%	
Light	0.123	−0.253	−103%	
D. Conscious rat brain, generalized sensory stimulation[e]				Madsen *et al*, 1999
Before stimulation				
Arterial blood (mmol/L)	6.81	0.50		
A–V difference (mmol/L)	0.68	−0.08	−6%	
Brain (μmol/g)	2.8	1.0		
After 5 minutes of stimulation				
Arterial blood level (mol/L)	7.81*	1.9*		
A–V difference (mmol/L)	0.60 *	+0.02*	+2% *	
Brain level (μmol/g)	3.1	1.9*		
E. Conscious human brain[e]	$Lactate_{Arterial}$ (mmol/L)	$(A-V)_{Lactate}$ (mmol/L)		
Rest	0.46	−0.04	−5%	Madsen *et al*, 1995
Cognitive activation + stress	0.47	−0.06***	−7%**	
Rest	0.94	−0.04	−4%	Dalsgaard, 2006
Light exercise	0.99	−0.05	−5%	
Moderate exercise	3.16	+0.12*	+11%*	
Maximal exercise	6.95	+0.50**	+41%**	
Early recovery	14.9	+0.71**	+44%**	

Positive arteriovenous differences (A−V) across the brain indicate uptake into brain, whereas negative values denote efflux from the brain. Tabulated data are mean values from the cited references.

*$P < 0.05$, **$P < 0.01$; ***$P < 0.001$ versus control.

[a]Lactate flux from or to the brain (in glucose equivalents) is expressed as percentage of glucose uptake, i.e., $100[(A-V)_{lactate}/2]/(A-V)_{glucose}$.

[b]Note that lactate was released from the brain in ammonia-loaded rats even though the brain lactate level is lower than that in blood. As lactate transport is passive and concentration gradient-driven, these results suggest locally high brain lactate levels that exceed the average value in tissue (see text).

[c]Efflux of ^{14}C-labeled lactate from the brain to blood during spreading cortical depression in the conscious rat was detectable within 2 minutes after an intravenous pulse of [6-^{14}C]glucose, and between 2 and 8 minutes after the pulse of [^{14}C]glucose the efflux of [^{14}C]lactate from brain was equivalent to that of unlabeled lactate indicating rapid equilibration with glycolytic intermediates and efflux of lactate derived from brain metabolism of blood-borne glucose (Cruz *et al*, 1999).

[d]The retina of the pig and cat is more metabolically active in the dark compared with light (contrasting the rabbit), presumably because light inhibits rod metabolism by inhibiting the dark current (Wang and Bill (1997) and references cited therein).

[e]Under 'resting' conditions, there is a slight efflux of lactate from the brain to blood as long as the blood lactate is relatively low (A, D; also see Madsen *et al* (1998); Linde *et al* (1999); Schmalbruch *et al* (2002)), in sharp contrast to the eye that releases large amounts of lactate under resting and activated conditions (C). When blood lactate increases above that in the brain during physical movement (D, rat) with moderate and strenuous exercise (E, human), lactate influx increases markedly and it becomes a significant brain fuel (Dalsgaard, 2006; Quistorff *et al*, 2008; van Hall *et al*, 2009).

release, but strenuous physical activity increases blood lactate level and brain uptake.

Extracellular Lactate as Fuel During Activation

Changes in extracellular metabolite levels can be measured with high temporal resolution using enzyme-linked sensors. Decreases in extracellular lactate level evoked by electrical stimulation (Hu and Wilson, 1997*a, b*) are assumed to be caused by neuronal lactate metabolism and are cited as evidence supporting the astrocyte-neuron lactate shuttle hypothesis (Bergersen, 2007; Pellerin *et al*, 2007). Metabolite levels reported by Hu and Wilson (1997*b*) are expressed as percentage of basal level, and percentage data hinder quantitative comparisons between glucose and lactate utilization because percentage changes do not account for differences in substrate concentration and delivery. Interpretation of percentage data in terms of relative consumption rates can be quite misleading, and these values were, therefore, converted to concentrations and used to calculate utilization rates (Table 7). Stimulation for 1, 2, 3, or 4 seconds had no or little effect on extracellular glucose and lactate levels, and only 5-second stimuli evoked changes (Hu and Wilson, 1997*b*). Minimal CMR_{glc} was calculated based on glucose delivered to the resting brain (hyperemic responses to activation are rapid but not quantified in this study; hence, the additional glucose delivered during a stimulus was not included in calculated CMR_{glc}) plus extracellular glucose consumed during the stimulus. The resulting rate during the first stimulus is $\sim$5-fold higher than resting CMR_{glc} (Table 7). This value is much higher than those evoked by strong physiologic stimuli ($\sim$50% to 100%), raising the possibility of seizure-like activity. Maximal lactate utilization rate during the first stimulus was only 4% of glucose plus lactate utilization. During subsequent stimuli, the extracellular lactate level increased and percentage decreases were larger, contrasting the lower baseline for extracellular glucose and lower percentage decreases during stimulation. Minimal CMR_{glc} increased 4.5- to 6-fold during subsequent stimuli, and maximal lactate utilization was $\sim$20% to 30% of the total (Table 7). Maximal lactate utilization contributed a trivial fraction to metabolism during the first episode and $<1/3$ of the total (ignoring upregulation of glucose delivery and utilization) during ensuing stimulus events.

To sum up, the static extracellular lactate content is unlikely to be a major brain fuel owing to its low level ($\sim$0.5 to 2 μmol lactate/g or $\sim$0.25 to 1 μmol glucose equivalents/g) and small extracellular fluid volume (20% of brain or $\sim$0.2 g/g brain). The overall glucose utilization rate for the brain is $\sim$0.7 μmol/g per min and is supported by a $>$1.5-fold excess of glucose influx from the blood. Total lactate in the brain could only meet glucose demand for $\sim$1 minute, and extracellular lactate for a much shorter time. For lactate produced in the brain to serve as a significant fuel, there must be a large transcellular flux through the lactate pool.

Changes in Metabolite Concentrations Need not Predict Flux Magnitude or Direction

Concentration changes arise from input–output differences, and without further information they cannot be used to evaluate shifts in metabolic rate. For example, during studies of sensory stimulation of nonfasted, conscious rats, the animals moved around, causing arterial plasma glucose and lactate levels to increase. These changes were accompanied by increases in brain glucose and lactate concentrations, presumably owing to transport down their concentration gradients (Table 8). Interpretation of increased brain glucose level as reflecting reduced CMR_{glc} would be wrong, because CMR_{glc} increased by 27% to 57% and glycogen turnover increased. Net accumulation of lactate in the brain corresponded to <2% of the pyruvate produced from glucose, and some lactate could be derived from glycogen (Table 8). To summarize, the large percentage changes in lactate concentration reflect small quantities and do not reflect glucose flux through the pyruvate pool.

Endogenous fluorescent compounds, NADH, NADPH, and FAD, are commonly used in microscopic studies to localize and evaluate redox changes during activation (Shuttleworth, 2010). Activation-induced changes in fluorescence ($\Delta F/F$) are generally very small (<10%) and are far below the responses to metabolic inhibitors (Table 8). The total concentrations of these redox compounds are quite low and the calculated cytoplasmic $NAD^+/NADH$ ratio is very high, indicating that most of this total cofactor pool is not fluorescent (Table 8). Thus, the baseline fluorescence (F) and the induced response (ΔF) correspond to only to a small fraction of the total amount of $NAD^+ + NADH$. Owing to low cofactor concentration and high glucose metabolic rates, cofactor oxidation-reduction turnover that accompanies pathway fluxes is high. Glycolytic or oxidative rate information cannot be obtained from $\Delta F/F$.

Glucose-Sparing Action of Alternative Substrates that Increase in Blood During Abnormal or Specific Conditions

In the 1960s, studies of human brain metabolism during prolonged starvation revealed that ketone body oxidation could account for $\sim$60% of the oxygen consumed. Ketone bodies spared glucose oxidation while permitting glycolysis and release of lactate and pyruvate from the brain (Table 9). Glucose-sparing effects of ketone bodies in different organs have been attributed, in part, to increased citrate levels and inhibition by citrate of phosphofructokinase, causing reduced glucose oxidation and release of lactate as gluconeogenic substrate

Table 7 Glucose utilization increases 5–6-fold and greatly exceeds lactate utilization after electrical stimulation of hippocampal dentate gyrus *in vivo*[a]

Experimental interval	*Glucose*	*Lactate*
Concentration in extracellular fluid (ECF), resting rat brain (μmol/mL)[b]	2.6	0.75
Resting glucose utilization rate (CMR_{glc}) in dentate gyrus *in vivo* (μmol/g per min)[c]	0.66	
12 seconds interval following the first 5 seconds electrical stimulation		
Percentage decrease in ECF concentration after stimulation[d]	20	7
Amount consumed from ECF (μmol/mL)[d]	0.52	0.05
Glc delivered to the resting brain from blood during 12 seconds (μmol/mL)[c]	0.13	–
Total Glc consumed from ECF + minimum Glc delivered; lac lost from ECF (all as Glc equivalents, μmol/mL)[e]	0.65	0.025
Calculated utilization rate during 12 seconds (all as glucose equivalents, μmol/g per min)	3.25	0.13
Minimum relative increase in CMR_{glc} after first stimulus compared with rest *in vivo*	4.9-fold	
Maximal lactate utilization as percentage of total lactate + glucose utilization	4%	
12 seconds interval following subsequent 5 seconds electrical stimuli		
Interinterval extracellular fluid concentration (μmol/mL)[f]	2.08	1.28
Percentage decrease in ECF concentration after stimulation[f]	10–20	28
Amount consumed from ECF (μmol/mL)[d]	0.21–0.42	0.36
Glc delivered to the resting brain from blood during 12 seconds (μmol/mL)[c]	0.13	–
Glc equivalent of net interinterval increase in ECF lactate level (μmol/mL) (i.e., glucose consumed that is converted to lactate and released to ECF)[f]	0.26	–
Total Glc consumed = (ECF loss + minimum Glc delivered + Lac accumulated in ECF); Lac consumed = loss from ECF (all as Glc equivalents, μmol/mL)[f]	0.60–0.81	0.18
Calculated utilization rate during 12 seconds (all as glucose equivalents, μmol/g per min)[f]	3.0–4.05	0.9
Minimum relative increase CMR_{glc} after subsequent stimuli compared with rest *in vivo*[f]	4.5–6.1-fold	
Maximal lactate utilization as percentage of total lactate + glucose utilization[e]	22–30%	

[a]Hu and Wilson (1997*b*) implanted glucose (Glc) and lactate (Lac) sensors into the dentate gyrus of the rat hippocampus, gave single or repeated electrical stimuli of 5-second duration, and measured temporal changes in extracellular glucose and lactate levels that were reported as percentage of the respective control values; actual concentrations were not stated. Shorter stimuli (1, 2, 3 seconds) did not produce detectable changes in lactate level, and those after 4 seconds were minor and variable.

[b]Extracellular fluid (ECF) brain glucose level is from Hu and Wilson (1997*a*) who used the same glucose sensor and experimental paradigm as did Hu and Wilson (1997*b*); absolute values for extracellular lactate were estimated as follows. Values for total brain tissue lactate level in various regions of normal resting rat brain range from $\sim$0.2 to 0.6 μmol/g in our laboratory (Dienel *et al*, 2002, 2007*a*; Cruz and Dienel, 2002); similar percentage increases also occur in the human brain (Mangia *et al*, 2007*b*). To allow for higher interlaboratory values up to $\sim$1 μmol/g, an intermediate value for resting brain [lac] = 0.75 was used for calculations in this table. For simplicity, extracellular and intracellular Glc and Lac levels are taken to be equivalent, with the caveat that extracellular Glc would be somewhat higher than intracellular Glc, whereas the converse would be true for Lac. All ECF lactate is assumed to be derived from metabolism of brain glucose. Where stated, lactate (Lac) concentration is divided by 2 to obtain glucose (Glc) equivalents of lactate.

[c]Glucose utilization rate is the average of mean values from four subregions of the dentate gyrus of conscious rats from the study of Wree *et al*, 1993. Glucose delivery to the resting brain is equal to glucose utilization rate at steady state; glucose delivered during a 12-second interval is (0.66 μmol/g per min)(0.2 minutes) = 0.13 μmol/g. This value does not include the additional stimulus-induced increases in delivery and metabolism of blood-borne glucose and of any brain glycogen consumed; calculated Glc utilization rates (CMR_{glc}) are, therefore, minimal values.

[d]Percentage changes in ECF concentrations at $\sim$12 seconds after the 5-seconds electrical stimulus are from Hu and Wilson, 1997*b*. Derived values were calculated from estimated initial concentrations and % changes.

[e]Minimal glucose utilization rate over a 12-second interval after a 5-second electrical stimulus was estimated as total Glc equivalents consumed (i.e., for the first stimulus: net decrease in extracellular Glc + Glc delivered to the resting brain; for subsequent stimuli: net Glc decrease + Glc delivered + Glc equivalents of Lac accumulated) divided by 0.2 minutes. It must be noted that minimal CMR_{glc} values after the first or subsequent stimuli are about 5–6-fold higher than normal resting rate. As this increase greatly exceeds values generally observed after very strong physiological stimuli (approximately 50–100%), the 5-second electrical stimulus may have induced local seizure activity. Calculated lactate utilization rate was based only on the net fall in lactate level, which may not be due only to metabolism, i.e., some lactate release to blood and lactate diffusion beyond the range of the sensor can contribute to a decrease in concentration; lactate utilization estimates are, therefore, maximal rates. Thus, calculated lactate utilization rates expressed as percentages of the calculated glucose plus lactate utilization rates are overestimates.

[f]The increase in extracellular lactate level during subsequent stimuli ranged from 140 to 200% of the basal level (Hu and Wilson, 1997*b*), and a mean of 170% was used to calculate the higher level after repeated stimuli; new basal ECF glucose level was set at 20% below the initial resting value, and transient decrements were set at 10–20% of the lower basal glucose level. The increase in basal lactate level is attributed to glucose metabolism to lactate and release to ECF. Calculations were made as described for the first stimulus, except that the glucose equivalent to the increase in ECF lactate is included in the CMR_{glc} total. This calculation also does not include the likelihood that CMR_{glc} increased between the subsequent stimuli, because interinterval ECF glucose level fell by 0.52 μmol/mL from 2.6 to 2.08 μmol/mL even though blood flow and glucose delivery probably also increased.

(Robinson and Williamson, 1980). High levels of ketone bodies (2.5 to 17 mmol/L) and lactate (4 to 8 mmol/L) also reduce glucose oxidation in brain slices and in infused, starved, or fat-fed rats (Table 9). Some studies report that exercising humans with elevated lactate levels (4 to 14 mmol/L) have reduced brain CMR_{glc}, whereas other studies find increased glucose and lactate metabolism during strenuous exercise (Table 9). Rats exercising at 85% of maximal respiratory rate had heterogeneous regional increases in CMR_{glc} and no decreases (Table 9). High levels of three oxidative substrates, lactate, glutamine,

Table 8 Changes in metabolite concentrations and pathway fluxes during brain activation in conscious rats and in brain slices

Metabolite	*Rest*	*Activation*[a]	*Net change (% of rest)*	*Reference*
Glucose[b]				Dienel *et al*, 2007*a*
Arterial plasma (mmol/L)	10.6	12.9*	2.4 (22%)	
Cerebral cortex (μmol/g)	2.38	2.82**	0.44 (19%)	
Inferior colliculus (μmol/g)	2.36	3.08**	0.62 (31%)	
Lactate[b]				
Arterial plasma (mmol/L)	0.92	1.31	0.39 (42%)	
Cerebral cortex (μmol/g)	0.34	0.85***	0.51 (150%)	
Inferior colliculus (μmol/g)	0.34	0.52*	0.18 (53%)	
Glycogen (unlabeled and ^{14}C-labeled)[b]				
Cerebral cortex (μmol/g)	4.60	3.96**	−0.64 (−14%)	
Inferior colliculus (μmol/g)	4.11	3.99	−0.12 (−3%)	
Cerebral cortex ([nCi/g]/ISA)	2.31	1.75*	−0.56 (−24%)	
Inferior colliculus ([nCi/g]/ISA)	2.35	1.96**	−0.39 (−17%)	
CMR_{glc} (μmol/min per g)[c]				
Overall cerebral cortex	1.07	1.36**	0.29 (27%)	
Sensory cortex	1.06	1.66***	0.60 (57%)	
Inferior colliculus	1.54	1.98***	0.44 (29%)	
Pyruvate formed (μmol/g during 10-minute interval)[d]				
Overall cerebral cortex	21.4	27.2	5.8 (27%)	
Sensory cortex	21.2	33.2	12.0 (57%)	
Inferior colliculus	30.7	39.6	8.9 (29%)	
Lactate accumulated/pyruvate formed (%)				
Overall cerebral cortex			1.9%	
Inferior colliculus			0.5%	
Lactate accumulated/glycogen consumed (%)[e]				
Overall cerebral cortex			40%	
Inferior colliculus			75%	
Brain tissue concentration (μmol/g)[f]				
NAD^+	0.2–0.4, 0.9[g]			Ranges are from values in frozen- or microwave-inactivated adult mouse, rat, guinea pig, or cat brain tissue from Lowry *et al* (1964), Bonavita *et al* (1970), Duffy *et al* (1975), Medina *et al* (1980), Welsh (1980), Garofalo *et al* (1988)
NADH	0.01–0.03			
$NADP^+$	0.01–0.02			
NADPH	0.005–0.01			
Calculated ratios[f]				Data from Veech *et al*, 1973; Miller *et al*, 1973; Howse and Duffy, 1975
Free cytoplasmic [NAD^+]/[NADH] (LDH reaction)	670–715			
Free cytoplasmic [$NADP^+$]/[NADPH] (IDH reaction)	0.01			
Mitochondrial [NAD^+]/[NADH] (GDH reaction)	0.5–1.5			
NAD(P)H fluorescence, brain slices (ΔF/Fo, %)	Cyanide, rotenone	Approximate ranges for NAD(P)H transients evoked by electrical stimulation		Representative values determined in brain slices by Kasischke *et al* (2004), Shuttleworth *et al* (2003), Brennan *et al* (2006), Galeffi *et al* (2007)
		Initial dip	Subsequent overshoot	
	+37 to +70%	−1% to −5%	+3% to +10%	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

[a]Brain activation was achieved by generalized sensory (gentle brushing of whiskers, head, back, tail), acoustic, and visual stimulation of conscious rats for 10 or 30 minutes. Values are group means from vehicle-treated control animals that were assayed during rest or activation.

[b]Metabolite levels were measured in arterial plasma and ethanol extracts of funnel-frozen brain from carefully handled, sequestered rats that were prelabeled for 30 minutes by an intravenous pulse of [1-^{14}C]glucose then given an additional 10 minutes of rest or activation. Unlabeled glycogen is reported as glucosyl units, whereas labeled glycogen is reported as nCi ^{14}C recovered in purified glycogen per gram tissue divided by the integrated specific activity (ISA) in arterial plasma to normalize each value to its exposure to labeled precursor.

[c]Local rates of glucose utilization were assayed during rest and activation with the fully quantitative deoxyglucose method using a 30-minute experimental interval.

[d]Pyruvate formed by the glycolytic pathway during the 10-minutes assay interval was calculated as (CMR_{glc})(2 pyruvate/glucose)(10 minutes).

[e]The net increase in lactate levels at the end of the 10-minute experimental period was divided by the amount of pyruvate formed from glucose or by the pyruvate equivalents of the glycogen consumed during the 10-minute time interval.

[f]Tissue concentrations are the sum of bound and free pyridine nucleotides. Cultured astrocytes are reported to have 50–100% higher NAD^+ levels than cultured neurons, but the mitochondrial NAD^+ levels are similar in both cell types, indicating that neurons have a greater proportion of NAD^+ in their mitochondria (Pieper *et al* (2000); Alano *et al* (2007) and references cited therein).

[g]The higher value for NAD^+ was in brain slices from 7-day-old rats from Zeng *et al* (2007) and is similar to the range of values in cultured brain cells (assuming 100 mg protein/g cultured astrocytes and neurons). Calculated ratios are estimates for the free cytoplasmic pools (based on components of the lactate dehydrogenase (LDH) or isocitrate dehydrogenase (IDH) reactions) and the mitochondrial pool (based on components of the glutamate dehydrogenase (GDH) reaction); the mitochondrial ratio is considered to be more reduced, but values are less reliable owing to complexities of mitochondrial metabolism and assumptions made for the calculation (see discussion in cited references and Siesjö (1978)).

Table 9 Utilization of alternative fuel and inhibition of glucose utilization by high concentrations of ketone bodies and lactate

Preparation[a]	*Treatment*[a]	*Response magnitude*[b]	*Reference*
Obese human	5–6 weeks starvation, arterial blood levels of ketone bodies: BHB = 6.7 mmol/L, AcAc = 1.2 mmol/L	Ketone body oxidation accounted for 60% of $CMRO_2$, spared glucose oxidation, with continued glycolysis and lactate/pyruvate release from the brain into the blood	Owen *et al*, 1967
Rat brain slices	5 mmol/L lactate or pyruvate	Reduced glucose uptake by 30% or 36%, respectively with no change in oxygen consumption	Rolleston and Newsholme, 1967
	5 mmol/L AcAc or 10 mmol/L BHB	No change in glucose uptake, decreased glucose oxidation 10 or 23%, increased lactate formation 21 or 46%, respectively; did not alter oxygen consumption	
	4.2 or 8.3 mmol/L lactate	Reduced 8.3 mmol/L [U-^{14}C]glucose $\rightarrow$ $^{14}CO_2$ by 28 or 41% respectively; 8.3 mmol/L increased glycogen 13%	Ide *et al*, 1969
	4.2 or 8.3 mmol/L BHB	Reduced 8.3 mmol/L [U-^{14}C]glucose $\rightarrow$ $^{14}CO_2$ by 33 or 49% respectively; 8.3 mmol/L increased glycogen 34%	
	4.2 or 16.7 mmol/L BHB	Reduced lactate oxidation 20–65%, depending on label position	
Adult rat	Up to 96 hours starvation or ketone body infusion; blood ketone levels up to 2.5–5 mmol/L	Increased arteriovenous difference across the brain with increasing ketone body concentration in blood	Hawkins *et al*, 1971
	48 hours starvation or 24 hours starvation + BHB infusion; blood ketone body level 2.5 or 6.5 mmol/L	Reduce glucose utilization 17–21%, increase lactate release to blood 800 or 1,700%, reduce calculated glucose oxidation 33 or 67%, increases O_2 consumption owing to ketone bodies from 0.6% to 25% or 60%, respectively, for the starved and infused animals. Brain citrate levels increased and may inhibit glycolysis owing to citrate inhibition of phosphofructokinase	Ruderman *et al*, 1974
	High fat diet raised blood BHB levels over the range 1–5.5 mmol/L	Decrease glucose utilization in anesthetized rat brain ~10% per mmol/L increase in plasma ketone body concentration	LaManna *et al*, 2009
	Lactate infusion increase plasma level from 1.3 ± 0.6 to 5.8 ± 1.6 mmol/L	Reduced glucose utilization in anesthetized rat by a mean of 38%, in dose-dependent manner	Wyss *et al*, 2011
	Running wheel exercise at 85% of maximal O_2 uptake	Heterogeneous increases in CMR_{glc} in most brain regions ranging from about 30–165%; no decreases observed in any structure during exercise (plasma glucose approximately 8.5–10 mmol/L; lactate not measured)	Vissing *et al*, 1996
Cultured cortical astrocytes (from PN1 rat, used at 25–30 DIV)	1, 2, 3, 5, 10 mmol/L lactate	Reduced glucose utilization by 15%, 25%, 40%, 50%, or 60%, respectively	Swanson and Benington, 1996
	1, 2, 3, 5, 10 mmol/L glutamine	Reduced glucose utilization by 15%, 20%, 45%, 40%, or 50%, respectively	
(from PN0–2 rat, used at 14 DIV)	0.5, 1, 2.5, 5 mmol/L lactate 0.25, 0.5, 1, 2.5 mmol/L pyruvate	Reduced glycolytic contribution to lactate labeling by 20%, 40%, 55%, 70% (lactate) or by 25%, 35%, 60%, 70% (pyruvate), respectively	Rodrigues *et al*, 2009
Cultured forebrain neurons (From PN0 rat; used at 5 DIV)	5.5 mmol/L [1-^{13}C]glucose + 1.1, 5.5. or 11 mmol/L lactate	55% and 68% decreases in fractional enrichment of glutamate C4 at 5.5 and 11 mmol/L extracellular lactate. Calculated contribution of 1.1, 5.5, and 11 mmol/L exogenous lactate is 46%, 90%, and 93%, respectively, of neuronal oxidative metabolism	Bouzier-Sore *et al*, 2003
	1.1 mmol/L glucose + 1.1 mmol/L lactate	Calculated relative contribution of lactate and glucose to neuronal oxidative metabolism is 75% and 25%, respectively	Bouzier-Sore *et al*, 2006
Human brain	[3-^{13}C]lactate infusion to produce ~0.5–3 mmol/L plasma lactate, corresponding to brain lactate levels of ~0.4 to 3 μmol/g	Plasma glucose levels were approximately 6–7 mmol/L and estimated brain glucose level is ~1–1.4 μmol/g, assuming a brain/plasma ratio of 0.2. Lactate oxidation contributes a maximum of about 4–8% of total oxidative metabolism in a concentration-dependent manner	Boumezbeur *et al*, 2010
	Lactate infused to raise plasma lactate from 0.6 to 4.1 mmol/L	Plasma glucose was about 5.3 mmol/L. Lactate infusion caused an ~17% $\downarrow$in whole brain CMR_{glc} assayed with [^{18}F]FDG (~5% per mmol/L increase in plasma lactate)	Smith *et al*, 2003
	Graded mild to exhaustive exercise and recovery	Assays of blood flow and arteriovenous differences for oxygen, glucose, and lactate across the brain at intervals during mild, moderate, and exhaustive exercise. Calculated cumulative uptake of glucose, lactate,	Quistorff *et al*, 2008

Table 9 *Continued*

Preparation[a]	*Treatment*[a]	*Response magnitude*[b]	*Reference*
		and oxygen progressively increase with time. $CMR_{O2}/CMR_{(glc+0.5\ lac)}$ ratio is substantially reduced during and after strenuous exercise	
	[1-^{13}C]lactate infusions during rest and cycling exercise (75% of maximal O_2 uptake) to achieve plasma lactate levels of 0.9, 3.9, and 6.9 mmol/L	Blood lactate contributed 8%, 19%, and 27%, at the respective plasma lactate levels, to cerebral energy metabolism	van Hall *et al*, 2009
	Cycling exercise at 30, 55, and 75% of maximal O_2 uptake. Plasma lactate rose from ~0.5 to 14 mmol/L	Brain CMR_{glc} assayed with [^{18}F]FDG. CMR_{glc} decreased with increased exercise intensity and fell in proportion to plasma lactate level (~3.5% per mmol/L increase in plasma lactate)	Kemppainen *et al*, 2005

[a]Ketone bodies, acetoacetate (AcAc) and β-hydroxybutyrate (BHB) levels in blood increase during prolonged fasting, starvation, and high fat diets. In many tissues including the brain, oxidative metabolism of ketone bodies spares glucose by reducing glucose oxidation, maintaining glycolysis, and releasing lactate, which can be converted back to glucose by gluconeogenesis (Robinson and Williamson, 1980). Although details of the mechanisms of regulation of glucose metabolism when alternative substrates are oxidized are not fully established, elevated ketone body metabolism is associated with an increased concentration of citrate, which, along with other metabolic regulators, is an inhibitor of brain phosphofructokinase within the physiological range (Passonneau and Lowry, 1963). Conceivably, increased lactate concentrations in blood and extracellular fluid could also increase intracellular citrate levels and contribute to glucose sparing by reduction of glycolysis and glucose oxidation. Brains were harvested for tissue culture at indicated postnatal (PN) age in days, and used for experiments after maintaining the cells in tissue culture for the indicated days *in vitro* (DIV). It must be noted that inhibition of glucose metabolism by lactate in cultured astrocytes and neurons is much higher than observed in normal brain *in vivo*, suggesting a strong influence of developmental stage, levels of glycolytic and oxidative enzymes and transporters, and relative glycolytic and oxidative fluxes in cultured cells compared with the normal adult brain. Many studies have shown that labeled glucose preferentially labels the large (neuronal) glutamate pool, but *in vivo* microdialysis studies have shown that fluorocitrate inhibits oxidation of lactate and glucose, indicating that astrocytes oxidize ~50% of interstitial lactate and ~35% of interstitial glucose (Zielke *et al*, 2007).
[b]Magnitude of response is expressed as approximate percentage change owing to treatment, 100[(treated−control)/control].

and pyruvate, in tissue culture media reduce glucose utilization in astrocytes and neurons in a dose-dependent manner (Table 9). When cultured forebrain neurons were incubated with lactate and glucose (1 mmol/L of each substrate), lactate was calculated to contribute 75% to total oxidative metabolism (Bouzier-Sore *et al*, 2006; Table 9). This conclusion sharply contrasts the quite small, 4% to 8%, contribution of lactate to oxidative metabolism in the brain of humans infused with lactate to achieve plasma and brain lactate levels of ~0.6 to 4.1 mmol/L and ~0.4 to 3 μmol/g, respectively (Boumezbeur *et al*, 2010). The responses of cultured neurons do not correspond to the adult human brain, presumably because of developmental differences affecting transport and metabolism. These findings indicate that translation of results of studies in immature cultured cells to the adult brain *in vivo* must establish similar metabolic and transport capabilities. Lactate can, but does not necessarily, reduce glucose utilization *in vivo*.

Evaluation of Roles of Lactate by Monocarboxylic Acid Transporter Inhibition

As lactate shuttling among brain cells is very difficult to evaluate, an MCT inhibitor (e.g., α-cyano-4-hydroxycinnamate or 4-CIN) is often used to assess effects of extracellular lactate on neuronal function, and decrements caused by transport blockade are inferred to reflect insufficient lactate fuel. However, these types of studies are difficult to interpret because low levels of 4-CIN severely inhibit pyruvate transport into the mitochondria from the rat heart and liver (<10 μmol/L) and brain (100 μmol/L), and 100 μmol/L markedly inhibits glucose-supported synaptosomal respiration during activation (Table 10). In addition to blocking plasma membrane lactate transport, 250 μmol/L 4-CIN also reduces oxidation of lactate and glucose owing to impairment of mitochondrial pyruvate transport (Table 10). Thus, the 10% compensatory increase in neuronal NBDG transport and 20% decrease in neuronal intracellular acidification in the presence of 5 mmol/L lactate plus 100 μmol/L 4-CIN (to preferentially inhibit neuronal MCT2 compared with astrocytic MCT1 or MCT4) (Erlichman *et al*, 2008; Table 10) could have arisen from reduced neuronal pyruvate oxidation, lactate uptake, or both. Even if the 4-CIN-evoked 10% increase in NBDG transport reflects only the magnitude of the astrocyte-neuron lactate shuttle hypothesis, the quantitative effect of blockade of lactate shuttling on neuronal glucose transport (and metabolism) is small.

Metabolic Modeling and Simulation Studies

Metabolic modeling is necessary to calculate glucose utilization and glucose oxidation rates from labeling studies carried out *in vivo*. The autoradiographic [^{14}C]deoxyglucose method uses a two-compartment

Table 10 Effects of α-cyano-4-hydroxycinnamate (4-CIN) on mitochondrial pyruvate oxidation and transport and oxidation of glucose and lactate

Preparation[a]	*4-CIN dose*	*Response magnitude*	*Reference*
Isolated mitochondria	1–100 μmol/L	6 μmol/L = Ki for pyruvate transport rat liver mitochondria; 1.5 μmol/L = Ki for pyruvate transport rat heart mitochondria 25 μmol/L almost complete inhibition of pyruvate oxidation in rat heart mitochondria 100 μmol/L almost complete inhibition of pyruvate oxidation in rat brain, adipose tissue, kidney cortex, or blowfly flight muscle mitochondria	Halestrap, 1975; Halestrap and Denton, 1974; Halestrap and Denton, 1975
Cultured astrocytes from PN0 rats used at 12–14 DIV and cortical neurons from E16 rats and used at 7 DIV	250 μmol/L	Reduced oxidation of lactate and glucose by 43% and 46%, respectively, in astrocytes and by 87 and 58%, respectively, in neurons. 4-CIN did not reduce glucose transport.	McKenna *et al*, 2001
Cortical synaptosomes	10–100 μmol/L 4-CIN	No effect on basal respiration rate with 15 mmol/L glucose substrate	Choi *et al*, 2009
	4 μmol/L FCCP + 10 μmol/L 4-CIN	35% inhibition of maximal FCCP-evoked respiration rate	
	+ 30 μmol/L 4-CIN	50% inhibition of maximal respiration rate	
	+ 100 μmol/L 4-CIN	60% inhibition of maximal respiration rate	
Oocytes transfected with MCT1 or MCT2	100 μmol/L	26% or 66% inhibition of lactate transport rate by MCT1 or MCT2, respectively. Ki for 50% lactate transport inhibition for MCT2 and MCT1 are 24 μmol/L and 425 μmol/L, respectively	Bröer *et al*, 1999
Brain slice	100 μmol/L	Astrocytes—no effect on 2-NBDG uptake; no effect on intracellular acidification when 5 mmol/L lactate was added to the aCSF perfusate containing 26 mmol/L bicarbonate and 10 mmol/L glucose Neurons—10% increase in 2-NBDG uptake; 20% reduction in intracellular acidification when 5 mmol/L lactate was added to aCSF	Erlichman *et al*, 2008

aCSF, artificial cerebrospinal fluid.

[a]Age at tissue harvest (embryonic, E, or postnatal, PN) and duration of culture (days *in vitro*, DIV) are indicated for cultured cells. The monocarboxylic acid transporter, MCT1, is predominantly in astrocytes, whereas the higher-affinity MCT2 is neuronal.

model (blood and brain) that takes into account the kinetic differences in rates of transport and phosphorylation of deoxyglucose and glucose (Sokoloff *et al*, 1977). The procedure assays the first irreversible step of glucose utilization, the hexokinase step, which corresponds to the overall rate of glucose consumption at steady state. [^{14}C]Glucose autoradiographic and biochemical assays evaluate labeled metabolites retained in the tissue at the end of the experimental period which must be short owing to label loss. ^{13}C-Magnetic resonance spectroscopic studies use programmed infusions to maintain constant arterial plasma [^{13}C]glucose concentrations, and to measure temporal profiles of incorporation of label from [^{13}C]glucose into amino acids derived from the TCA cycle. Compartmental modeling enables calculation of glucose oxidation rates in neurons and astrocytes, glutamate–glutamine cycling, and rates of other pathways, depending on the label position and precursor (Mason and Rothman, 2004). ^{13}C-Magnetic resonance spectroscopic assays focus on the oxidative pathways because the glycolytic pools (glucose to pyruvate/lactate) quickly equilibrate with arterial plasma [^{13}C]glucose, and once this occurs, no kinetic information can be obtained from these compounds to estimate glycolytic rate.

In their revised, more comprehensive model for coupling of glucose metabolism with synaptic activity, Hyder *et al*, 2006 predict (see their Figure 5) that most of the glucose consumed during activation is used glycolytically by astrocytes, with significant lactate shuttling to neurons and lactate oxidation by neurons. This model also predicts very little (a few percent) lactate release from the brain, contrasting the much greater label release (~50%; see above, 'Underestimation of metabolic activation with labeled glucose') based on autoradiographic and biochemical studies of brain activation in conscious rats assayed in parallel with [6-^{14}C]glucose and [^{14}C]deoxyglucose (Collins *et al*, 1987; Ackermann and Lear, 1989; Adachi *et al*, 1995; Cruz *et al*, 1999, 2007). The basis for the quantitative differences in

the fate of lactate in the ^{14}C- and ^{13}C-magnetic resonance spectroscopic assays remains to be established. Assays of total glucose metabolized and rates of glycolytic, glycogenolytic, oxidative, and anaplerotic (i.e., biosynthetic) pathways are required to have a fuller understanding of brain metabolic activation and roles of lactate.

Metabolic modeling and computer-based simulations are also very useful to predict pathway fluxes in neurons and astrocytes under various test conditions. Calculated rates and predicted outcomes are critically influenced by model assumptions that define the metabolic capabilities and energetic demands of neurons and astrocytes and their subcellular compartments, the magnitude of metabolic activation, cellular concentrations of glucose and lactate transporters, kinetic properties of the endothelial, neuronal, and astrocytic nutrient transporters, and other factors. Model assumptions govern the predicted cellular consumption of glucose, the cellular origin of lactate, and the direction of lactate shuttling (i.e., the astrocyte to neuron or neuron to astrocyte), and the magnitude and duration of lactate concentration changes. Different models, modeling principles, and model assumptions underlie discordant conclusions related to the roles of lactate and glucose in brain activation derived from computer-based simulation studies. Interested readers are referred to studies by Aubert *et al* (2005, 2007), Aubert and Costalat (2007), Simpson *et al* (2007), DiNuzzo *et al* (2010*a*,*b*), Mangia *et al* (2009*b*), Barros and Deitmer (2010), Occhipinti *et al* (2010), and Calvetti and Somersalo (2011) and commentaries by Jolivet *et al* (2010) and Mangia *et al* (2011) for detailed discussions of assumptions and limitations of transport and metabolic models and of simulations derived from them.

Concluding Comments

A wealth of data obtained over several decades in many laboratories shows that cultured neurons and synaptosomes are capable of greatly increasing glucose transport, glycolysis, and glucose-supported respiration under many experimental conditions that increase energy demand. The emphasis of this review is on measured data that directly or indirectly relate to brain lactate metabolism. Modeling and simulation studies are also very useful to predict outcomes, as well as to suggest and design critical experiments. Data in Tables 1 to 10 identify strong trends and some discordant findings, and elucidation of the basis for apparently discrepant results will help understand important characteristics of brain cells. Incorporation of results from *in vitro* studies into models describing the cellular basis of glucose utilization must accommodate these major data sets, as well as two very different physiologic situations involving brain lactate transport and metabolism *in vivo*, outward and inward lactate concentration gradients.

Outwardly Directed Lactate Concentration Gradient from the Activated Brain to Blood in Physically Inactive Subjects with Low Plasma Lactate Levels

Brain activation usually causes disproportionately greater increases in CBF and CMR_{glc} compared with CMR_{O2} (Dienel and Cruz, 2004, 2008). Glycolytic activation increases intracellular lactate concentration, causing lactate to diffuse down its concentration gradient to extracellular fluid. Lactate can then be avidly taken up into astrocytes (Gandhi *et al*, 2009), channeled through the astrocytic syncytium through gap junctions, and discharged from astrocytic endfeet to perivascular fluid and the vasculature, where it may serve as a signaling molecule for blood flow regulation. Rapid efflux of labeled lactate from the brain during activation contributes to the $\sim$50% underestimates of CMR_{glc} by labeled glucose in autoradiographic and positron emission tomographic studies. Generation and release of unlabeled lactate contributes to the decrease in CMR_{O2}/CMR_{glc} ratio during activation. It must be noted that the small increases in CMR_{O2}, if any, during activation reflect oxygen consumed by oxidation of all compounds. As lactate utilization must consume oxygen, the maximal contribution of any increase in lactate shuttling to total oxidation during activation cannot exceed the ΔCMR_{O2}. For example, if CMR_{glc} increases by 50% and CMR_{O2} increases 20%, this increase in CMR_{O2} corresponds to oxidation of the additional pyruvate derived from glucose, glycogen, and lactate, and oxidation of any other compounds in neurons and astrocytes. If neurons account for half of the additional oxygen consumed by direct metabolism of glucose-derived pyruvate, then lactate shuttling cannot exceed half of the net increase in CMR_{O2}, or 10% in this example.

Inwardly Directed Lactate Concentration Gradient from the Blood to the Entire Brain in Subjects with High Blood Lactate Levels

Lactate flooding during lactate infusions and strenuous exercise (and *in vitro* assays) eliminates local lactate concentration gradients in tissues arising from focal activation and enables lactate to serve as an opportunistic, supplemental fuel for cells throughout the entire brain. However, lactate oxidation during flooding conditions and partial inhibition of glucose utilization by lactate do not prove directed cell-to-cell lactate shuttling or its use as a major fuel under other situations. Biochemical regulatory mechanisms take place and can modulate glucose utilization by different mechanisms. Lactate uptake with H^+ and H^+ production by the LDH (Figure 1) can reduce intracellular pH (depending on buffering capacity), lactate conversion to pyruvate reduces NAD^+ availability for glycolysis, and lactate oxidation generates ATP and citrate. Acidification, ATP, and citrate can inhibit phosphofructokinase in a very complex, concentration-dependent manner that

is influenced by other modulators of this enzyme and can reduce CMR_{glc}. Notably, some studies have shown that glucose uptake and utilization does increase (Table 9) during strenuous exercise in rats (Vissing *et al*, 1996) and in humans who also have increased lactate uptake and oxidation (Quistorff *et al*, 2008). High lactate levels that arise during strenuous exercise or hypoxic episodes may be 'biologically intended' to be glucose-sparing, similar to ketone bodies during starvation.

Cell-Type Specific Production of Lactate, Cell-to-Cell Lactate Shuttling, and Lactate Oxidation or Release

Many studies carried out in different laboratories over several decades show the high glycolytic and respiratory capacity of nerve endings and cultured neurons. These findings are consistent with high neuronal glucose utilization *in vivo* and they negate assertions that neurons cannot upregulate glucose transport and glycolytic metabolism. During low-level lactate infusions into resting humans, lactate oxidation by the brain contributes < 8% to total TCA cycle flux. Moreover, blockade of lactate-pyruvate transporters in brain slices with 4-CIN evokes only a 10% increase in neuronal NBDG uptake. Although modeling predicts significant lactate shuttling, direct, strong *in vivo* evidence for astrocyte-to-neuron lactate shuttling coupled to local neuronal lactate oxidation as a major fuel is lacking.

Taken together, many independent lines of evidence obtained *in vivo* and *in vitro* support the conclusion that glucose, not lactate, is the major brain fuel during activation and that neurons may be a major source of lactate during activation. Small or no increases in CMR_{O2} during activation compared with CBF and CMR_{glc} indicate preferential upregulation of nonoxidative metabolism of glucose, but most of the ATP generated during activating conditions comes from the oxidative pathway. In pulse-labeling assays, CMR_{glc} is greatly underestimated when assayed with labeled glucose owing to rapid label loss arising from lactate efflux, decarboxylation reactions, and label spreading (Cruz *et al*, 1999, 2007). Most lactate generated from glucose microinfused into the brain is not locally oxidized (Ball *et al*, 2010). Lactate dispersal and release can be mediated by astrocytes (Gandhi *et al*, 2009), and blockade of lactate transporters and gap junctions increase focal label retention in activated structures (Cruz *et al*, 2007). Strong Ca^{2+} signals in neuronal mitochondrial reduce MAS activity, which would increase neuronal lactate production and reduce any neuronal lactate utilization (Bak *et al*, 2009; Contreras and Satrústegui, 2009). Specific neuronal structures and activities depend on glycolysis, including dendritic spines that lack mitochondria (Li *et al*, 2004; Bourne and Harris, 2008), the plasma membrane calcium pump (Ivannikov *et al*, 2010), and glutamate loading into synaptic vesicles (Ikemoto *et al*, 2003). The cost for a neuron to package one glutamate is one ATP, which is half that required by astrocytes for glutamate–glutamine cycling (one ATP for sodium extrusion and one for glutamine synthesis). In cultured glutamatergic neurons, glucose, not lactate, utilization is enhanced by NMDA-induced glutamate release (Bak *et al*, 2009). These findings support neuronal upregulation of glycolysis during excitatory neurotransmission, and strong compartmentation of glycolysis in astrocytes during brain activation is considered unlikely.

Failure of glutamate transport blockade to reduce stimulus-evoked lactate increases, and metabolic activation in the cerebellum (as does an AMPA receptor blocker; Caesar *et al* (2008)) is consistent with the low predicted ATP cost for astrocytic participation in glutamate–glutamine cycling compared with postsynaptic and other signaling events (Attwell and Laughlin, 2001). Astrocytes increase glycogenolysis and oxidative metabolism during activation, besides their presumed use of blood glucose. Glycogenolysis generates glucose-6-phosphate that serves as fuel for astrocytes and can also inhibit astrocytic hexokinase activity, providing a mechanism to divert blood-borne glucose for use by neurons (DiNuzzo *et al*, 2010*b*). Small increases in astrocytic oxidative metabolism during activation *in vivo* produce substantial portion of the total increase in ATP generated in astrocytes during activation. The sites and processes consuming the ATP are not known, but fine perisynaptic processes of astrocytes contain mitochondria, endowing these structures with high oxidative capacity that can be used to power many processes linked to neurotransmission, including glutamate uptake and sodium extrusion, regulation of extracellular $[K^+]$ (Hertz *et al*, 2007), and glutamate-evoked calcium waves (Cornell-Bell *et al*, 1990*a*). Astrocytic processes are dynamic structures, their formation is stimulated by glutamate exposure, and they advance and retract from active synapses by actin-dependent mechanisms that involve ATP hydrolysis (Cornell-Bell *et al*, 1990*b*; Reichenbach *et al*, 2010). Further work is required to include these processes in the energetics of working astrocytes *in vivo*.

Release of lactate from the resting and activated brain even though it can serve as an oxidative fuel is an important, unresolved issue that probably involves many factors, including the following: (1) the rapid-onset hyperemic response delivers more fuel to the brain; (2) glucose supply to brain exceeds demand by a factor of at least 1.5 in normoglycemic subjects over a wide range of CMR_{glc} and glucose levels in rats (Cremer *et al*, 1983; Hargreaves *et al*, 1986) and humans (Shestov *et al* (2011) and references cited therein); (3) lactate release to perivascular fluid may enhance the hyperemic response during activation by causing vasodilation; (iv) spatial–temporal interactions of increased energy demand (e.g., plasma membrane ion pumps) during activation may preferentially depend on glycolysis and channeling of lactate, with its discharge from astrocytic endfeet to perivascular space. Excess glucose delivery and

high-capacity neuronal glucose transport and metabolism support the conclusion that neurons do not need lactate as supplemental fuel under normal activating conditions. In contrast, lactate flooding of the brain owing to elevated blood levels would normally occur when whole-body glycolytic metabolism may exceed overall oxidative metabolism, such as during strenuous physical work, exercise, and hypoxia. High blood lactate levels abolish brain lactate efflux gradients and can evoke glucose-sparing responses in brain and other organs. Alternative substrates can also substantially contribute to brain energetics when glucose supply is inadequate, e.g., during hypoglycemia or intense brain activity. Most *in vivo* evidence supports the brain's use of glucose as its major fuel under normal activating conditions in sedentary or modestly physically active subjects.

In conclusion, detailed studies of brain energy metabolism and neurotransmission and their interrelationships during the past 40 years have substantially increased our understanding of the cellular contributions to brain function, imaging, and spectroscopic studies. Development of new approaches to resolve discordant results and extend current technologies is expected to have a high impact on the use of metabolic imaging techniques to assess cellular functions *in vivo* and to evaluate human brain diseases.

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Conflict of interest/Disclosure

The author declares no conflict of interest.

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