Activity-Dependent Regulation of Energy Metabolism by Astrocytes: An Update

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ABSTRACT

Astrocytes play a critical role in the regulation of brain metabolic responses to activity. One detailed mechanism proposed to describe the role of astrocytes in some of these responses has come to be known as the astrocyte-neuron lactate shuttle hypothesis (ANLSH). Although controversial, the original concept of a coupling mechanism between neuronal activity and glucose utilization that involves an activation of aerobic glycolysis in astrocytes and lactate consumption by neurons provides a heuristically valid framework for experimental studies. In this context, it is necessary to provide a survey of recent developments and data pertaining to this model. Thus, here, we review very recent experimental evidence as well as theoretical arguments strongly supporting the original model and in some cases extending it. Aspects revisited include the existence of glutamate-induced glycolysis in astrocytes in vitro, ex vivo, and in vivo, lactate as a preferential oxidative substrate for neurons, and the notion of net lactate transfer between astrocytes and neurons in vivo. Inclusion of a role for glycogen in the ANLSH is discussed in the light of a possible extension of the astrocyte-neuron lactate shuttle (ANLS) concept rather than as a competing hypothesis. New perspectives offered by the application of this concept include a better understanding of the basis of signals used in functional brain imaging, a role for neuron-glial metabolic interactions in glucose sensing and diabetes, as well as novel strategies to develop therapies against neurodegenerative diseases based upon improving astrocyte-neuron coupled energetics. © 2007 Wiley-Liss, Inc.

The idea that lactate (provided by astrocytes or other sources) could be a supplementary fuel (in addition to glucose) for activated neurons has polarized opinion with regard to the sole use of glucose as the neuronal energy substrate (for contrasting views, see Korf, 2006; Schurr, 2006). More precisely, the neurometabolic community seems to have been divided into two schools of thought: those who claim that glucose is the sole energy substrate for brain cells (referred as “glucoseniks”), and the others who support the concept that lactate could be an important albeit supplementary fuel for neurons (the “lactatians”). However, an important point that seems to have been forgotten in the heated debate must be underlined: it has never been claimed that glucose is not a neuronal substrate, nor that neurons consume exclusively either glucose or lactate, but rather that lactate (synthesized among others by astrocytes) is used by neurons as an aerobic substrate under precise circumstances. Few seemed to have correctly grasped this message (but see Gladden, 2004) whereas many others have clearly expressed their opposition to what appears to be an incorrectly perceived and/or portrayed view (Chih and Roberts, 2003; Chih et al., 2001; Dienel and Cruz, 2004; Dienel and Hertz, 2001; Hertz, 2004). Notwithstanding, it is our opinion that several important issues concerning the astrocyte-neuron lactate shuttle hypothesis (ANLSH) in general have been misrepresented, incorrectly interpreted, or simply have diverged with time from the original proposal during the recent years. In a previous review article (Pellerin and Magistretti, 2003), we redefined more precisely the status of the concept. Some of the key features are illustrated in Figures 1 and 2 of this review. Figure 1 describes the first part of the original concept that was proposed in 1994 related to the basal (nonactivated) state whereas Figure 2 details the second part pertaining to the activation and adds new results that flesh out the ANLSH. To provide all the arguments necessary for the correct assessment of the validity of this hypothesis, it is important to dissipate a number of unfounded criticisms and re-establish the value of solid experimental and theoretical demonstration. For this reason, recent experimental data and theoretical developments are reviewed below providing further support to the concept.

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under normal conditions, and O2 are supplied via the blood circulation. Glucose, the main cerebral energy substrate in the adult brain, is transported into the cell by the sodium-dependent glucose transporter (GLUT1) and is metabolized in the presence of 5 or 25 mM glucose (Pellerin and Magistretti, 1994; Takahashi et al., 1995). However, astrocytes cultured in 25 mM glucose or less. One consequence could have been a difference in glycogen stores. Indeed, different glycogen levels have been observed between astrocytes maintained in the presence of 5 or 25 mM glucose (Pellerin et al., 1997). However, astrocytes cultured in 25 mM glucose were preincubated in a medium containing 5 mM glucose for 2–4 h prior to any experiment evaluating metabolic responses to glutamate, allowing a return of glycogen levels to a value equivalent to the one measured in astrocytes prepared in 5 mM glucose (Pellerin et al., 1997). Thus, a confounding effect of glycogen levels can be excluded. Notwithstanding, based on a comparison of the level of oxidative metabolism measured as CO2 production between one of the initial studies (Takahashi et al., 1995) and unpublished data from one of the groups that did not observe a similar effect (Hertz, 2004), it was concluded that high glucose levels must be the culprit since they apparently reduced oxidative metabolism in astrocytes. Indeed, it was already shown that glucose concentration in the culture medium has a strong influence on the oxidative capacity of astrocytes in vitro (Abe et al., 2006). However, concomitant variations in glucose concentration in the presence and absence of glutamate were never experimentally tested by the ANLSH detractors. Indeed, a complete demonstration should include experiments in which astrocytes in culture prepared in media with low (<7.5 mM) and high (>25 mM) glucose concentrations display not only different metabolic profiles but also opposite responses upon glutamate exposure. Indeed, experiments were independently done by other groups with astrocyte cultures prepared in a medium containing 5.6 mM glucose (Hamai et al., 1999; Keller et al., 1996). Results showed an increase in glucose utilization triggered by glutamate very similar to the response observed with cultures prepared in 25 mM glucose. Furthermore, some of us directly addressed this question in a recent study (Brunet et al., 2004). Isolated stem cells were differentiated into astrocytes in a medium containing either 5 or 33 mM glucose. Results showed that the glycolytic response to glutamate was the same, and if any better, in cells differentiated in 5 mM glucose. This study also demonstrated that as stem cells differentiated into astrocytes, they acquired their glycolytic phenotype and their capacity to respond to glutamate by enhancing aerobic glycolysis. This finding was further supported by the observation that expression of all the different components involved in the mechanism increased together with the differentiation process. We also extended our demonstration later showing that astrocytes prepared from different brain regions all gave rise to the same capacity to enhance glycolysis upon glutamate stimulation (Pellerin and Magistretti, 2005). Thus, this property appears to be an intrinsic feature of astrocytes and is not specifically linked either to culture conditions or cell origin.

**DEMONSTRATION OF GLUTAMATE-INDUCED AEROBIC GLYCOLYSIS IN CULTURED ASTROCYTES**

On the basis of a few in vitro studies reporting either no effect (Hertz et al., 1998; Peng et al., 2001) or a different metabolic response (Liao and Chen, 2003; Swan-son et al., 1990), it has been argued that the previously described activation of aerobic glycolysis by glutamate in astrocytes (Pellerin and Magistretti, 1994), which was confirmed by others (Hamai et al., 1999; Keller et al., 1996; Takahashi et al., 1995), must be a culture artifact (Hertz, 2004). Indeed, some researchers did not manage to reproduce such results and explained this by the difference in glucose concentration present in the culture medium. Although initial studies reporting enhanced glycolysis were performed on astrocyte cultures prepared in a medium containing 25 mM glucose (Pellerin and Magistretti, 1994; Takahashi et al., 1995), those who were not able to reproduce this finding had prepared their cultures in a medium containing 7.5 mM glucose or less. One consequence could have been a difference in glycogen stores. Indeed, different glycogen levels have been observed between astrocytes maintained in the presence of 5 or 25 mM glucose (Pellerin et al., 1997). However, astrocytes cultured in 25 mM glucose were preincubated in a medium containing 5 mM glucose for 2–4 h prior to any experiment evaluating metabolic responses to glutamate, allowing a return of glycogen levels to a value equivalent to the one measured in astrocytes prepared in 5 mM glucose (Pellerin et al., 1997). Thus, a confounding effect of glycogen levels can be excluded. Notwithstanding, based on a comparison of the level of oxidative metabolism measured as CO2 production between one of the initial studies (Takahashi et al., 1995) and unpublished data from one of the groups that did not observe a similar effect (Hertz, 2004), it was concluded that high glucose levels must be the culprit since they apparently reduced oxidative metabolism in astrocytes. Indeed, it was already shown that glucose concentration in the culture medium has a strong influence on the oxidative capacity of astrocytes in vitro (Abe et al., 2006). However, concomitant variations in glucose concentration in the presence and absence of glutamate were never experimentally tested by the ANLSH detractors. Indeed, a complete demonstration should include experiments in which astrocytes in culture prepared in media with low (<7.5 mM) and high (>25 mM) glucose concentrations display not only different metabolic profiles but also opposite responses upon glutamate exposure. Indeed, experiments were independently done by other groups with astrocyte cultures prepared in a medium containing 5.6 mM glucose (Hamai et al., 1999; Keller et al., 1996). Results showed an increase in glucose utilization triggered by glutamate very similar to the response observed with cultures prepared in 25 mM glucose. Furthermore, some of us directly addressed this question in a recent study (Brunet et al., 2004). Isolated stem cells were differentiated into astrocytes in a medium containing either 5 or 33 mM glucose. Results showed that the glycolytic response to glutamate was the same, and if any better, in cells differentiated in 5 mM glucose. This study also demonstrated that as stem cells differentiated into astrocytes, they acquired their glycolytic phenotype and their capacity to respond to glutamate by enhancing aerobic glycolysis. This finding was further supported by the observation that expression of all the different components involved in the mechanism increased together with the differentiation process. We also extended our demonstration later showing that astrocytes prepared from different brain regions all gave rise to the same capacity to enhance glycolysis upon glutamate stimulation (Pellerin and Magistretti, 2005). Thus, this property appears to be an intrinsic feature of astrocytes and is not specifically linked either to culture conditions or cell origin.

**NEW EXPERIMENTAL EVIDENCE FOR A GLYCOLYTIC RESPONSE OF ASTROCYTES UPON GLUTAMATERGIC STIMULATION FROM IN VITRO, EX VIVO, AND IN VIVO APPROACHES**

Using novel and completely different approaches, several groups have recently provided strong support for a prominent glycolytic response in astrocytes upon activation, obtained either by direct application of glutamate...
or stimulation of glutamatergic pathways. This is the case for Felipe Barros and colleagues who have used a fluorescent technique combined with confocal microscopy to monitor glucose transport in cultured astrocytes and neurons with a time resolution of a few seconds. They reported that glutamate induced a rapid (within a few seconds) increase in glucose transport in neurons, which was accompanied by a decrease in glucose utilization (Porras et al., 2004). This suggests that activation of AMPA receptors leads to a rapid increase in glucose uptake and utilization in neurons.

Fig. 2. Neuroenergetics upon glutamatergic activation. A: *Early phase.* Activation of glutamatergic afferents leads to synaptic release of glutamate, AMPA receptor (AMPAR) activation and generation of an excitatory postsynaptic potential (EPSP) caused by Na\(^+\) entry within the postsynaptic spine [1]. Depolarization propagates to the dendrite and causes opening of voltage-sensitive Na\(^+\) channels, leading to further Na\(^+\) entry. Reestablishment of ion gradients is accomplished by the Na\(^+\)/K\(^+\) ATPase [2] that creates considerable energy expenditure. As a consequence, oxidative phosphorylation is activated [3] and mitochondrial NADH levels first decrease (Kasischke et al., 2004). Then, enhanced TCA cycle activity will ensue [4] to supply NADH for oxidative phosphorylation and support ATP production. As pyruvate utilization in the TCA cycle increases and its cytoplasmic levels decrease, the conditions become favorable for both enhanced glucose and lactate use.

Surprisingly, activation of AMPA receptors and coupled Na\(^+\) entry lead to a reduction in glucose uptake and utilization in neurons [5] (Porras et al., 2004), thus further favoring lactate utilization as a preferential oxidative substrate [6] (Bouzier-Sore et al., 2003). This would cause a transient drop in extracellular lactate levels as measured in vivo (Hu and Wilson, 1997; Mangia et al., 2003). B: *Late phase.* Glutamate released in the synaptic cleft is taken up by astrocytes to be recycled via the specific glutamate transporters GLAST and GLT1 [1]. A large Na\(^+\) influx caused by glutamate uptake takes place and activates the Na\(^+\)/K\(^+\) ATPase [2] (Pellerin and Magistretti, 1997), glucose transport [3] (Loaiza et al., 2003) and [4] glucose utilization (Pellerin and Magistretti, 1994) in astrocytes. The enhancement of aerobic glycolysis in astrocytes first causes a large increase in cytosolic NADH (Kasischke et al., 2004) that normalizes with the conversion of pyruvate into lactate and its release via monocarboxylate transporters expressed on astrocytes (mainly MCT1 and 4) [5]. Such a lactate release following glutamatergic activation corresponds to the increase in extracellular lactate levels measured in vivo (Demestre et al., 1997; Hu and Wilson, 1997).

Lactate produced by astrocytes during this later phase of activation not only replenishes the extracellular pool but also could help sustain neuronal energy needs as activation persists. Metabolic events occurring in the early and late phases described earlier constitute the so-called ANLS and its importance grows with the degree of glutamatergic activation. Such a view is supported by a series of experiments conducted in vivo (Serres et al., 2003, 2004, 2005). C: *Intense and prolonged stimulation.* Upon strong and long-lasting stimulation that occurs in certain conditions, glucose utilization becomes very important, in part, due to intense glutamate reuptake in astrocytes, that extracellular glucose levels are insufficient to sustain such uptake [1]. In such a situation, glycogen present in the astrocyte is mobilized to provide the necessary glycosyl units [2] as previously demonstrated in vivo (Swanson et al., 1992). Glycolysis is the predominant pathway [3] and lactate is produced [4] to maintain the high glycolytic rate. Resynthesis of glycogen will cause additional glucose uptake that might contribute to create a mismatch between glucose utilization and oxygen consumption, a phenomenon known as "uncoupling" (Fox and Raichle, 1986).
of astrocytes in the glutamate/glutamine cycle is essential to replenish the neuronal glutamate pool and maintain neurotransmission. Despite clear evidence that glutamate recycling into glutamine predominates (Fonseca et al., 2005; Lebon et al., 2002), it does not rule out that a small fraction of glutamate would not be recycled through this pathway. Part of this glutamate could be oxidized and would have to be resynthesized de novo from glucose by astrocytes, as demonstrated previously (Hertz et al., 1999). However, there is no solid experimental evidence demonstrating that glutamate uptake and recycling in astrocytes is supported solely by glutamate oxidation, especially in vivo, nor is there any reason to rule out the participation of glycolytic ATP to support at least part of this process.

In vivo demonstration, in which a major part of the increase in glucose utilization observed in an activated brain region is due to astrocytic glycolysis, was provided previously (Cholet et al., 2001; Herard et al., 2005; Voutsinos-Porche et al., 2003a,b). Even if these data were obtained in part on transgenic animals (with all possible caveats associated with the use of such animals), several controls were done to exclude any compensation or modification of either their normal glutamatergic neurotransmission or their metabolism. First, no difference in expression of several components of the glutamatergic pathway could be observed in GLT1 and GLAST KO compared to wild-type animals, including glutamate receptors, the other nontargeted glutamate transporters, and the enzyme glutamine synthetase. Moreover, no change in excitatory postsynaptic potential characteristics could be detected in hippocampal slices prepared from GLT1 and GLAST KO animals to ensure that their metabolism was not impaired (Voutsinos-Porche et al., 2003a). It was demonstrated that astrocytes from glial glutamate transporter KO animals exhibit a glycolytic response similar to the one observed with astrocytes from wild-type animals if the glutamate transporter step is bypassed. Thus, allowing glutamate to cause a massive Na+ influx via its receptors (by preventing their desensitization) rather than via its transporters produced the same response, demonstrating that it is only the absence of glutamate transporters that precludes the metabolic activation and not a metabolic defect. Finally, a decrease in the response was also observed in mature transgenic animals (Herard et al., 2005; Voutsinos-Porche et al., 2003b) as well as in adult rats treated to obtain a GLAST glutamate transporter knockout (Cholet et al., 2001), refuting the argument of a developmentally restricted or phenotype-associated phenomenon.
LACTATE AS A PREFERENTIAL OXIDATIVE SUBSTRATE FOR NEURONS

Over several decades, dozens of studies performed in vitro, ex vivo, and in vivo have repeatedly demonstrated that lactate constitutes an excellent oxidative substrate for neurons (for review, see Pellerin, 2003). It is unnecessary here to mention them all but, considering the large number of independent reports using so many different preparations and approaches, it is important to point out that they all came to the same conclusion, i.e., lactate is efficiently oxidized by neurons. Thus, the role of lactate as an important neuronal energy substrate can hardly be questioned. This large body of work cannot be omitted in discussions focusing on the lactate shuttle concept. Within the brain, astrocytes undoubtedly represent an important lactate source both at rest and upon stimulation, as discussed earlier (see also Figs. 1 and 2). Lactate accumulated in the extracellular space but also intracellularly in astrocytes constitutes a pool readily available for neurons upon increased energy demands. As demonstrated by Kasischke and coworkers, a rapid decrease in mitochondrial NADH occurs in dendrites upon activation followed by a recovery of the signal at this location (Kasischke et al., 2004). This pattern was interpreted as an activation of oxidative metabolism that causes a decrease in NADH and then an increase in the tricarboxylic acid cycle activity to replenish the mitochondrial NADH pool (see Fig. 2a). Considering that both glucose and lactate are available to neurons, the question arises as to what their preferential oxidative substrate would be under such circumstances.

In two independent studies, using different approaches, Sokoloff and colleagues as well as Bouzier-Sore and colleagues determined in a quantitative manner the proportion of glucose and lactate used oxidatively by neurons (Bouzier-Sore et al., 2003; Itoh et al., 2003). Both studies came to the same conclusion that lactate is the predominant oxidative substrate over glucose in cultured neurons. A criticism that was addressed to it. Moreover, in a more recent study (Bouzier-Sore et al., 2006), a similar substrate competition was performed on astrocytic as well as on neuronal cultures using glucose and lactate at physiological concentrations (1.1 mM each). Results confirmed that lactate was preferentially consumed by neurons, in contrast to astrocytes. More surprisingly, even in the presence of lactate in the medium, neurons produced lactate, indicating an intracellular compartmentation of lactate in neurons. Such a compartmentation was already observed in muscle (Kam and Milligan, 2006).

One argument put forward against the use of lactate by neurons is the fact that if glycolysis is very active in neurons, on the basis of the observation that neurons express glucose transporters and hexokinase, this should prevent lactate use (Chih and Roberts, 2003). Since both glycolysis (at the glyceraldehyde phosphate dehydrogenase-catalyzed step) and lactate dehydrogenase require NAD⁺ and might compete for it, glycolysis would take away all the cytosolic NAD⁺ available. This postulate forms the so-called redox switch concept proposed by Cerdan and coworkers (Cerdan et al., 2006; Cruz et al., 2001). Felipe Barros and his colleagues recently offered an elegant resolution of this paradox. They measured and compared glucose transport and utilization in astrocytes and neurons within the same culture at rest and upon glutamate exposure (Porras et al., 2004). In contrast to the increase in glucose transport observed in astrocytes, glutamate caused a reduction of glucose transport in neurons.

On the basis of the activation of AMPA receptors and Na⁺ influx, the effect was reversible and affected glucose transporters directly. This reduction in glucose transport by neurons upon glutamate stimulation has two main implications. First, reduction in glycolytic flux in neurons upon stimulation alleviates the redox switch problem by preserving cytosolic NAD⁺ for use by the lactate dehydrogenase to convert lactate into pyruvate. Thus, under glutamatergic stimulation, lactate utilization by neurons is favored (see Fig. 2a). The inhibition of neuronal glucose transport by glutamate was even stronger in the presence of lactate (Porras et al., 2004), which is consistent with the reduction in glucose utilization observed in vivo in humans by fluorodeoxyglucose PET when providing lactate to the brain by raising its plasma level (Smith et al., 2003). Second, as glutamate increases the ratio of glucose transport between astrocytes and neurons by 12 fold, the neurotransmitter will redistribute the sugar from neurons to astrocytes. As a result, more glucose can be converted by astrocytes to lactate. In parallel, since neurons have a reduced glucose uptake capacity but increased energy needs upon glutamate exposure, lactate oxidation will be favored.

Preferential use of lactate as an oxidative substrate does not preclude the use of glucose by neurons and the importance of glycolysis to sustain at least some neuronal functions remains. Indeed, it was shown that glycolysis seems essential to ensure proper vesicular filling (Ikemoto et al., 2003) and maintain neurotransmitter homeostasis (Bak et al., 2006) at glutamatergic synapses. Moreover, it appears to be involved in maintaining
some GABAergic receptor activity (Laschet et al., 2004). Nevertheless, it was also shown that lactate can efficiently promote vesicular accumulation and release of GABA from synaptosomes (Tarasenko et al., 2006) as well as sustain synaptic vesicle turnover in cultured cortical neurons (Morgenthaler et al., 2006). Whether glycolysis is essential for a certain type of activity (pre-versus postsynaptic) and/or a specific neuronal subtype (glutamatergic versus GABAergic) remains to be established.

**EVIDENCE FOR A NET LACTATE TRANSFER BETWEEN ASTROCYTES AND NEURONS IN VIVO**

Evidence exists in vivo for enhanced lactate utilization by brain parenchymal cells upon activation. Thus, Hu and Wilson reported that the sustained activation of the perforant path led first to a decrease in extracellular lactate concentrations, as detected by a microsensor implanted in the hippocampal dentate gyrus, followed by a massive increase (Hu and Wilson, 1997). The initial decrease in lactate concentration, observed at the beginning of a sustained activation but also at the onset of every stimulation upon repetitive activation, most likely represents lactate utilization by brain cells. Similarly, MR spectroscopic observation of an initial dip in extracellular lactate concentration was reported in humans (Mangia et al., 2003), thus refuting the criticism that animal results could be biased by the invasive approach of implanted biosensors. Mangia and colleagues interpreted this initial dip as an argument against ANLSH, but this interpretation appears unlikely (see the Insight from mathematical modeling section below for a discussion of these points). The pattern of lactate concentration changes over time with sustained activation (first a decrease, followed by an «overshoot») is also consistent with the scenario described by Kasischke and coworkers based on a subcellular NADH signal (Kasischke et al., 2004). In the first few seconds of activation, an increase in neuronal oxidative metabolism takes place that could be fueled by extracellular lactate already present, thus leading initially to a decrease in extracellular lactate concentration. After about 10 s, glycolysis in astrocytes is activated and replenishes the extracellular pool, causing even a transient peak. If neuronal activation is activated and replenishes the extracellular pool, causing a &oversign;overshoot«) is also consistent with the aforementioned experimental observations of a redistribution of glucose away from neurons toward the astrocytes upon increased activity, and in return with enhanced astrocytic glycolysis to provide lactate for fulfilling ongoing neuronal energy needs upon sustained activation. Interestingly, such a scenario has been recognized recently as most likely and quantitatively important based on several NMR studies performed in vivo (Hyder et al., 2006).

The observation made in vivo by MR spectroscopy based on [3-13C]lactate metabolism that lactate is more specifically a neuronal substrate has also been questioned (Hertz, 2004). The initial conclusion of a predominantly neuronal lactate metabolism was based on the finding that glutamine C2 and C3 enrichments were similar, indicating that there was no entry of 13C into the astrocytic TCA cycle via the pyruvate carboxylase (PC) pathway (Bouzier et al., 2000; Hassel and Brathe 2000; Tyson et al., 2003). In contrast, it has been argued that such a result could simply be the consequence of a rapid equilibration between oxaloacetate and fumarate (Hertz, 2004). Such reasoning was mainly based on a previous in vitro study (Merle et al., 1996) that showed that in cultured astrocytes, a fraction of oxaloacetate was equilibrated with fumarate. Extrapolating these data, it was hypothesized that, in vivo, the equilibration yield of oxaloacetate with fumarate would be so great that the difference in enrichment between glutamine C2 and C3 linked to PC activity would be totally masked when [3-13C]lactate is the substrate (Hertz, 2004). However, there is no evidence to assume that the astrocytic metabolism of [3-13C]pyruvate via PC would lead to different enrichments of glutamine C2 and C3 when this compound derives from glycolysis (for example, when starting from [1-13C]glucose), and to similar enrichments when it derives from [3-13C]lactate. Paradoxically, an argument invoked as supporting rapid equilibration is a study by Gruetter et al. (2001), who used a mathematical model of brain metabolism in which there was in fact no equilibration between oxaloacetate and fumarate. Thus, previous conclusions that lactate metabolism in vivo is predominantly neuronal remain entirely valid and are further supported by the most recent results (Serres et al., 2003, 2004, 2005), suggesting a net transfer of lactate from astrocytes to neurons in vivo that increases with the level of activity.

**INSIGHTS FROM MATHEMATICAL MODELING**

Although mathematical modeling cannot per se identify among controversial hypotheses the correct one, it
can give a coherent, quantitative framework for the discussion, suggest possible mechanisms or, conversely, emphasize the contradictions or implausibility of some hypotheses. In this regard, the model of Gjedde and Marrett (2001) gathers, in one simple equation, the main components of pyruvate and lactate metabolism: glycolysis, pyruvate entry into mitochondria, near-equilibrium of the LDH-catalyzed reaction, and export of lactate through the blood brain barrier. On this basis, the authors suggested that the glycolysis in neurons but not in astrocytes is responsible for the delay in oxidative metabolism that they observed in human visual cortex using PET, so that PET data could be interpreted as dismissing the ANLSH. Although this model is potentially interesting, the authors make very strong hypotheses. In particular, they assume that the lactate/pyruvate ratio is specific to each cell type, neurons versus astrocytes, and does not vary upon stimulation. In fact, it is well known that the lactate/pyruvate ratio critically depends on the NADH/NAD⁺ ratio (see, e.g., Berg et al., 2002). Although this latter ratio may effectively differ between neurons and astrocytes at rest, recent experimental data from Kasischke et al. (2004) unambiguously show that the NADH/NAD⁺ ratio varies upon the stimulation in both cell types, while earlier studies had shown changes of this ratio in neural tissue (e.g., Dóra et al., 1984). Thus, it appears that the hypothesis of constant lactate/pyruvate ratios, which is central to this reasoning, does not fit with these data and is thus very unlikely.

Information can also be drawn from theoretical studies of lactate kinetics. Kuhr et al. (1988) elaborated a model of exchanges of lactate between extracellular and intracellular compartments, using Michaelis–Menten kinetics to describe the carrier-mediated transport of lactate. On this basis, they interpreted their measurements of extracellular, arterial, and venous lactate in rats in vivo, under various circumstances: electrically induced shocks, cardiac arrest, and intravenous administration of lactate. Although their model does not include the blood brain barrier, careful analysis of both experimental and theoretical results suggests that lactate clearance from brain tissue by the bloodstream plays only a minor role, while most tissue lactate is likely recycled to pyruvate. More recently, Leegsma-Vogt et al. (2004) developed a compartmental model of the kinetics of cerebral and extracerebral lactate, and compared the theoretical results to continuous sampling of arterial and venous blood concentrations. In a preceding article, these authors (Leegsma-Vogt et al., 2003) had concluded that lactate does not replace glucose as a cerebral metabolic substrate. In their more recent study, although their model was plausible without assuming any cerebral lactate consumption, they obtained a better fit by including a brain lactate sink, which again is in favor of the utilization of lactate as an energy substrate. These authors also suggest that when the intracerebral lactate level becomes high enough, at least part of the lactate formed by the astrocytes may leave the brain. Interestingly, this latter suggestion is not contradictory with the ANLS hypothesis. Thus, both the models of Kuhr and colleagues and Leegsma-Vogt and colleagues are consistent with the hypothesis of lactate metabolism by brain tissue. However, neither of these studies describes the compartmentalization between neurons and astrocytes, so that they do not directly address the question of the ANLS.

On the basis of a previous mathematical model of the relationships among brain electrical activity, energy metabolism, and hemodynamics (Aubert and Costalat, 2002), some of us developed a model of compartmentalized energy metabolism between neurons and astrocytes to test the ANLS hypothesis from a theoretical point of view (Aubert and Costalat, 2005). This model deliberately relies upon hypotheses that are unfavorable to ANLS. Especially, it is assumed that no ANLS is present at the resting state, and parameter values for neurons and astrocytes are chosen in such a way that ANLS is not favored: for instance, we took the same values in neurons and astrocytes for maximal transport rates and Michaelis constants for MCT1s, parameters for the regulation of hexokinase and phosphofructokinase, or the Michaelis constant of mitochondrial respiration for ADP. The only assumed difference between neurons and astrocytes is a moderate lactate production by astrocytes at rest, owing to a slightly lower value of mitochondrial maximal activity, this production being balanced by lactate efflux through the blood brain barrier, as suggested by Gjedde et al. (2002). Notwithstanding, simulations clearly show ANLS to occur, at least during the first tens of seconds of a sustained activation, and during the poststimulus period. The occurrence of ANLS during the later phase of a sustained activation depends on the relative astrocytic versus neuronal stimulus intensity, which can be related to the notion, introduced by Collins (1997) and Gjedde et al. (2002), of differentiated “white” and “red” responses of neuronal tissue to stimulation. A “white” response would correspond to a “simple” stimulation, with low information content, and might induce an important increase in presynaptic and astrocytic glycolysis, while postsynaptic neuron activity would not be greatly enhanced, so that their mitochondria would be poorly stimulated; in this case, simulations suggest that ANLS is still present during the later phase of a sustained stimulation. In the case of a “red” response, a stimulus with high information content would result, besides the stimulation of astrocytes and presynaptic neurons, in a significant depolarization of postsynaptic neurons, resulting in a marked increase in CMRO₂; in this case, simulations suggest that ANLS need not occur during the later phase of a sustained stimulation. In all cases, the simulated evolution of extracellular and tissue lactate concentrations is largely consistent with the data of Hu and Wilson (1997) and Mangia et al. (2003), with an initial dip at the beginning of the stimulation period, which clearly indicates that the MRS data of Mangia and colleagues cannot be viewed as an argument against ANLS. Furthermore, a parameter study performed using a model of lactate kinetics (Aubert et al., 2005) unambiguously shows that the lactate initial dip can be
explained neither by a lactate washout due to the CBF increase nor by pH changes: this makes early lactate consumption by cells very likely. These findings are fully consistent with the simulations of the aforementioned model (Aubert and Costalat, 2005), where lactate is drawn by neurons from the extracellular space during the initial dip. Most interestingly, simulation curves for the NADH/NAD⁺ ratio in astrocytes and neurons are highly consistent with the recent data of Kasischke et al. (2004), with an early neuronal oxidation and a somewhat delayed astrocytic reduction. Thus, theoretical results suggest the following mechanisms, at least during the first tens of seconds of a sustained stimulation. (i) At the beginning of the stimulation, neurons might undergo an increased mitochondrial activity, with a NADH decrease, which might consume lactate, part of which would be supplied by the extracellular pool. This need not be contradictory with a possible concomitant increase in neuronal glycolysis. (ii) Astrocytic lactate may slightly decrease due to ANLS, but this phenomenon is progressively overwhelmed by the stimulation of astrocytic glycolysis, which is responsible for the slightly delayed increase in astrocytic NADH. Thus, modeling clearly shows that the biphasic behavior of both lactate and NADH/NAD⁺ kinetics can be put together within a quantitative framework that is fully consistent with the ANLS hypothesis. Note that these theoretical results are supported by recent experimental data showing an intracellular mitochondrial heterogeneity in astrocytes (Waagepetersen et al., 2006).

GLYCOGEN: JUST ANOTHER LACTATE RESERVE?

Another interesting case is glycogen. It has been proposed that glycogen mobilization occurring during intense sensory stimulation could account for the uncoupling phenomenon described previously by Raichle and colleagues (Fox and Raichle, 1986), and as such it was presented as being inconsistent with the existence of the ANLSH (Dienel and Cruz, 2003). Our previous work dating back 25 years (Magistretti et al., 1981) testifies the fact that we strongly support the idea of an important role for glycogen in brain energy metabolism (Magistretti, 1988; Magistretti and Pellerin, 1997; Magistretti et al., 1993; Tsacopoulos and Magistretti, 1996). Although we agree that glycogen mobilization and its subsequent resynthesis, for which we have made a number of contributions to the understanding of the underlying molecular mechanisms (Allaman et al., 2000, 2003, 2004; Cardinaux and Magistretti, 1996; Hof et al., 1988; Magistretti et al., 1981, 1986; Pellegrini et al., 1996; Sorg and Magistretti, 1991, 1992; Sorg et al., 1995), could be one factor involved in the uncoupling phenomenon, it is necessary, however, to replace this observation in a proper perspective. Under basal conditions, the ratio of O₂ consumption over glucose utilization is close to six in the brain, indicating that glucose is almost entirely oxidized to provide the energy necessary to support brain function. A change in this ratio as occurring in the experiments of Raichle and colleagues (Fox and Raichle, 1986), whereby an increase in glucose utilization was not accompanied by a commensurate enhancement in O₂ consumption upon activation, is considered as uncoupling. A survey of the different stimulation paradigms tested, and their degree of coupling/uncoupling has been compiled (Rothman et al., 2003). Interestingly, uncoupling represents more the exception than the rule and seems associated more specifically with the conditions of intense somatosensory stimulations. More complex cognitive activations rather give rise to concomitant increases in glucose and oxygen consumption of a comparable magnitude, keeping a ratio consistent with coupling. Thus, it could be envisaged that an increase in astrocytic glycolysis together with enhanced neuronal oxidative lactate utilization could take place under most activation paradigms that involve glutamatergic stimulation, giving rise to a coupling situation in most cases. In the few situations of intense somatosensory stimulation (see Fig. 2c), the additional mobilization and resynthesis of glycogen might occur, further promoting the emergence of an uncoupling condition (Shulman et al., 2001). This view is supported by experiments performed in an optic nerve preparation where it was shown that glial glycogen is mobilized only upon high intensity stimulation under normal glucose concentrations (Brown et al., 2003, 2004). In other words, the two processes (glycolysis and glycogenolysis in astrocytes) could be complementary and most likely activated successively as the intensity of activation increases. Such a mechanism would ensure that astrocytes maintain their capacity to recycle glutamate at high intensity of stimulation as extracellular glucose would become a limiting factor. As can be seen, glycogen mobilization and activation of glial glycolysis are not mutually exclusive and can simply represent two steps in a graded process to deal with different levels of activation. In addition, it is most likely that glycogen can be mobilized under specific circumstances for other purposes. It is important to remember that the major product from glycogenolysis released by astrocytes is lactate (Dringen et al. 1993). Previous reports have shown that some neurotransmitters and neuromodulators such as noradrenaline, adenosine, ATP, or vasoactive intestinal peptide enhance glycogenolysis in astrocytes (Sorg and Magistretti, 1991; Sorg et al., 1995) but not glutamate. A suggested role for this effect is also to provide lactate as an additional energy substrate for active neurons (Magistretti et al., 1993; Tsacopoulos and Magistretti, 1996). Furthermore, independently from synaptic transmission, it has been proposed that lactate provided by glycogenolysis in astrocytes could serve to sustain action potential propagation in axons (Brown et al., 2003, 2004, 2005; Tekkók et al., 2005). Thus, from our point of view, glycogen mobilization can be seen more as an extension of the ANLS concept rather than as a competing hypothesis. Interestingly, a number of observations remain enigmatic when not considered within the context of the ANLSH. Although they do not constitute by themselves a demonstration of the existence of this mechanism, they
deserve to be integrated in a coherent description of brain energy metabolism. Thus, the selective distribution of lactate dehydrogenase isoforms (Bittar et al., 1996; Laughton et al., 2000) as well as monocarboxylate transporters or MCTs (reviewed in Pellerin et al., 2005) between astrocytes and neurons indicates that lactate metabolism occupies a central place in brain energy metabolism. The possibility of lactate transfer between these cell types would be highly facilitated by this distribution. Similarly, the presence of the monocarboxylate transporter MCT2 at postsynaptic densities of glutamatergic synapses together with suggestions of a possible translocation mechanism is rather intriguing (Bergersen et al., 2001, 2005), but would make sense if upon activation lactate transport becomes a limiting factor for its utilization in neurons, as has been suggested (Hertz and Dienel, 2005). Evidence for this point has already been provided in cultured neurons, for which it was shown that overexpression of MCT2 allowed neurons to increase their lactate consumption upon glutamate exposure (Bliss et al., 2004). Overexpression was also observed for MCT1 but not for GLUT1 under inflammatory conditions, and for MCT4 under hypoxia (Ullah et al., 2006), providing a more rapid release of lactate that might contribute to a protective mechanism for neurons. The description of the selective presence of the α2 subunit of the Na+, K+ ATPase in astrocytic processes surrounding glutamatergic synapses and its colocalization with the glial glutamate transporters GLAST and GLT1 is another important observation requiring to be considered in the context of the mechanisms governing glucose utilization in astrocytes (Cholet et al., 2002). Moreover, the absence of mitochondria in these fine astrocytic processes too small to accommodate them (Rohmann and Wolff, 1996) raises the question of the metabolic pathway (glycolysis and/or glycogenolysis?) that fuels the α2 subunit of the Na+, K+ ATPase at this location. Finally, the observation that upon differentiation into astrocytes, all the different components participating in the glycolytic response to glutamate (the glutamate transporter GLAST, the α2 subunit of the Na+, K+ ATPase, the monocarboxylate transporter MCT1 are upregulated concomitantly (Brunet et al., 2004), pleads for a coordinated process that represents a constitutive feature of astrocytes. These observations cannot be simply dismissed and the only coherent explanation to date is afforded by the ANLSH.

**PERSPECTIVES**

It is likely that the debate regarding the ANLSH will persist for some time and further experiments will be needed to test its validity under various conditions. For the moment, the model put forward proposes a coherent explanation for the cellular and molecular events giving rise to some metabolic signals observed in vivo following the activation of glutamatergic pathways, considering that glutamate represents the major neurotransmitter in the central nervous system. It is likely that it will require further modifications and refinement to account for most observations. An interesting extension that was recently proposed by Cerdan and coworkers focuses on redox coupling between astrocytes and neurons (Cerdan et al., 2006). It is suggested that the lactate transfer between astrocytes and neurons occurring as part of the classical ANLS concept serves not only the purpose of fueling the neuronal TCA cycle but also provides reducing equivalents to the neuron. In this manner, the intracellular compartments of astrocytes and neurons are coupled and the directionality of the lactate transfer is determined by the redox state of each cell, predominantly oxidized in neurons due to the predominance of oxidative metabolism while rather reduced in astrocytes owing to the high glycolytic activity. Operation of the previously described redox switch in this scheme perfectly accounts for both the reduction in neuronal glycolysis and the favored lactate utilization by neurons upon stimulation.

It is important to underline that, in the actual context, few other models have emerged offering similarly interesting perspectives. Exploring the possible implications of this concept in different areas of the neurosciences and evaluating its potential/usefulness represents a unique opportunity for further progress. A first field concerned by the evolution of our knowledge about brain energy metabolism is functional brain imaging. For example, it has been suggested that GABAergic activation does not directly contribute to the glucose utilization signal since GABA did not enhance aerobic glycolysis in astrocytes (Chatton et al., 2003). Thus, glutamatergic activity remains the major (and perhaps the sole) pathway responsible for this metabolic signal. Moreover, it was shown that a glucose utilization signal can propagate through an astrocytic syncytium, forming a metabolic wave (Bernardinelli et al., 2004). This would mean that the metabolic signal recorded by brain imaging could emanate from a much larger area than the original neuronal network activated. This is critical to know when determining the areas activated based on the origin of the signal. The concept that astrocytes, via their uptake of glutamate, represent the source of different imaging signals has now been extended to imaging based on intrinsic optical signals. In the olfactory bulb, it was shown that the enhancement of the intrinsic optical signal caused by an odor was abolished by a glutamate transporter inhibitor but not by glutamate receptor antagonists (Gurden et al., 2006). Quite importantly, it becomes possible now to propose a coherent framework to explain the origin of most brain imaging signals based on our knowledge of metabolic and hemodynamic events. As it was clearly shown recently, both neuronal and astrocytic responses determine imaging signals, while the ANLS constitutes an integral component of such responses (Aubert et al., 2007).

The concept of the ANLS has also found some unexpected applications. This is the case in the field of glucose sensing and diabetes. It was shown that the detection of circulating glucose levels depends on the conversion of glucose into lactate by astrocytes in the hypothalamus (Lam et al., 2005). Then, the use of...
lactate by hypothalamic neurons leads to the activation of their ATP-sensitive potassium channels, reducing their firing and the signal to promote glucose production from the liver. Consistent with a role of lactate shuttling in glucose sensing, Rutter and coworkers have shown that while only astrocytes responded to extracellular glucose changes with alterations in their intracellular ATP concentrations, hypothalamic neurons responded to changes in lactate levels instead (Ainscow et al., 2002). Another important and promising outcome of the application of the principles revealed by the ANLSH concerns neuroprotective strategies. Functional brain imaging has shown that several neurodegenerative diseases are characterized by metabolic impairments that often precede the onset of the symptoms (Feigin et al., 2001; Kennedy et al., 1995). For years, it was thought that improving energy metabolism in neurons, notably by enhancing glucose uptake, would be one of the most valuable strategies for neuroprotection (Sapolsky, 2003). However, it was realized that based on the ANLSH, enhancing glucose uptake in astrocytes and lactate uptake in neurons could be an alternative approach. An experimental demonstration that it is indeed the case was provided using a system of cocultures with astrocytes and neurons overexpressing glucose and monocarboxylate transporters, respectively (Bliss et al., 2004). Additionally, progress has been made in the development of cognitive enhancers that could be useful for the treatment of various conditions ranging from mild cognitive impairments to Alzheimer’s disease (Marshall, 2004). Several of the agents identified (e.g., the ampakines) act impairs to Alzheimer’s disease (Marshall, 2004).

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