Metabolic Coupling between Glia and Neurons

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The concept of a metabolic coupling between neurons and glia, particularly astrocytes, geared at maintaining energy metabolism homeostasis in the brain has been entertained for some time. The first element of this concept emerged from the analysis of the cytological relationships existing among capillaries, astrocytes, and the neuropil (Fig. 1). Indeed, based on the observation that specialized astrocytic processes, the end-feet, are interposed between virtually all brain capillaries and neuronal processes, it was postulated over a century ago that astrocytes represented the primary route for the transfer of metabolic substrates from the circulation to the neuropil (Golgi, 1886; Sala, 1891). A lucid formulation of this concept was presented by Andriezen (1893): "The development of a felted sheath of neuroglia fibers in the ground-substance immediately surrounding the blood vessels of the Brain seems therefore . . . to allow of the free passage of lymph and metabolic products which enter into the fluid and general metabolism of the nerve cells." Although this appealing concept has been discussed critically throughout the literature over decades (Nicholls et al., 1992), functional evidence for specific molecular and cellular processes supporting this concept has been lacking, and the nature of the metabolic substrates distributed by astrocytes to neurons remains elusive. Because glucose is the almost exclusive energy source of the brain, an unanswered question has been whether the function of astrocytes is restricted to glucose uptake and to its distribution, without further metabolic processing, to neurons, or whether astrocytes release a metabolic intermediate of glucose. The major obstacle for functional studies aimed at resolving this question has been the difficulty of achieving the cellular resolution necessary to monitor metabolic fluxes between the highly intermingled neuronal and glial processes in the intact brain. This obstacle has been overcome in recent years by using simple and well compartmentalized neural systems such as the retina, or purified cellular preparations of neurons and glia.

In this article, we will review the evidence obtained from studies carried out (1) in primary cultures of astrocytes or neurons prepared from mouse cerebral cortex, and (2) in the honeybee drone retina and in the juvenile guinea pig retina. The view that emerges from these studies confirms, and in fact extends, the concept put forward over a century ago. Thus, astrocytes are indeed the

primary site of glucose uptake during neuronal activity; however, glucose does not transit unmetabolized through astrocytes for distribution to the neuropil, as was postulated by Sala (1891) and by Golgi (1886). Rather, glucose is processed glycolytically in astrocytes, which release lactate (or alanine in the insect retina) as the metabolic substrate to be used by neurons. These experimental data indicate a remarkable metabolic compartmentation between astrocytes and neurons, which is corroborated by the selective distribution of certain enzymatic activities in either cell type. In addition, they provide the cellular basis for unexpected findings obtained by functional-brain imaging techniques, such as positron emission tomography (PET) (Fox and Raichle, 1986; Fox et al., 1988) and ¹H magnetic resonance spectroscopy (MRS) (Prichard et al., 1991), which consistently indicate the occurrence of glycolysis during neuronal activity. The converging set of results obtained under diverse experimental conditions and in different cellular preparations provides strong arguments for revising the view that glial cells simply represent a cellular mass filling the spaces between neurons and providing a preferential, but metabolically inert, route for the passage of glucose from the circulation to neurons. That glycogen, the storage form of glucose, is localized almost exclusively in astrocytes is further evidence for an active metabolic processing of glucose by this cell type (for review, see Magistretti et al., 1993). Glycogenolysis in astrocytes is under tight control of specific neurotransmitters that can mobilize it, via receptor-mediated mechanisms, during neuronal activity (Magistretti et al., 1993).

Uptake and phosphorylation of glucose by glial cells during neuronal activity

The first evidence for and quantitative assessment of the prevalent uptake and phosphorylation of glucose by glial cells during neuronal activity was provided in the honeybee drone retina. A unique feature of this preparation is its crystal-like structure and metabolic compartmentation. In this neural preparation, modular, rosette-like structures, made up of six photoreceptor neurons that respond directly to light stimulation, are surrounded by one type of glial cell, the outer pigment cell (Fig. 2). In addition to this structural segregation, the metabolic compartmentation of glia and neurons is remarkable. Photoreceptor neurons are enriched in mitochondria and virtually devoid of glycogen, whereas the reverse is the case in glial cells (Tsacopoulos et al., 1987). Energy metabolism in photoreceptors is exclusively aerobic, because anoxia or the inhibitor of mitochondrial respiration amobarbital abolish their light-induced electrical activity in a few seconds (Dimitracos and Tsacopoulos, 1985).

Uptake and phosphorylation of glucose by a tissue can be determined quantitatively by monitoring the accumulation of

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Figure 1. Schematic representation of the cytological relationships existing among intraparenchymal capillaries, astrocytes, and the neuropil. Astrocyte processes surround capillaries (end-feet) and ensheath synapses; in addition, receptors and uptake sites for neurotransmitters are present on astrocytes. These features make astrocytes ideally suited to sense synaptic activity (*A*) and to couple it with uptake and metabolism of energy substrates originating from the circulation (*B*).

[^3H]2-deoxyglucose (2-DG) (Sokoloff et al., 1989). 2-DG is an analog of glucose, which is transported into cells through glucose transporters and phosphorylated by hexokinase. The [^3H]2-DG-phosphate (2-DG-P) that is formed is not metabolized further and accumulates intracellularly, thus providing a quantitative assessment of glucose utilization by a tissue (Sokoloff et al., 1977). Using this technique, glucose uptake and phosphorylation have been determined in honeybee drone retinal slices maintained in a physiological solution. The accumulation of 2-DG-P proceeds linearly over 60 min at a rate of $6 \mu\text{mol/hr}$ and shows a K_m (combined for uptake and phosphorylation) of 1.6 mM, a value of the same order of magnitude as that found in the mammalian brain (Tsacopoulos et al., 1988). Most importantly, quantitative autoradiography unequivocally indicates that the accumulation of ^3H label (verified by HPLC to correspond to 2-DG-P) occurs exclusively in glial cells. Photoreceptors, by contrast, are labeled only at background levels (Fig. 2*a*). These observations indicate that the uptake and phosphorylation of glucose occur almost exclusively in the glial cells of this nervous tissue. Indeed, subcellular localization of hexokinase and measurement of hexokinase activity in purified suspensions of freshly isolated glial cells or photoreceptors indicated that nearly all hexokinase activity is confined in the cytosolic fraction of glial cells (Veuthey et al., 1994). During photostimulation, this metabolic compartmentation is maintained. Photoreceptor neurons, not glial cells, contain the visual pigment rhodopsin and, therefore, are the only cells in the retina directly excitable by light. Accordingly, repetitive photostimulation increases oxygen consumption of photoreceptors from 38 to $160 \mu\text{l of O}_2 \cdot \text{ml of photoreceptor}^{-1} \cdot \text{min}^{-1}$; yet there is a 50% increase in 2-DG uptake and phosphorylation exclusively localized in the glial cells (Tsacopoulos and Poitry, 1982; Brazitikos and Tsacopoulos, 1991). This observation raises two important questions. First, because glial cells cannot be activated directly by light, is there a signal released from the activated photoreceptors to trigger glucose uptake and phosphorylation in the glial cells? Second, is the increased oxygen consumption of photoreceptor neurons accounted for by their oxidation of a metabolic substrate released by the glial cells? The first question has not been answered definitively for the honeybee drone retina, although NH_4^+ is a strong candidate for a signal released by the photoreceptors (Tsacopoulos and Poitry, 1995). In mammalian neural tissue, strong evidence points to glutamate as the coupling signal between neuronal activation and glucose uptake by astrocytes (Pellerin and Magistretti, 1994; Takahashi et al., 1995). Regarding the second question, alanine has been identified as the metabolic substrate released by glial cells of the honeybee drone

retina during photoreceptor activation. Evidence supporting this will be detailed below.

A similar metabolic coupling between glial cells and neurons has been demonstrated in the guinea pig retina. Although not as completely compartmentalized from the structural viewpoint as the honeybee drone retina, astrocytes (Müller cells) can be identified easily in histological sections of this mammalian central nervous system structure. Using a combination of biochemical and autoradiographic techniques described previously for the honeybee drone retina, evidence has been obtained for a predominant 2-DG uptake into Müller cells under both basal and light-stimulated conditions (Poitry-Yamate and Tsacopoulos, 1991). These observations, obtained in whole retina, were confirmed in a highly purified preparation consisting exclusively of Müller cells freshly isolated from juvenile guinea pig retinas. This preparation showed intensive labeling of individual cells exposed to 2-DG that was subject to inhibition by the glycolysis inhibitor iodoacetate (Poitry-Yamate and Tsacopoulos, 1992).

Further clarification of the biochemical mechanisms underlying cell-specific metabolic fluxes between glia and neurons has been provided recently in purified preparations consisting of primary cultures of astrocytes or neurons isolated from rodent brain. In general, the basal rate of glucose utilization has been reported to be higher in astrocytes than in neurons (Lopes-Cardozo et al., 1986); in mouse cerebral cortical cultures, values are $\sim 20 \text{ nmol} \cdot \text{mg of protein}^{-1} \cdot \text{min}^{-1}$ for astrocytes and $\sim 10 \text{ nmol} \cdot \text{mg of protein}^{-1} \cdot \text{min}^{-1}$ for neurons (Pellerin et al., 1995). These values are of the same order of magnitude as those determined *in vivo* for cortical gray matter using the 2-DG autoradiographic technique (Sokoloff et al., 1977).

The contribution of astrocytes to glucose utilization during neuronal activity is even more striking. Indeed, astrocytes are ideally positioned to provide coupling between neuronal activity and glucose uptake. As noted in the introductory remarks, astrocyte end-feet surround intraparenchymal capillaries, which are the source of glucose. This cytoarchitectural arrangement implies that astrocytes form the first cellular barrier that glucose entering the brain parenchyma encounters, and it makes astrocytes a likely site of primary glucose uptake (Fig. 1). Another feature of astrocytes is the existence of processes that ensheath synaptic contacts (Peters et al., 1991). The recent demonstration of neurotransmitter receptors and uptake sites on astrocytes suggests that astrocytes are geared to sense synaptic activity (Barres, 1991; Murphy, 1993). In fact, two well established functions of astrocytes are the clearance of potassium and the uptake of glutamate, both of which increase in the extracellular space in conjunction with synaptic

Figure 2. *a*, Autoradiograph of a slightly oblique section prepared from a slice of honeybee drone retina previously incubated with [^3H]2-DG for 60 min and then washed for 30 min. Only glial cells wrapping the photoreceptors (rosettes) are heavily stained with [^3H]2-DG-P (magnification, 720 $\times$). *b*, Light micrograph of a section almost perpendicular to the axes of the ommatidia. The *rosettes* are the retinulae, clusters of six photoreceptor cells. The *dark lozenge* at the center of each retinula is the rhabdom, made of microvilli contributed by each of the photoreceptors. The visual pigment rhodopsin is packed in the microvilli. The *pale cells* between the retinulae are glial cells. Staining was done with Toluidine blue. (magnification, 640 $\times$; scale bar, 20 μm). *c*, Electron micrograph showing a cross-section through part of an intact retina, nearly perpendicular to the long axes of six large photoreceptor cells forming one retinula. The photoreceptors contain many mitochondria oriented at the periphery of the cell. Glial cells surrounding photoreceptors contain almost no mitochondria (magnification, 1200 $\times$). *d*, Slightly oblique section. The use of OsO_4 in Millonig buffer (Veuthey et al., 1994) reveals the glycogen granules filling a glial process. Note the vicinity of mitochondria and glycogen granules, separated by the cytoplasmic membranes of the photoreceptors and glial cells (magnification, 11,000 $\times$).

activation (Kuffler et al., 1966; Barres, 1991). During physiological stimulation of a given cortical area, glutamate is released from the axon terminals of activated excitatory pathways (Fonnum, 1984; Danbolt, 1994). *In vitro*, activation, therefore, can be mimicked by exposure of cells to relevant concentrations of glutamate, and glucose uptake and phosphorylation can be monitored via the 2-DG technique. Indeed, glutamate stimulates 2-DG uptake and phosphorylation by astrocytes, with an EC_{50} of $\sim 70 \mu\text{M}$ (Pellerin and Magistretti, 1994). This effect is not mediated by the glutamate receptors present on astrocytes (Pearce, 1993); rather, stim-

ulation involves activation of glutamate transporters (Pellerin and Magistretti, 1994; Takahashi et al., 1995). Three main types of glutamate transporters have been characterized (Kanner, 1993; Danbolt, 1994; Kanai et al., 1994): EAAC1, which is neuron-specific ($\text{EC}_{50} = 1 \mu\text{M}$); GLT1, which is present exclusively on astrocytes ($\text{EC}_{50} = 10 \mu\text{M}$); and GLAST, which is present on both neurons and astrocytes ($\text{EC}_{50} = 50\text{--}70 \mu\text{M}$). The uptake of glutamate by these transporters is coupled to the inward transport of 2–3 Na^+ ions per glutamate molecule (Bouvier et al., 1992). Thus, glutamate uptake into astrocytes causes an increase in the intra-

Figure 3. Schematic representation of the mechanism for glutamate-induced glycolysis in astrocytes during physiological activation. At glutamatergic synapses, presynaptically released glutamate depolarizes postsynaptic neurons by acting at specific receptor subtypes. The action of glutamate is terminated by an efficient glutamate-uptake system located primarily in astrocytes. Glutamate is cotransported with Na^+ , causing an increase in the intra-astrocytic concentration of Na^+ , leading to an activation of the astrocyte Na^+/K^+ -ATPase. Activation of Na^+/K^+ -ATPase stimulates glycolysis, i.e., glucose utilization and lactate production (Parker and Hoffman, 1967; Lipton and Heimbach, 1978; Paul et al., 1979; Lynch and Balaban, 1987). Lactate, once released by astrocytes, can be taken up by neurons and serve them as an adequate energy substrate (for graphic clarity, only lactate uptake into presynaptic terminals is indicated; however, this process also could occur at the postsynaptic neuron). In accord with recent evidence, glutamate receptors also are shown on astrocytes (Pearce, 1993). This model, which summarizes *in vitro* experimental evidences indicating glutamate-induced glycolysis, reflects cellular and molecular events occurring during activation of a given cortical area (arrow labeled *A* for activation). Direct glucose uptake into neurons under basal conditions also is shown (arrow labeled *B* for basal conditions): Pyr, pyruvate; Lac, lactate; Gln, glutamine; G, G-protein. [Taken from Pellerin and Magistretti (1994) with permission.]

cellular concentration of Na^+ , which can reach 10–20 mM (Bowman and Kimelberg, 1984; Kimelberg et al., 1993). The stimulation of astrocyte glucose uptake is inhibited by preincubation of the astrocytes with the specific glutamate transporter inhibitor DL-threo- β -hydroxyaspartate (THA); replacement of Na^+ by choline in the medium also inhibits glucose uptake (Pellerin and Magistretti, 1994). These results clearly indicate a tight coupling between Na^+ -dependent glutamate uptake and glucose utilization by astrocytes (Fig. 3). A similar coupling between Na^+ -dependent glutamate uptake and glucose utilization has been confirmed recently in rat astrocytes by Sokoloff and colleagues (Takahashi et al., 1995). Analysis of the intracellular molecular mechanism(s) of this coupling points at a critical involvement of Na^+/K^+ -ATPase, as indicated by the complete inhibition by ouabain of glutamate-evoked 2-DG uptake in astrocytes (Pellerin and Magistretti, 1994). The astrocytic Na^+/K^+ -ATPase responds predominantly to increases in intracellular Na^+ , for which it shows a K_m of ~ 10 mM (Kimelberg et al., 1978; Erecinska and Dagani, 1990). Because in cultured astrocytes, the intracellular Na^+ concentration ($[\text{Na}]_i$) ranges from 10 to 20 mM (Kimelberg et al., 1993), Na^+/K^+ -ATPase is set to be activated when $[\text{Na}^+]_i$ rises concomitantly with glutamate uptake (Bowman and Kimelberg, 1984). Indeed, an increase in $[\text{Na}^+]_i$ evoked by the Na^+ ionophore monensin stimulates 2-DG uptake into astrocytes in an ouabain-sensitive manner (Yarowsky et al., 1986), indicating that a major determinant of glucose utilization by astrocytes is the activity of Na^+/K^+ -ATPase (Brookes and Yarowsky, 1985). In this context, it is important to

note that *in vivo*, the mechanism that accounts primarily for the 2-DG uptake induced by neuronal activity is the Na^+/K^+ -ATPase activity (Mata et al., 1980).

There is ample evidence in the literature indicating that in a variety of tissues, including the brain, kidney, vascular smooth muscle, and erythrocytes, activation of Na^+/K^+ -ATPase activity stimulates glycolysis, i.e., glucose uptake and lactate production (Parker and Hoffman, 1967; Paul et al., 1979; Lipton and Robacker, 1983; Lynch and Balaban, 1987). That glutamate stimulates lactate production and release by astrocytes (with an EC_{50} of 70 μM) (Pellerin and Magistretti, 1994) is consistent with this evidence.

Nature of the metabolite produced from glucose by glial cells to fuel neurons

The view that emerges from the experiments reviewed thus far is that glucose uptake and phosphorylation in the nervous system occur predominantly in glial cells. Neurons also take up and phosphorylate glucose and express glucose transporters (Maher et al., 1994; Nagamatsu et al., 1994) and hexokinase (Kao-Jen and Wilson, 1980). However, as reviewed below, the preferred substrate for the tricarboxylic acid (TCA) cycle in neurons appears to be lactate (or alanine for the honeybee drone retina), implying that glucose taken up and phosphorylated by neurons is preferentially processed through other metabolic pathways, such as glycolysis or the pentose phosphate pathway (PPP). Supporting this view is evidence that in rod outer segments, the PPP is

essential for the maintenance of the visual cycle (Hsu and Molday, 1994). In addition, the production of reducing equivalents, namely reduced nicotinamide-adenine dinucleotide phosphate (NADPH), through the PPP appears to be a significant mechanism to scavenge oxygen free radicals produced by the oxidative activity of neurons (Ben-Yoseph et al., 1994). Here, we will review in more detail the evidence that the glycolytic products lactate (in the mammalian central nervous system) and alanine (in the honeybee drone retina) are released by glial cells and provide adequate energy substrates for neurons.

There is ample experimental evidence indicating that lactate and pyruvate are adequate energy substrates for brain tissue *in vitro* (McIlwain, 1953; Ide et al., 1969; Teller et al., 1977; Schurr et al., 1988). Thus, synaptic activity can be maintained in cerebral cortical slices with lactate or pyruvate as the only substrate provided (McIlwain, 1953; McIlwain and Bachelard, 1985; Schurr et al., 1988). Neurodegeneration of hippocampal slices induced by glucose removal is prevented by addition of lactate to the perfusing medium (Izumi et al., 1994).

Quantitatively, lactate is the primary metabolic intermediate released by cultured astrocytes, at a rate of 15–30 nmol · mg of protein⁻¹ · min⁻¹ (Walz and Mucke, 1988; Dringen et al., 1993a). Other, quantitatively less important intermediates released by astrocytes are pyruvate (at a rate ~10-fold lower than lactate), α -ketoglutarate, citrate, and malate (Shank and Campbell, 1984; Selak et al., 1985; Sonnewald et al., 1991; Shank et al., 1993). Fluxes of lactate between astrocytes and neurons have been quantified *in vitro*, showing lactate utilization even in the presence of glucose (Larrabee, 1983, 1992, 1995). As noted earlier, when activation is mimicked *in vitro* by exposing cultured astrocytes to glutamate, a marked release of lactate and, to a lesser degree, pyruvate is observed (Pellerin and Magistretti, 1994). Glutamate evokes lactate release with the same potency and time course that it stimulates glucose utilization, which indicates that glutamate stimulates the glycolytic processing of glucose.

Studies in the well compartmented honeybee drone retina and in acutely isolated cell preparations from guinea pig retina corroborate such metabolic fluxes by which glucose taken up by glia is converted into glycolytic products that fuel neurons. The development of a simple preparation of the guinea pig retina, consisting of acutely isolated Müller cells (the predominant glial element in the vertebrate retina) still attached to photoreceptors, was of particular value for the analysis of such metabolic fluxes (Poitry-Yamate et al., 1995). The interest in this preparation comes from observation that the structural and functional relationships and the polarity of both glia and photoreceptors are preserved after isolation and can be analyzed for several hours *in vitro*. This cell model has been used to obtain quantitative experimental evidence supporting the metabolic flux of lactate and amino acids between glial cells and neurons. Thus, under aerobic conditions and in the presence of glucose in the medium, Müller cells intensely transform glucose to lactate, causing massive lactate release into the extracellular space (Poitry-Yamate et al., 1995). In addition, experiments using ¹⁴C-labeled lactate or glucose indicate that freshly isolated photoreceptor neurons preferentially utilize lactate rather than glucose as an energy substrate. This is illustrated by the fourfold higher rate of ¹⁴CO₂ production from [¹⁴C]lactate than from [¹⁴C]glucose (Poitry-Yamate et al., 1995). Quantitative analysis of radiolabeled substrate fluxes indicated that lactate produced from glucose by Müller cells and released into the extracellular space fuels the mitochondrial oxidative metabolism of photoreceptor neurons. Thus, >90% of the lactate produced by

solitary Müller cells from exogenous glucose is found in the bathing solution. The specific radioactivity (s.a.) of lactate released into the bathing solution by the cells is considerably higher than the s.a. of lactate remaining inside the cells. Lactate also is synthesized from glycogen present in the Müller cells. However, the lactate produced from glucose in the Müller cells is preferentially released into the extracellular space. These data demonstrate that in a purified preparation consisting of structurally and functionally coupled glial cells (Müller cells) and neurons (photoreceptors), stimulation increases the consumption of lactate by the neurons in a manner that is quantitatively in accord with the increase in their O₂ consumption (Haugh et al., 1990), which shows that lactate provides a preferred substrate for oxidative phosphorylation.

The model of a metabolic compartmentation in which glucose taken up by astrocytes is converted glycolytically into lactate, which is then released into the extracellular space to be utilized by neurons, is consistent with the available biochemical and electrophysiological observations. This array of experimental evidence supports the model of cell-specific metabolic regulation illustrated in Figure 3. Several additional observations made in various cellular systems by using complementary techniques further support this model. For example, for lactate to be an adequate metabolic substrate for neurons, particularly during their stimulation, two additional conditions have to be fulfilled. First, an active lactate uptake by neurons has to exist; second, the enzymatic machinery allowing lactate to enter the TCA cycle has to be present in neurons. Both features recently have been shown to occur. Thus, in addition to the evidence for a preferential utilization of lactate by photoreceptors isolated from guinea pig retina, a lactate uptake system has been demonstrated in pure neuronal cultures of mouse cerebral cortex (Dringen et al., 1993b). Also, after conversion to pyruvate via a reaction catalyzed by lactate dehydrogenase (LDH), 1 mole of lactate can provide 18 moles of ATP via oxidative phosphorylation. Five isozymes of LDH exist. One of them is the M₄ (muscle) homotetramer present in tissues that produce lactate glycolytically, and a second is the H₄ (heart) homotetramer, which is enriched in tissues that use lactate as a substrate. The other three isozymes are heterotetramers composed of different combinations of the M and H subunits and have intermediate functional properties. There is evidence indicating that the M subunit is localized predominantly in astrocytes, whereas neurons are enriched in the H subunit (Tholey et al., 1981). This observation, which has been confirmed recently in the human brain (Bittar et al., 1995), is consistent with a predominant lactate production by astrocytes and lactate utilization by neurons.

In vivo studies support the idea that during activation of a given brain area, glycolysis may transiently exceed the rate of oxidative phosphorylation. Thus, increases in lactate levels in the rat somatosensory cortex after forepaw stimulation have been demonstrated (Hossmann and Linn, 1987; Ueki et al., 1988). In addition, lactate levels assayed *in vivo* by microdialysis in freely moving rats increase in hippocampus and striatum after somatosensory stimulation (Schasfoort et al., 1988; Fellows et al., 1993). Studies with PET and ¹H-MRS to be discussed below confirm the occurrence of glycolysis during neuronal activation.

Quantitative analyses performed in honeybee drone retina preparations by HPLC and HPLC-mass spectroscopy (HPLC-MS) further confirm the utilization by neurons of glycolytic intermediates produced by glial cells. In these preparations, glucose-6-phosphate formed predominantly in the glial cells is converted to pyruvate and subsequently transaminated to form alanine. (The

Figure 4. Simplified representation of the metabolic pathways of glucose, with particular emphasis of the fate of pyruvate, the end-product of glycolysis.

honeybee drone retina does not produce lactate.) As shown in Figure 4, pyruvate that does not enter the TCA cycle can be either converted to lactate or transaminated to form alanine; this latter reaction is catalyzed by the combined action of alanine aminotransferase (ALAT) and cytosolic glutamate dehydrogenase (GDH) (Fig. 4). Both metabolic pathways of pyruvate are necessary to regenerate nicotinamide adenine dinucleotide (NAD^+) and, therefore, maintain the redox potential of the cell. This is particularly crucial for honeybee retinal glia because with the virtual absence of mitochondria in these cells, glycolysis proceeds anaerobically in the dark as well as during photostimulation (Brazitikos and Tsacopoulos, 1991). Thus, maintenance of an appropriate NADH/NAD^+ ratio is essential to sustain a high glycolytic flux. Alanine synthesized in glia is released into the interstitial space and taken up by photoreceptors by a Na^+ -dependent transport mechanism (Tsacopoulos et al., 1994). Quantitative determination of alanine in the interstitial fluid of the retina of live honeybee drones reveals a concentration of 31 mM, a value well within the range of the K_m of the alanine uptake system (Cardinaud et al., 1994). In the photoreceptors, alanine is deaminated to pyruvate via a reaction catalyzed by the neuronal isoform of ALAT (Rusca et al., 1982). Pyruvate then can enter the TCA cycle for complete oxidation (Fig. 4).

In summary, although plasma lactate does not represent an adequate metabolic substrate for the brain because of its limited permeability across the blood-brain barrier (Pardridge and Oldendorf, 1977), lactate formed within the brain parenchyma via glutamate-activated glycolysis in astrocytes can fulfill the energetic needs of neurons.

Incorporation of glucose into glycogen in astrocytes and mobilization of glycogen during brain activity

In the brain, glycogen is localized almost exclusively in astrocytes (Peters et al., 1991). Despite its low concentration in the central nervous system (between 20 and 40 nmol/mg of protein), the turnover rate of glycogen is extremely rapid and its levels are

Table 1. Induction of glycogenolysis by neurotransmitters in primary astrocyte cultures of mouse cerebral cortex

Neurotransmitter	EC_{50} (nM)
VIP	3
PHI	6
Secretin	0.5
PACAP	0.08
Noradrenaline	20
Adenosine	800
ATP	1300

PHI, Peptide histidine isoleucine; PACAP, pituitary adenylate cyclase-activating peptide.

finely tuned by synaptic activity. For example, marked decreases in synaptic activity during barbiturate-induced anesthesia cause marked increases in the glycogen content of the brain (Phelps, 1972). Extensive evidence for a very active and tightly regulated metabolism of glycogen in astrocytes has become available, further supporting the idea that astrocytes are the site of intense glucose metabolism. Thus, mouse cerebral cortical astrocytes synthesize glycogen from glucose at a rate of $5\text{--}10 \text{ nmol} \cdot \text{mg}$ of protein $^{-1} \cdot \text{min}^{-1}$, causing steady-state levels of $30\text{--}60 \text{ nmol/mg}$ of protein, which are very similar to glycogen levels measured in the rodent brain (Sagar et al., 1987; Sorg and Magistretti, 1991; Sorg and Magistretti, 1992). Experiments had indicated that specific neurotransmitters promote glycogenolysis in slices prepared from different brain areas, including the cerebral cortex. Thus, the monoamines noradrenaline (NA), serotonin, and histamine, the peptide vasoactive intestinal peptide (VIP), and the purine adenosine all promote glycogenolysis in mouse cerebral cortical slices, the rate of which is dependent on transmitter concentration (Quach et al., 1978, 1980, 1982; Magistretti et al., 1981, 1986). The respective morphological characteristics of the monoaminergic afferents that globally innervate the cerebral cortex, on one hand, and those of the local and radially oriented intracortical VIP neurons, on the other hand, indicate the existence of two complementary neuronal systems that can mobilize glycogen in the cortex, one global, spanning functionally distinct cortical regions, and the other local, exerting its metabolic activity within cortical columns (Magistretti and Morrison, 1988; Magistretti, 1990). Similar observations were made in primary cultures of mouse astrocytes, in which an induction of glycogenolysis by NA, VIP, analogs of VIP peptide histidine isoleucine (PHI) and pituitary adenylate cyclase-activating peptide (PACAP), adenosine, and ATP was demonstrated (Sorg and Magistretti, 1991; Magistretti et al., 1993; Sorg et al., 1995) (Table 1). The initial rate of glycogenolysis induced by VIP and NA in astrocytes ranges from 5 to $10 \text{ nmol} \cdot \text{mg}$ of protein $^{-1} \cdot \text{min}^{-1}$ (Sorg and Magistretti, 1991), a value that is remarkably close to the rate of glucose utilization of gray matter, as determined by the 2-DG autoradiographic method (Sokoloff et al., 1977). This concordance indicates that the glycosyl units mobilized in response to the two glycogenolysis-inducing neurotransmitters can provide quantitatively adequate substrates for the energy demands of the brain parenchyma. It is not clear yet whether the glycosyl units mobilized by glycogenolysis are used by astrocytes to meet their own energy demands during brain activity, or whether they are metabolized to a substrate such as lactate (Dringen et al., 1993a), which then is released for the use by neurons. Experimental results clearly indicate that glycogenolysis does not cause cultured astrocytes to release glucose (Dringen et al., 1993a).

The honeybee drone retina also shows activity-coupled glycolysis. As noted earlier, glycogen in this nervous tissue is localized exclusively in glia (at a concentration of ~90 mg/ml of glia) (Evequôz-Mercier and Tsacopoulos, 1991). When a retinal slice is superfused with a medium lacking glucose, photoreceptors still respond to photostimulation and increase their oxygen consumption fourfold (Tsacopoulos and Poitry, 1982; Tsacopoulos et al., 1987), whereas the glycogen content of the slices is reduced (Evequôz-Mercier and Tsacopoulos, 1991). This type of experiment, which can be repeated over several hours, indicates that energy substrates can be mobilized from glial cells and oxidized in photoreceptor neurons, as indicated by the increase in oxygen consumption, to sustain their electrical activity. This inference has been confirmed by additional experiments in which photostimulation of photoreceptors bathed in a glucose-containing medium triggers a large rise in glycogen turnover (Evequôz-Mercier and Tsacopoulos, 1991). Similarly, in the mammalian retina, glycogen and glycogen-metabolizing enzymes are concentrated primarily in Müller cells. Acutely isolated Müller cells from juvenile guinea pig retina contain ~180 nmol of glycosyl units/mg of protein, a value three- to sixfold higher than that measured in cultured astrocytes (Swanson et al., 1990; Sorg and Magistretti, 1991), and rodent brain (Sagar et al., 1987). The glycogen content of purified Müller cells maintained in the absence of glucose from the bathing solution decreases by ~50% in 30 min (Poitry-Yamate and Tsacopoulos, 1992).

Glycogenolysis also has been demonstrated *in vivo* in rat brain after physiological activation of a modality-specific pathway (Swanson et al., 1992). This activity-dependent glycogenolysis was revealed by a newly developed autoradiographic technique for the detection of glycogen (Swanson et al., 1992). Thus, repeated stimulation of the facial vibrissae caused a marked decrease in the density of glycogen-associated autoradiographic grains in the barrel fields of the rat somatosensory cortex and in their afferent thalamic nuclei (Swanson et al., 1992). These observations clearly indicate that the physiological activation of specific neuronal circuits causes the mobilization of glycogen in glial cells.

Relevance of metabolic findings for functional brain imaging

The 2-DG autoradiographic technique developed by Sokoloff and his colleagues has revealed two fundamental features of brain-energy metabolism: its regional heterogeneity, and its tight coupling to neuronal activity (Sokoloff, 1991). However, despite the tremendous gains in our knowledge of brain function both in experimental animals and, because of the development of the [^{18}F]2-DG PET method, in humans, the cellular localization and the biochemical mechanisms underlying the 2-DG-based signal had been unresolved. Indirect evidence suggested that the activity-dependent uptake of 2-DG occurs in the neuropil, i.e., in regions that are enriched in axon terminals, dendrites, and synapses ensheathed by astrocytic processes, rather than in regions hosting neuronal perikarya (Kadekaro et al., 1985; Sokoloff, 1991). Given the complex intertwining of neural processes in the nervous system, this observation is particularly apparent in those circuits in which a clear-cut polarization of neural process exists (Roland, 1993). Thus, a striking example was provided by a study of Sokoloff and colleagues, who showed that when the sciatic nerve of anesthetized rats is stimulated, a frequency-dependent increase in 2-DG uptake occurs in the dorsal horn of the spinal cord (where afferent sensory axon terminals make synaptic contacts with second-order interneurons), but not in the dorsal root

ganglion, where the cell bodies of the sensory neurons are localized (Kadekaro et al., 1985). Other evidence for a selective uptake of the tracer in the neuropil was obtained in well laminated central nervous system structures, such as the primate visual cortex and the hippocampus (Kennedy et al., 1976). The evidence obtained in simple and well compartmentalized nervous systems and in purified cell cultures clearly indicates that 2-DG uptake during neuronal activity occurs in glial cells. This conclusion does not question, of course, the validity of 2-DG-based techniques for mapping neuronal activity. Rather, it provides a cellular and molecular basis for these *in vivo* imaging procedures.

PET analyses indicate the occurrence of metabolic uncoupling during neuronal activation, in which an increased glucose utilization is not matched by a commensurate increase in oxygen consumption (Fox and Raichle, 1986; Fox et al., 1988). Accordingly, lactate production is observed by ^1H -MRS during neuronal activation in humans (Prichard et al., 1991; Sappey-Marini et al., 1992). These *in vivo* data converge to indicate that neuronal activation is linked to glycolysis leading to lactate production. The set of observations reviewed in this article provides a cellular localization for the site of lactate production—astrocytes metabolize glucose to lactate (or alanine in the insect retina) and release it as a metabolic substrate for neurons.

In this article, we have attempted to integrate recent experimental results obtained in a variety of preparations and using various experimental approaches. They converge to indicate the existence of cell-specific metabolic fluxes occurring in the brain, particularly during neuronal activation. The data highlight a pivotal role for glial cells in coupling neuronal activity to energy metabolism and in transforming blood-borne glucose into metabolic substrates that can fuel neurons. In addition, the data provide a cellular and molecular model for the interpretation of unexpected findings obtained recently with functional-brain imaging techniques such as PET and ^1H -MRS.

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