Cellular Bases of Brain Energy Metabolism and Their Relevance to Functional Brain Imaging: Evidence for a Prominent Role of Astrocytes

Survey of Brain Energy Metabolism at the Organ and Regional Levels

Organ Level

Fundamental observations on brain energy metabolism at the organ level culminated over 40 years ago, in particular through the pioneering work of Schmidt and Kety (1948). By determining arteriovenous (A-V) differences of a number of metabolic substrates the view emerged that, except under certain nonphysiological conditions, glucose is the obligatory energy substrate for the brain (Edvinsson et al., 1993). Glucose utilization by the brain is 31 μmol/100 gm/min, while oxygen consumption is 160 μmol/100 gm/min; since CO₂ production is almost identical, the respiratory quotient (RQ) of the brain is nearly 1, indicating that carbohydrates are the substrates for oxidative metabolism (Sokoloff, 1960). With a global blood flow of 57 ml/100 gm/min, the brain extracts approximately 50% of oxygen and 10% of glucose from the arterial blood. Given a theoretical stoichiometry of 6 μmol of oxygen consumed for each μmol of glucose, the expected brain glucose utilization should in theory be 26.6 μmol/100 gm/min rather than the measured 31 μmol/100 gm/min; thus, an excess of 4.4 μmol/100 gm/min of glucose follows other metabolic fates. These include the production of lactate and pyruvate which do not necessarily enter the tricarboxylic acid cycle, but rather, can be released into the circulation. Glucose can also be incorporated into lipids, proteins, and glycogen, and it is the precursor of certain neurotransmitters such as GABA, glutamate, and acetylcholine (Sokoloff, 1989; Edvinsson et al., 1993).

It should also be noted that a limited proportion of oxygen is actually utilized for purposes other than direct energy generation. Neural cells contain oxidases and hydroxylases, which are key regulatory enzymes in the metabolic pathways of a number of neuroactive molecules. Examples of such oxygen-requiring enzymes are cyclooxygenases and lipoxygenases involved in the synthesis of eicosanoids from arachidonic acid, tyrosine and tryptophan hydroxylases, dopamine-B-hydroxylase, and monoamine oxidase, which are all enzymes that regulate the metabolism of monoamine neurotransmitters such as GABA, glutamate, and acetylcholine (Sokoloff, 1989; Edvinsson et al., 1993).

Certain metabolic intermediates, under particular conditions, can substitute for glucose as alternative substrates for brain energy metabolism (Sokoloff, 1989). Thus starvation, diabes, or breast-feeding in neonates all lead to increased plasma levels of the ketone bodies acetoacetate and β-hydroxybutyrate, which can be used by the brain as metabolic substrates (Sokoloff, 1989). Mannose, which is not normally present in the blood and cannot therefore be considered a physiological substrate, can sustain normal brain function in the absence of glucose. Lactate and pyruvate can sustain synaptic activity in vitro (McIlwain and Bachelard, 1985; Schurr et al., 1988). Because of their limited permeability across the blood-brain barrier, they cannot adequately substitute for plasma glucose to maintain brain function (Pardridge and Oldendorf, 1977). However, if formed inside the brain parenchyma, they are useful metabolic substrates for neural cells (McIlwain, 1953; McIlwain et al., 1969; Teller et al., 1977). Net release of lactate and pyruvate (negative A-V difference) is occasionally measured in normal individuals, and more frequently in aged subjects or during convulsions (Kety, 1957; Cohen et al., 1967; Folbergrova, 1974). Since steady-state arteriovenous (A-V) differences provide indirect evidence that a substance can be either used as a substrate by the brain (positive A-V difference) or produced by the brain (negative A-V difference) these observations indicate a variable degree of glycolytic glucose processing by the brain (Fig. 1).

Regional Level

Studies at the organ level, while revealing the global substrate requirements for the brain and their stoichiometry, failed to provide the appropriate level of resolution to appreciate two major features of brain energy metabolism: (1) its regional heterogeneity, and (2) its tight relationship with the functional activation of specific pathways. A major technical advance that brought the regional resolution to studies of brain energy metabolism and that laid the ground for visualization of neural activity, is the autoradiographic 2-deoxyglucose method (2-DG) developed by Sokoloff and colleagues. With this technique, local rates of glucose utilization (LCMRglu) can be determined with a spatial resolution of approximately 50–100 μm (Sokoloff et al., 1977; Sokoloff, 1981).

Using the 2-DG autoradiographic technique, LCMRglu have been determined in virtually all structurally and functionally defined brain regions during various physiological and pathological states. Activation of pathways subserving specific modalities, such as visual, auditory, olfactory, or somatosensory stimulations as well as motor activity has also been revealed in the pertinent brain structures by the 2-DG technique (Sharp et al., 1975; Kennedy et al., 1976; Wolf et al., 1983; Melzer et al., 1985; Ginsberg et al., 1987). A consistent finding has been that the increase in 2-DG uptake linked to functional activation occurs in the neuropil, that is, in regions that are enriched in axon terminals, dendrites, and synapses enshrouded by astrocytic processes, and not where neuronal perikarya are located (Kadekarao 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 showing 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 axon terminals make synaptic contacts with second order neurons) but not in the dorsal root ganglion, where the cell body of the sensory neurons is localized (Kadekarao et al., 1985). As another example, increases in glucose utilization in the well-laminated monkey primary visual cortex elicited by appropriate visual stimuli, are most pronounced in layer IV, which is poor in perikarya but...
Glycogen

Glucose-6-phosphate

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**Pathway**

**GLYCOLYSIS**

Pentose Phosphate,

Pyruvate

Lactate

TCA Cycle

CO₂

H₂O

Figure 1. Metabolic pathways for glucose. Glycolysis is shown with thick arrows.

**Cytological Relationships between Neurons and Astrocytes**

When considering brain energy metabolism the focus is predominantly, if not exclusively, placed on neuronal energy metabolism. However, other cell types, namely glia and vascular endothelial cells, play an active role in the flux of energy substrates to neurons. The arguments are both quantitative and qualitative. First, while it is arduous to provide a definitive ratio between neurons and non-neuronal cells, given the variability in figures obtained in various species, brain areas, and developmental ages using often methods that are not easily comparable, it is clear that neurons contribute at most 50% of cerebral cortical volume (O'Kusky and Colonnier, 1982; Kimmelberg and Norenberg, 1989; Bignami, 1991). Estimates of astrocyte:neuron ratios of 10:1 have been put forward (Bignami, 1991). In addition, there is clear evidence indicating that the astrocyte:neuron ratio increases with increasing brain size (Tower and Young, 1973); this is an important consideration when considering the cellular bases of brain energy metabolism in humans. Second, particular astrocytic profiles, the 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 them a likely site of prevalent glucose uptake (Fig. 2). This latter structural feature has long been suggested as evidence indicating a role of astrocytes in the distribution of substances from blood to other brain cells (Sala, 1891; Andriezen, 1893). Finally, astrocyte processes are wrapped around synaptic contacts, implying that they are ideally positioned to sense and be functionally coupled to increased synaptic activity. 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 activation (Barres, 1991).

**Comparison of Glucose Utilization by Astrocytes and Neurons**

**Basal Glucose Utilization**

Basal glucose utilization of the gray matter as determined by the 2-DG technique varies, depending on the brain structure,
between 50 and 150 µmol/100 gm wet weight/min in the rat (Sokoloff et al., 1977). If a protein content of 10% of wet weight is assumed, a value of 5-15 nmol/mg prot/min is obtained. These values are approximately 50% lower in the primate brain (Kennedy et al., 1978). Physiological activation of specific pathways results in a 1.5-3-fold increase in LCMRglu, as determined by the 2-DG technique (Miyaoaka et al., 1979; McCulloch et al., 1980). Glucose utilization can be determined with the 2-DG technique in celluloarly homogeneous preparations of astrocytes or neurons such as primary cultures prepared from neonatal or embryonic mouse cerebral cortex. The basal rate of glucose utilization in astrocytes is higher than in neurons, with a rate in astrocytes of 22.3 nmol/mg prot/min and in neurons of 6.3 nmol/mg prot/min. These values are of the same order as those determined in vivo for cortical gray matter, with the 2-DG autoradiographic technique (Sokoloff et al., 1977). Thus, in view of this difference and of the quantitative preponderance of astrocytes compared to neurons in the gray matter, these data reveal a significant contribution of astrocytes to basal glucose utilization as determined by 2-DG autoradiography or PET.

**Glucose Utilization during Activation**

The contribution of astrocytes to glucose utilization during activation is even more striking. Thus, since during activation of a given cortical area the concentration of glutamate in the extracellular space increases considerably due to its release from the axon terminals of activated pathways (Fonnum, 1984), the effect of glutamate application on glucose utilization by astrocytes and neurons in culture was examined. As shown in Figure 3, t-glutamate stimulates 2-DG uptake and phosphorylation by astrocytes in a concentration-dependent manner, with an EC₅₀ of 80 µM (Pellerin and Magistretti, 1994; Takahashi et al., 1995). This effect is not mediated by specific glutamate receptors known to be present on astrocytes (Pearce, 1993), since it is not inhibited by any of the specific antagonists tested nor is it mimicked by agonists specific for each receptor subtype such as NMDA, AMPA, quisqualate, or t-ACPD. The effect of glutamate is stereospecific, with only the L-isomer being active, and dependent on the presence of extracellular sodium. This pharmacological profile is characteristic of the glutamate transporter (Kanner, 1993). Indeed, the increase in glucose utilization evoked by glutamate is completely abolished by preincubation of the cultures with the potent glutamate transporter inhibitor L-threo-β-hydroxyaspartate (THA; Pellerin and Magistretti, 1994). These results clearly indicate that a tight coupling between form rosette-dependent glutamate uptake and glucose utilization by astrocytes (Fig. 4). The intracellular molecular mechanism(s) of this coupling are presently being investigated; a critical involvement of the Na⁺/K⁺-ATPase is likely, since ouabain completely inhibits the glutamate-evoked 2-DG uptake by astrocytes (Pellerin and Magistretti, 1994). The astrocytic Na⁺/K⁺-ATPase responds predominantly to increases in intracellular Na⁺ for which it shows a Kₘ of about 10 mM (Kimelberg et al., 1978; Erecinska, 1989). Since in cultured astrocytes, the (Na⁺), concentration ranges between 10 and 20 mM (Kimelberg et al., 1993), Na⁺/K⁺-ATPase is set to be readily activated when (Na⁺), raises concomitantly with glutamate uptake (Bowman and Kimelberg, 1984). In this context, it is important to note that in vivo, the main mechanism that accounts for the activation-induced 2-DG uptake is represented by the activity of the Na⁺/K⁺-ATPase (Mata et al., 1980).

In contrast to what is observed in astrocytes, glutamate does not stimulate 2-DG uptake in cultured neurons, despite the fact that glutamate can elicit functional responses in these cells, which are mediated by ionotropic and metabotropic receptors. Examples of functional responses in these neuronal cultures, which are totally devoid of glial markers, are the NMDA and AMPA/kainate-mediated increases in intracellular calcium (Stella et al., 1995), stimulation of arachidonic acid formation (Stella et al., 1995), and induction of c-fos expression (Pellegri et al., 1994), as well as the metabotropic receptor-mediated stimulation of phospholipase C (Stella et al., 1995). Cultured neurons also possess an active glutamate reuptake system, which has, however, a maximal capacity almost 80 times inferior to that of astrocytes (Vₘₐₓ in neurons 0.74 nmol/mg prot/min; Vₘₐₓ in astrocytes 57.4 nmol/mg prot/min).

A similar compartmentalization of glucose uptake has been described in a series of elegant experiments carried out by Marcos Tsacopoulos and his colleagues in the honeybee drone retina and, more recently, in the guinea pig retina. The honeybee drone retina is a crystal-like structure that is structurally and metabolically compartmentalized (Tsacopoulos and Veuthey, 1993). Thus, in this highly organized nervous tissue preparation, photoreceptor cells form rosette-like structures that are surrounded by glial cells. In addition, mitochondria are exclusively present in the photoreceptor neurons. Upon activation of the photoreceptors by light, an increase in 2-DG uptake can be visualized in the glial cells surrounding the rosettes, but not in the photoreceptors (Tsacopoulos et al., 1988). An increase in O₂ consumption is, nonetheless, measured in photoreceptors. These experiments suggest that following activation of photoreceptors by light, glucose is predominantly taken up by glial cells, which then release a metabolic substrate to be oxidized by photoreceptor cells. Similar results have been observed in guinea pig retina, in which 2-DG uptake is visualized by autoradiography only in Müller cells (Poitry-Yamate and Tsacopoulos, 1992).

**Lactate Released by Astrocytes Is a Metabolic Substrate for Neurons**

The fact that during activation the increase in glucose uptake can be ascribed predominantly, if not exclusively, to astrocytes, implies that energy substrates other than glucose must be released by astrocytes. As indicated earlier, lactate and pyruvate are adequate substrates for brain tissue in vitro (McIlwain, 1953; Ide et al., 1969; Teller et al. 1977; McIlwain and O'Connor, 1978). This was investigated in a series of elegant experiments carried out by Marcos Tsacopoulos and his colleagues in the honeybee drone retina and, more recently, in the guinea pig retina. The honeybee drone retina is a crystal-like structure that is structurally and metabolically compartmentalized (Tsacopoulos and Veuthey, 1993). Thus, in this highly organized nervous tissue preparation, photoreceptor cells form rosette-like structures that are surrounded by glial cells. In addition, mitochondria are exclusively present in the photoreceptor neurons. Upon activation of the photoreceptors by light, an increase in 2-DG uptake can be visualized in the glial cells surrounding the rosettes, but not in the photoreceptors (Tsacopoulos et al., 1988). An increase in O₂ consumption is, nonetheless, measured in photoreceptors. These experiments suggest that following activation of photoreceptors by light, glucose is predominantly taken up by glial cells, which then release a metabolic substrate to be oxidized by photoreceptor cells. Similar results have been observed in guinea pig retina, in which 2-DG uptake is visualized by autoradiography only in Müller cells (Poitry-Yamate and Tsacopoulos, 1992).
observed (Pellerin and Magistretti, 1994). This glutamate-marked release of lactate, and to a lesser degree, pyruvate, is in vitro cently shown. Thus, when activation is mimicked in astrocytes must be demonstrated. Both mechanisms have been re-

lease by astrocytes increases; second, lactate uptake by neu-
trons during activation, two additional conditions have to be fulfilled. First, that, indeed, during activation lactate re-

pyruvate) to be an adequate metabolic substrates for neurons, glucose (Larrabee, 1983, 1992). However, in order for lactate (or 
cs between astrocytes and neurons have been quantified
betalactate (Dringen et al., 1993b). Furthermore, fluxes of endogenous lactate
reduced by transporters of the Na+/K+ ATPase (Bachelard, 1985; Schurr et al., 1988). Indeed, recent evidences obtained in purified neuronal cultures indicates the
take up by neurons as an energy fuel. Indeed, recent evi-
tation implies that during activation lactate may normally be

tained in cerebral cortical slices with only lactate or pyruvate as a substrate (Mcllwain, 1953; Mcllwain and

ton vasoconstriction, indicating increases in lactate levels in the rat somatosensory
tissue stimulation were demonstrated (Schasfoort et al., 1988; Fellows et al., 1993). Interestingly, the rate of lactate clearance from the extracellular space was markedly slowed in the presence of tetrodotoxin, a specific blocker of the neuronal
age-sensitive sodium channels responsible for the generation of action potentials (Fellows et al., 1993). This latter observation implies that during activation, lactate may normally be taken up by neurons as an energy fuel. Indeed, recent evidence obtained in purified neuronal cultures indicates the presence of a saturable and specific transport system for lactate (Dringen et al., 1993b).

Thus, a metabolic compartmentation whereby glucose taken up by astrocytes is metabolized glycolytically to lactate, which is then released in the extracellular space to be utilized by neurons, is consistent with the available biochemical and electrophysiological observations. This array of in vitro and in vivo experimental evidence supports the model of cell-specific metabolic regulation illustrated in Figure 4. In particular, as far as glutamate-induced glycolysis is concerned, this model is taken to reflect cellular and molecular events occurring during activation of a given cortical area. Direct neuronal glucose uptake could still take place under these conditions, as it does in the basal state. It should also be noted that a reciprocal relationship appears to exist between aerobic gly-

Figure 4. Schematic representation of the mechanism for glutamate-induced glycolysis in astrocytes during physiological activation. At glutamatergic synapses, glutamate depolarizes 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+, resulting in an increase in the intracellular concentration of Na+, leading to an activation of the Na+/K+ ATPase. The pump, fueled by ATP provided by membrane-bound glycolytic enzymes (possibly phosphoglycerate kinase, PGK; see Proverbio and Hoffman, 1977), activates glycolysis, that is, glucose utilization and lactate production, in astrocytes. Lactate, once released, can be taken up by neurons and serve as an adequate energy substrate. For graphic clarity, only lactate uptake into presynaptic terminals is indicated. However, this process could also occur at the postsynaptic neuron. This model, which summarizes in vitro experimental evidences indicating glutamate-induced glycolysis, is taken to reflect cellular and molecular events occurring during activation of a given cortical area (arrow labeled A: activation). Direct glucose uptake into neurons under basal conditions is also shown (arrow labeled B: basal conditions). P: pyruvate; Lac, lactate; Gin, glutamine; G, G-protein. (Taken with permission from Pellerin and Magistretti, 1994).
glycogen is due to the inhibition of neuronal activity, stressing localized in astrocytes in vivo (Phelps, 1972); interestingly however, the glycogen content of synaptic activity is markedly attenuated, glycogen levels raise sharply during barbiturate anesthesia, a condition in which synaptic activity (Magistretti et al., 1993). Thus, for example, extremely rapid and glycogen levels are finely coordinated with the M subunit is predominant in astrocytes, while neurons are enriched with the H subunit (Tholey et al., 1981), an observation consistent with lactate production by astrocytes and utilization by neurons. Interestingly, a similar metabolic exchange between cell types has been described in the testis (Mita and Hall, 1982). In this case, active glycolysis in Sertoli cells produces lactate, which is the preferred metabolic substrate for round spermatids.

Glycogenolysis Occurs during Activation and Is Localized in Astrocytes

Glycogen is the single largest energy reserve of the brain; it is mainly localized in astrocytes, although ependymal and choroid plexus cells, as well as certain large neurons in the brain stem contain glycogen (Magistretti et al., 1993). When compared to liver or muscle, the glycogen content of the brain is exceedingly small, about 100 and 10 times inferior, respectively. However, the brain can hardly be considered a glycogen storage organ and here the function of glycogen should be viewed as that of providing a metabolic buffer during physiological activity. Indeed, glycogen turnover in the brain is extremely rapid and glycogen levels are finely coordinated with synaptic activity (Magistretti et al., 1993). Thus, for example, during barbiturate anesthesia, a condition in which synaptic activity is markedly attenuated, glycogen levels rise sharply (Phelps, 1972); interestingly however, the glycogen content of cultures containing exclusively astrocytes is not increased by general anesthetics (Swanson et al., 1989); this observation indicates that the \textit{in vivo} action of barbiturates on astrocytic glycogen is due to the inhibition of neuronal activity, stressing the existence of a tight coupling between synaptic activity and astrocytic glycogen. In addition, reactive astrocytes, which develop in areas where neuronal activity is decreased or absent as a consequence of injury, contain high amounts of glycogen (Shimizu and Hamuro, 1958; Wolfe et al., 1962; Watanabe and Passonneau, 1974). Glycogen levels are tightly regulated by various neurotransmitters (Magistretti et al., 1993). Thus, we have shown that Vasointestinal Peptide (VIP), a neurotransmitter contained in a homogeneous population of bipolar, radially oriented neurons (Magistretti and Morrison, 1988) could promote a cAMP-dependent glycogenolysis in mouse cerebral cortical slices (Magistretti et al., 1981). In view of the morphology and arborization pattern of VIP-containing neurons (Fig. 5), we proposed that these cells could regulate the availability of energy substrates locally, within cortical columns (Magistretti et al., 1981; Magistretti and Morrison, 1988). A similar effect had been previously described for NA (Quach et al., 1978), serotonin and histamine (Quach et al., 1980, 1982). The noradrenergic system is organized according to principles strikingly different from those of VIP neurons: the cell bodies of NA-containing neurons are localized in the locus coeruleus in the brain stem from where axons project to various brain areas including the cerebral cortex; here, they enter the rostral end and progress caudally with a predominantly horizontal trajectory, across a vast rostrocaudal expanse of cortex (Morrison et al., 1978). Given these morphological features we suggested that, in contrast to VIP-containing intracortical neurons, the noradrenergic system could regulate energy homeostasis globally, spanning across functionally distinct cortical areas (Magistretti et al., 1981; Magistretti and Morrison, 1988; Fig. 6).

The glycogenolytic effect of VIP and NA is exerted in astrocytes, as indicated by studies in primary astrocyte cultures...
ATP are also glycogenolytic in astrocytes (Table 1). The initial rate of glycogenolysis activated by VIP and NA is between 5 and 10 nmol/mg prot/min (Sorg and Magistretti, 1991), a value that is remarkably close to glucose utilization of the gray matter, as determined by the 2-DG autoradiographic method (Sokoloff et al., 1977). This correlation indicates that the glycosyl units mobilized in response to the two glycogenolytic neurotransmitters can provide quantitatively adequate substrates for the energy demands of the brain parenchyma. At this stage, it is not yet clear whether the glycosyl units mobilized through glycogenolysis are used by astrocytes to face their energy demands during activation, or whether they are metabolized to a substrate such as lactate (Dringen et al., 1993a), which is then released for the use of neurons. A well-established fact is that glucose is not released by astrocytes, at least in vitro (Dringen et al., 1993a), supporting the view that the activity of glucose-6-phosphatase in astrocytes is very low (Sorg and Magistretti, 1991). This correlation indicates that the glycosyl units mobilized in response to the two glycogenolytic neurotransmitters can provide quantitatively adequate substrates for the energy demands of the brain parenchyma. At this stage, it is not yet clear whether the glycosyl units mobilized through glycogenolysis are used by astrocytes to face their energy demands during activation, or whether they are metabolized to a substrate such as lactate (Dringen et al., 1993a), which is then released for the use of neurons. A well-established fact is that glucose is not released by astrocytes, at least in vitro (Dringen et al., 1993a), supporting the view that the activity of glucose-6-phosphatase in astrocytes is very low (Sorg and Magistretti, 1991; Fishman and Karnovsky, 1980).

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<thead>
<tr>
<th>Substance</th>
<th>EC_{50} (mM)</th>
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<tbody>
<tr>
<td>VIP</td>
<td>3</td>
</tr>
<tr>
<td>PHI</td>
<td>6</td>
</tr>
<tr>
<td>Secretin</td>
<td>0.5</td>
</tr>
<tr>
<td>PACAP</td>
<td>0.08</td>
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<tr>
<td>Noradrenaline</td>
<td>20</td>
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<tr>
<td>Isoproterenol (β)</td>
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<tr>
<td>Methoxamine (α)</td>
<td>600</td>
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<tr>
<td>Adenosine</td>
<td>600</td>
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<tr>
<td>ATP</td>
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Another action of NA on energy metabolism is the marked stimulation of 2-DG uptake in primary astrocyte cultures (Yu et al., 1993). This action is functionally coordinated with glycogenolysis, since the same extracellular signal (NA) results in an increased availability of glycosyl units for ATP production in astrocytes. In contrast to NA, VIP does not influence glucose uptake by astrocytes (Yu et al., 1993).

Glycogenolysis, revealed by a newly developed autoradiography technique for glycogen, has been demonstrated also in vivo following physiological activation of a modality-specific pathway (Swanson et al., 1992). Thus, repeated stimulation of the vibrissae resulted in a marked decrease in the density of glycogen-associated autoradiographic grains in the somatosensory cortex of rats (barrel field) as well as in the relevant thalamic nuclei (Swanson et al., 1992). These observations indicate that the physiological activation of specific neuronal circuits results in the mobilization of glial glycogen stores.

Further evidence supporting a role of astrocytic glycogen as a metabolic buffer in the early stages of activation has been provided in hippocampal slices (Lipton, 1988). Electrical stimulation of the slice results in an immediate and marked increase in NADH fluorescence, an index for the activation of glycolysis (Lipton, 1973). This increase in NADH fluorescence is observed in a well-oxygenated medium containing adequate supplies of glucose, and occurs at the onset of synaptic activity. However, the signal disappears when the glycogen content of the slices is depleted by a glycogenolytic pretreatment (Lipton, 1973). This observation further suggests that an activation of glycogenolysis occurs at the onset of synaptic activity.

### Correlations with Functional Brain Imaging

The data reviewed thus far on the cell-specific metabolic events that take place during activation strongly suggest an activity-dependent glucose uptake into astrocytes, which then release lactate as the metabolic substrate to fuel neuronal energy metabolism. Evidence has also been provided for an activation-induced glycogen mobilization in astrocytes. This set of observations is consistent with some now well-established but originally unexpected findings obtained in functional

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Table 1

Glycogenolytic neurotransmitters in primary cultures of mouse cortical astrocytes

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PHI, Peptide histidine isoleucine; PACAP, pituitary adenylate cyclase activating peptide.
brain imaging studies, for which they provide, in fact, some cellular and molecular bases. With the advent of Positron Emission Tomography (PET) and the use of positron-emitting isotopes such as 18F, local glucose utilization has been studied in humans with 2-[(18F)fluoro-2-deoxyglucose (Phelps et al., 1979; Raichle et al., 1979; Reivich and Alavi, 1983). Similarly, local oxygen consumption and changes in blood flow can be studied in humans by PET using 15O and H3O (Frackowiak et al., 1980; Raichle et al., 1983). PET studies in which blood flow, oxygen consumption, and glucose utilization were determined in the same subject have now clearly established that focal physiological cortical activation results in a metabolic uncoupling whereby the increases in blood flow and glucose utilization are not matched by a commensurate increase in oxygen consumption, indicating nonoxidative glucose utilization (Fox and Raichle, 1986; Fox et al., 1988). These in vivo observations are consistent with the model of metabolic fluxes summarized in Figure 4, which is based on observations made in purified cellular preparations and in situ normal and compartmentalized neural systems. In particular, the glutamate-induced glycolysis recently reported in astrocytes provides a cellular basis for the metabolic uncoupling demonstrated in PET studies, which has led to the notion of activation-induced glycolysis (Fox et al., 1988).

Another important issue that emerges from studies at the cellular level is that glucose utilization, as visualized during physiological activation in humans by PET using 18F-labeled deoxyglucose or in laboratory animals with the 2-DG autoradiography technique may reflect predominantly uptake of the tracer into astrocytes. This conclusion does not question the validity of deoxyglucose-based techniques to map neuronal activity; rather, it provides a cellular and molecular basis for the interpretation of data obtained with PET.

Evidence for glycolysis as the principal metabolic pathway operational during activation of a given brain area has also been provided by in vivo 1H-NMR spectroscopy studies (Prichard et al., 1991; Sappey-Marinier et al., 1992). Such analyses, which are consistent with the previously reviewed evidences obtained in microdialysis studies (Fellows et al., 1993), show increases in the lactate signal in primary visual cortex following physiological activation (Prichard et al., 1991; Sappey-Marinier et al., 1992). These in vivo 1H-NMR spectroscopy studies taken together with the PET data indicating a metabolic uncoupling, reveal a previously unrecognized prevalence of glycolysis over oxidative phosphorylation during activation of the possible role of at least one of the two components has been identified. The first one is the absorption by hemoglobin over almost the entire wavelength range from 400 and 940 nm, which appears to correlate with changes in blood volume. The second, which becomes predominant between 600-630 nm, appears to correspond to the transition of oxyhemoglobin to deoxyhemoglobin, and would thus reflect oxygen delivery (Frostig et al., 1990).

Changes in tissue optical properties that correlated with metabolic activity were first described almost 70 years ago (Kelin, 1925). Since these intrinsic optical signals were relatively small, it is not until recently that, with the advent of appropriate amplification procedures, they have been usefully exploited to study brain activation. Although it was shown that transmission of infrared light could be used to monitor blood volume changes and hemoglobin oxygenation state in the intact brain (Jöbsis, 1977), the technique using the reflectance signal from light at wavelengths between 480 and 940 nm has been more extensively studied and developed (Grinvald et al., 1988). The nature of the events giving rise to the reflected light signal has been identified. One at least two components have been identified. The first one is the absorption by hemoglobin over almost the entire wavelength range from 480 and 940 nm, which appears to correlate with changes in blood volume. The second, which becomes predominant between 600-630 nm, appears to correspond to the transition of oxyhemoglobin to deoxyhemoglobin, and would thus reflect oxygen delivery (Frostig et al., 1990).

When the time course of the reflectance signal obtained at different wavelengths following activation is compared, it becomes clear that the signal detected at 600 nm precedes the signal at either 570 or 840 nm (Frostig et al., 1990). This was interpreted as an indication that oxygen delivery begins before the increase in blood volume takes place. Since the early signal, which has a latency between 100 and 300 msec is currently used as an index of neuronal activation (Bonhoeffer and Grinvald, 1993), the notion of the signal detected in optical imaging studies is deoxyhemoglobin has been the prevailing one. A point that is important to note is the fact that, after the initial rise, the signal at 600 nm decreases while the signal at 570 and 840 nm begins to increase (Fig. 7). Even more striking is the fact that if the stimulus causing the activation is of short duration, the signal at
Much less is known about the mechanism(s) responsible for oxygen delivery. One possibility is that local increases in oxygen consumption by the active neuromotor trigger oxygen release from hemoglobin by establishing a PO2 gradient. However, PET studies show only marginal increases in oxygen consumption in activated brain areas (Fox et al., 1988). In peripheral tissues such as muscle, a well-known mechanism responsible for oxygen release during increased metabolic demands is the Bohr effect (Stryer, 1988). Actively metabolizing tissues, such as muscle, produce large amounts of CO2 and H+. Both agents bind to hemoglobin and decrease its affinity for oxygen, thus causing a local oxygen delivery. A similar mechanism is likely to take place in the brain. In particular, lactate, produced as a consequence of glycolysis-induced activation (Fox et al., 1988; Prichard et al., 1991; Pellerin and Magistretti, 1994) could provide the necessary H+. One problem with this mechanism is that it appears too slow to account for the rapid oxygen delivery signal observed with optical imaging. Another possibility which, to our knowledge, has not been considered so far, is NO production. This gas is produced during activation (Akgören et al., 1994; Iadecola et al., 1994) and diffuses rapidly through biological tissues (Vanderkooi et al., 1994). NO has been shown both in free oxyhemoglobin solutions or in erythrocyte suspensions to react with oxyhemoglobin (Henry et al., 1993). Indeed, based on this well-characterized interaction, hemoglobin is routinely used as an inhibitor of NO’s actions since it prevents NO to interact with other molecular targets (Gibson and Roughton, 1957; Martin et al., 1986; Moncada et al., 1991). The interaction of NO with hemoglobin can yield two products: methemoglobin or nitrosylhemoglobin (Henry et al., 1993). Methemoglobin results from the oxidation of oxyhemoglobin by NO (in this reaction, the iron atom in the heme ring is oxidized from Fe2+ to Fe3+); indeed, the spectral changes of oxyhemoglobin to methemoglobin are used to determine NO formation in tissues (Kelm and Schrader, 1990). In contrast, nitrosylhemoglobin results from the binding of NO to the heme ring of either deoxyhemoglobin or methemoglobin (Kanner et al., 1992). NO was also shown to bind with a much greater affinity to the hemoprotein than O2 (Henry et al., 1993). An isosbestic point, that is, the wavelength at which two molecules have identical absorbance properties, exists among nitrosylhemoglobin, methemoglobin, and deoxyhemoglobin at around 600 nm (Van Assendelft, 1970; Kanner et al., 1992). This is the wavelength at which the signal detected by optical imaging is interpreted as oxygen delivery (Frostig et al., 1990). However, a signal detected at 600 nm can be due to an increase in either nitrosylhemoglobin, methemoglobin, or deoxyhemoglobin, or a combination of them (Fig. 8). Since the early reflectance signal during activation is largest at 605 ± 10 nm (Bonhoeffer and Grinvald, 1993), the possibility should be considered that the optical signal may arise from nitrosylhemoglobin and/or methemoglobin formed by the re-action of NO with hemoglobin. This point is particularly worth considering in view of the fact that PET studies indicate only a marginal increase in oxygen consumption upon activation, hence, making the formation of significant amounts of deoxyhemoglobin unlikely. These considerations bring into perspective a possible role of NO in the early signal.

600 nm shows an undershoot (Grinvald et al., 1986). Since the 600 nm signal is taken to reflect predominantly the ratio of deoxyhemoglobin over oxyhemoglobin, the decrease and following undershoot could be interpreted either as the reox-ygenation of hemoglobin, or more likely, as the arrival of large amounts of oxyhemoglobin overcoming the smaller desatur-ation process taking place. This, of course, would be brought about by the increase in blood volume that occurs upon ac-tivation (Fox and Raichle, 1986; Fox et al., 1988). As a net result, during the undershoot phase, it can be predicted that the blood leaving the activated area would have a higher oxyhemoglobin content than at rest. This is precisely what is observed with functional magnetic resonance imaging (fMRI), a tech-nique based, as noted earlier, on the difference in magnetic susceptibility between oxyhemoglobin and deoxyhemoglobin (Cohen and Bookheimer, 1994; Raichle, 1994). The latency of fMRI is longer than optical imaging, that is, a few seconds compared to a few hundred milliseconds (Frostig et al., 1990; Cohen and Bookheimer, 1994; Menon et al., 1994); this would explain why fMRI monitors only the increase in the oxyhemoglobin signal concomitant with the increase in blood vol-ume (Fig. 7). This interpretation on the origin of the reflectance and fMRI signals would agree with PET measurements of blood flow and O2 consumption (Fox and Raichle, 1986; Fox et al., 1988) which indicate an important increase in blood flow upon activation with only a small increase in O2 consumption.

The mechanism that gives rise to both the early increase in oxygen delivery and to the subsequent increase in blood volume recorded by optical imaging still remains to be fully elucidated. Since the initial proposal by Roy and Sherrington (1890) suggesting that products of cerebral metabolism could be responsible for the coupling of neuronal activity to blood flow, a number of candidates have been identified, including H+, K+ and adenosine (Edvinsson et al., 1993). The latest po-tential candidate on the list is the gas nitric oxide (NO), al-though variable results were obtained from studies in which inhibition of NO synthesis was achieved to prevent the activa-tion-induced increase in local blood flow (Iadecola et al., 1994). However, recent evidence has provided indication that NO, formed through the action of glutamate released during neuronal activation, can, indeed, couple activation to blood flow in the cerebellum (Akgören et al., 1994).

Figure 7. Temporal characteristics of reflectance signals obtained by optical imaging and the fMRI signal. The optical imaging signal recorded at 600-630 nm is taken to reflect primarily the ratio of HbO2:Hb. Thus, an increase in signal is interpreted as net "oxygen delivery." However, these wavelengths the signal could theoretically originate from deoxyhemoglobin (Hb) as well as from either nitrosylhemoglobin (HbNO) or methemoglobin (metHHb; see text and Fig. 8, for treatment of this point). The optical signal recorded either at 570 or 840 nm is accounted for by the increase in total hemoglobin (Hb + HbO2), a likely consequence of increased blood volume. The fMRI signal is interpreted as an increase in the ratio of HbO2:Hb, a consequence of the increased blood volume with little or no increase in oxygen consumption by the tissue during activation, which leads to a higher oxyhemoglobin content in the venous blood.

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detected by optical imaging techniques (Grinvald et al., 1986; Frostig et al., 1990).

Concluding Remarks

The focus of this article has been to try to highlight the latest observations of brain energy metabolism at the cellular and molecular levels and their relationships with the recent developments in functional brain imaging. From the convergence of in vitro and in vivo observations, a novel emerging concept is that the brain resorts to glycolysis to face increased energy demands during activation. Astrocytes appear to be the predominant cellular locus of this process. The activation-induced glycolysis provides the signal for in vivo FDG studies, 1H-NMR lactate spectroscopy, and, ultimately, fMRI. Therefore, while the cellular and even molecular basis for these three signals appears to be reasonably clarified, the nature of the molecular mechanism(s) that underlie the coupling between activation and increased blood flow is still elusive, even 100 years after the seminal work of Roy and Sherrington (1890).

Notes

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