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Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging

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Despite striking advances in functional brain imaging, the cellular and molecular mechanisms that underlie the signals detected by these techniques are still largely unknown. The basic physiological principle of functional imaging is represented by the tight coupling existing between neuronal activity and the associated local increase in both blood flow and energy metabolism. Positron emission tomography (PET) signals detect blood flow, oxygen consumption and glucose use associated with neuronal activity; the degree of blood oxygenation is currently thought to contribute to the signal detected with functional magnetic resonance imaging, while magnetic resonance spectroscopy (MRS) identifies the spatio-temporal pattern of the activity-dependent appearance of certain metabolic intermediates such as glucose or lactate. Recent studies, including those of neurotransmitter-regulated metabolic fluxes in purified preparations and analyses of the cellular localization of enzymes and transporters involved in energy metabolism, as well as *in vivo* microdialysis and MRS approaches have identified the neurotransmitter glutamate and astrocytes, a specific type of glial cell, as pivotal elements in the coupling of synaptic activity with energy metabolism. Astrocytes are ideally positioned to sense increases in synaptic activity and to couple them with energy metabolism. Indeed they possess specialized processes that cover the surface of intraparenchymal capillaries, suggesting that astrocytes may be a likely site of prevalent glucose uptake. Other astrocyte processes are wrapped around synaptic contacts which possess receptors and reuptake sites for neurotransmitters. Glutamate stimulates glucose uptake into astrocytes. This effect is mediated by specific glutamate transporters present on these cells. The activity of these transporters, which is tightly coupled to the synaptic release of glutamate and operates the clearance of glutamate from the extracellular space, is driven by the electrochemical gradient of Na^+. This Na^+-dependent uptake of glutamate into astrocytes triggers a cascade of molecular events involving the Na^+/K^+-ATPase leading to the glycolytic processing of glucose and the release of lactate by astrocytes. The stoichiometry of this process is such that for one glutamate molecule taken up with three Na^+ ions, one glucose molecule enters an astrocyte, two ATP molecules are produced through aerobic glycolysis and two lactate molecules are released. Within the astrocyte, one ATP molecule fuels one 'turn of the pump' while the other provides the energy needed to convert glutamate to glutamine by glutamine synthase. Evidence has been accumulated from structural as well as functional studies indicating that, under aerobic conditions, lactate may be the preferred energy substrate of activated neurons. Indeed, in the presence of oxygen, lactate is converted to pyruvate, which can be processed through the tricarboxylic acid cycle and the associated oxidative phosphorylation, to yield 17 ATP molecules per lactate molecule. These data suggest that during activation the brain may transiently resort to aerobic glycolysis occurring in astrocytes, followed by the oxidation of lactate by neurons. The proposed model provides a direct mechanism to couple synaptic activity with glucose use and is consistent with the notion that the signals detected during physiological activation with ^{18}F-deoxyglucose (DG)-PET may reflect predominantly uptake of the tracer into astrocytes. This conclusion does not question the validity of the 2-DG-based techniques, rather it provides a cellular and molecular basis for these functional brain imaging techniques.

Keywords: astrocytes; deoxyglucose; PET; lactate; glutamate

1. INTRODUCTION

The central principle underlying all functional imaging techniques is that local changes in brain activity can be visualized by monitoring the changes in blood flow and energy metabolism that are associated with activity of specific neuronal circuits (Raichle 1994). Thus, positron emission tomography (PET) can monitor the increase in cerebral blood flow (CBF), in glucose use and in oxygen consumption (Phelps *et al*. 1979; Frackowiak *et al*. 1980); the degree of blood oxygenation yields the signals that are detected with functional magnetic resonance imaging

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(fMRI) (Ogawa *et al*. 1992). The assumption is that the energetic requirements associated with synaptic function represent the indicators detected with functional brain imaging techniques. Indeed, PET and fMRI do not detect synaptic activity directly but do measure signals that reflect activity-dependent energy consumption. Questions currently awaiting a response are (i) is this assumption correct, and if so, what are the mechanisms that underlie this coupling, and (ii) what are the specific components of synaptic transmission that trigger the metabolic and blood flow responses? An enlightening perspective was already presented by Charles Sherrington in a seminal article published over 100 years ago in the *Journal of Physiology* (Roy & Sherrington 1890). He postulated that '[the brain possesses] intrinsic mechanisms by which its vascular supply can be varied locally in correspondence with local variations of functional activity'; he postulated also that 'chemical products of cerebral metabolism ... cause variations of calibre of the cerebral vessels'. The basis for neurovascular coupling was laid. The development of the autoradiographic 2-deoxyglucose (2-DG) method by Sokoloff and colleagues (Sokoloff *et al*. 1977), provided the demonstration for neurometabolic coupling, that is the coupling existing between synaptic activity and glucose use. These findings and the hypotheses derived from them, substantiated the existence of a relatively tight coupling between the activity of modality-specific neuronal circuits with blood flow and energy metabolism. However, the nature of the cellular and molecular mechanisms underlying these couplings has long remained elusive. In this article we review recent evidence at the cellular and molecular levels, with a particular focus on the emerging evidence for a central role of astrocytes in coupling activity with metabolism.

2. THE CENTRAL ROLE OF ASTROCYTES IN COUPLING SYNAPTIC ACTIVITY WITH ENERGY METABOLISM

Camillo Golgi and his associates suggested that, in view of their cytological characteristics, astrocytes should play a central role in the distribution of energy substrates from the circulation to neurons (Golgi 1886; Sala 1891; Andriezen 1893). Structural and functional arguments for such a role of astrocytes have been accumulated in recent years. Thus, while the ratio between neurons and non-neuronal cells depends on species, brain areas or developmental ages, it is a well-established fact that neurons contribute at most 50% of cerebral cortical volume (O'Kusky & Colonnier 1982; Kimelberg & Norenberg 1989; Bignami 1991). An astrocyte : neuron ratio of 10:1 is a feature of most brain regions (Bignami 1991). Astrocytes are stellate cells (hence their name) with multiple fine processes, some of which are in close apposition to capillary walls. In fact the entire surface of intraparenchymal capillaries is covered by these specialized processes called astrocytic end-feet (Peters *et al*. 1991). 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 (figure 1). In addition, glucose transporters of the GLUT1 type are expressed on astrocytic end-feet (Morgello *et al*. 1995). Other astrocyte processes are wrapped around synaptic contacts which possess receptors for a variety of neurotransmitters as well as reuptake sites, in particular for glutamate. In fact there is evidence obtained from quantitative morphometric studies supporting the notion that astrocytes are polarized cells, with one process contacting a cell of mesodermal origin (most frequently an endothelial cell of the capillary) and a multitude of processes that are intertwined within the neuropil ensheathing synaptic contacts. These synaptically targeted lamellar processes represent approximately 80% of an astrocyte membrane surface and are devoid of organelles (Rohlmann & Wolff 1996). Overall, these features imply that astrocytes are ideally positioned to sense increases in synaptic activity and to couple them with energy metabolism.

3. SYNAPTICALLY RELEASED GLUTAMATE TRIGGERS GLUCOSE USE IN ASTROCYTES

Glutamate is the main excitatory neurotransmitter of the cerebral cortex. Activation of afferent pathways by specific modalities (e.g. somatosensory, visual, auditory) or of corticocortical association circuits results in a spatially and temporally defined local release of glutamate from the activated synaptic terminals (Fonnum 1984). The released glutamate exerts profound effects on the excitability of target neurons, which are mediated by specific subtypes of glutamate receptors (Gasic & Hollmann 1992). The action of glutamate on postsynaptic neurons is rapidly terminated by an avid reuptake system present on astrocyte processes (Bergles & Jahr 1998), which ensheath synaptic contacts (Peters *et al*. 1991). This is in fact one of the best established physiological functions of astrocytes (Barres 1991).

The removal of glutamate from the synaptic cleft is operated through specific glutamate transporters, two of which are predominantly, if not exclusively, glial specific (Danbolt 1994; Robinson & Dowd 1997). These are GLT-1 and GLAST. The third glutamate transporter subtype, EAAC-1, is exclusively localized in neurons, but does not appear to be involved in the clearance of synaptically released glutamate (Rothstein *et al*. 1994). Glutamate uptake into astrocytes is driven by the electrochemical gradient of Na^+; it is indeed an Na^+-dependent mechanism with a stoichiometry of three Na^+ ions co-transported with one glutamate molecule (figure 1). The coupling between synaptic glutamate release and its re-uptake into astrocytes is so tight that determination of the Na^+ current generated in astrocytes by the co-transport of glutamate and Na^+ through the glutamate transporter provides an accurate reflection of glutamate release from the synapse (figure 1; Bergles & Jahr 1997). Once in astrocytes, glutamate is predominantly converted to glutamine through an ATP-requiring reaction catalysed by the astrocyte-specific enzyme glutamine synthase (Martinez-Hernandez *et al*. 1977). Glutamine is then released by astrocytes and taken up by neurons to replenish the neurotransmitter pool of glutamate (figure 1).

A second, unexpected consequence of transporter-mediated glutamate uptake into astrocytes is the stimulation of aerobic glycolysis in these cells, i.e. of glucose use and lactate production. This metabolic effect of glutamate is expressed with an EC_{50} of approximately 80 μM (Pellerin &

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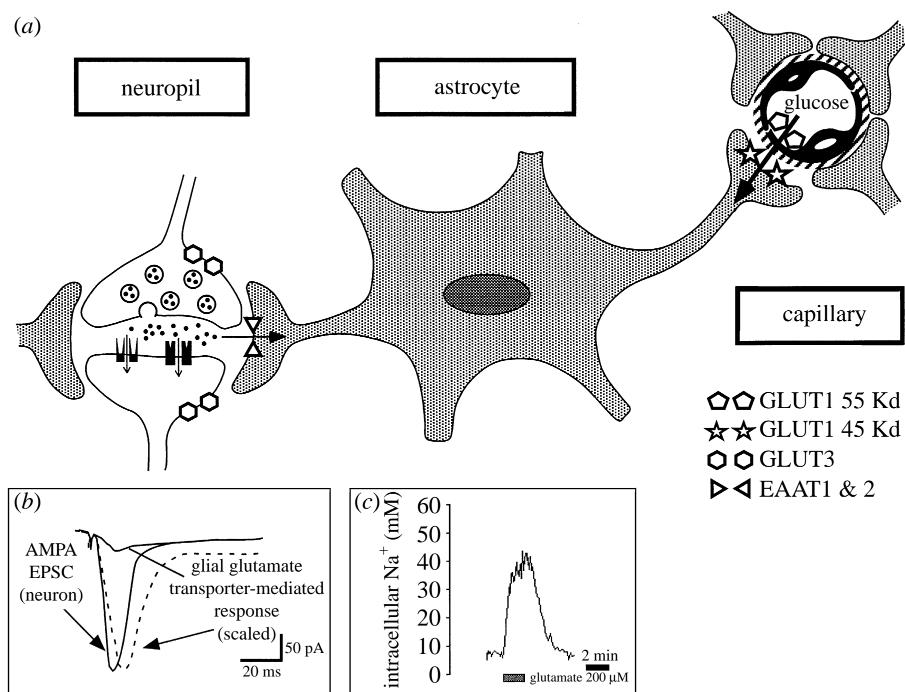

Figure 1. (*a*) Cytological relationships of astrocytes with neurons and capillaries. Specialized astrocytic processes, the end-feet, cover virtually all capillary endothelial walls. GLUT1 glucose transporters are present both on endothelial cells (55 kDa form) and on astrocytic end-feet (45 kDa form). Other astrocytic processes ensheath synaptic contacts. These processes are enriched in glutamate transporters (EAAT1 and 2). Neurons also express glucose transporters (GLUT3). (*b*) Tight coupling between glutamate-mediated synaptic responses and glutamate reuptake into hippocampal astrocytes. The Nα-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor-mediated excitatory postsynaptic current (EPSC) monitored in the postsynaptic neuron following glutamate release is tightly coupled to an Na^+ current generated by the co-transport of glutamate and Na^+ through the glial glutamate transporter. The glial glutamate transporter-mediated current (dotted line) has been scaled to the EPSC. Note that both the neuronal and glial currents begin at the same time, while the glial response shows a slower kinetics. (Redrawn and adapted from Bergles & Jahr (1997).) (*c*) Glutamate-mediated increase in $[Na^+]_i$ in cultured astrocytes. The $[Na^+]_i$ was determined by fluorescence microscopy using the specific fluorescent dye SBFI (Na^+-binding benzofuran isophthalate). Note that following glutamate application (200 µM) the intracellular Na^+ concentration rapidly rises from about 8 to 40 µM. (Kindly provided by Dr Jean-Yves Chatton, Institute of Physiology, University of Lausanne.)

Magistretti 1994). Pharmacological evidence that this effect of glutamate is not mediated by specific glutamate receptors was provided by the absence of effect by agonists, specific for each glutamate receptor subtype. Consistent with this finding, receptor antagonists do not inhibit the effect of glutamate (Pellerin & Magistretti 1994). Similar conclusions on the role of glutamate transporters in triggering glucose use by astrocytes were later confirmed by Sokoloff and colleagues (Takahashi *et al.* 1995).

Glutamate-stimulated increase in glucose uptake and phosphorylation into astrocytes is abolished in the absence of Na^+ in the extracellular medium (Pellerin & Magistretti 1994), consistent with the necessity for an electrochemical gradient for the ion to drive glutamate uptake. In addition, L- and D-aspartate, but not D-glutamate, mimic the effect of L-glutamate, the physiological agonist. Such a specific pharmacological profile provides the signature for a phenomenon mediated by the glutamate transporter. Finally, the specific glutamate transporter inhibitor β-threohydroxyaspartate inhibits the glutamate-stimulated glucose use (Pellerin & Magistretti 1994).

Central to the coupling between glutamate transporter activity and glucose uptake into the astrocyte is the activation of the Na^+/K^+-ATPase as indicated by the fact that ouabain completely inhibits the glutamate-evoked 2-DG uptake by astrocytes (Pellerin & Magistretti 1994). The astrocytic Na^+/K^+-ATPase responds predominantly to increases in intracellular Na^+ concentration ($[Na^+]_i$) for which it shows a K_m of about 10 mM (Kimelberg *et al.* 1993). Since in cultured astrocytes, the $N[Na^+]_i$ ranges between 10 and 20 mM (Kimelberg *et al.* 1993), Na^+/K^+-ATPase is set to be readily activated when $N[Na^+]_i$ rises concomitantly with glutamate uptake (Bowman & Kimelberg 1984). Indeed, recent evidence obtained in our laboratory using ^{86}Rb uptake to directly monitor the activity of the pump, shows that glutamate activates the Na^+/K^+-ATPase (Pellerin & Magistretti 1996, 1997). This effect of glutamate is due to the mobilization of a subunit of the pump which is highly sensitive to ouabain, probably the α_2-subunit (Pellerin & Magistretti 1997).

There is ample evidence from studies in a variety of cellular systems including the brain, kidney, vascular smooth muscle and erythrocytes, that increases in the activity of the Na^+/K^+-ATPase stimulate glucose uptake and glycolysis (Parker & Hoffman 1967; Lipton & Robacker 1983). Consistent with this view, glutamate stimulates the glycolytic processing of glucose in astrocytes, as indicated by the increase in lactate release (Pellerin & Magistretti 1994).

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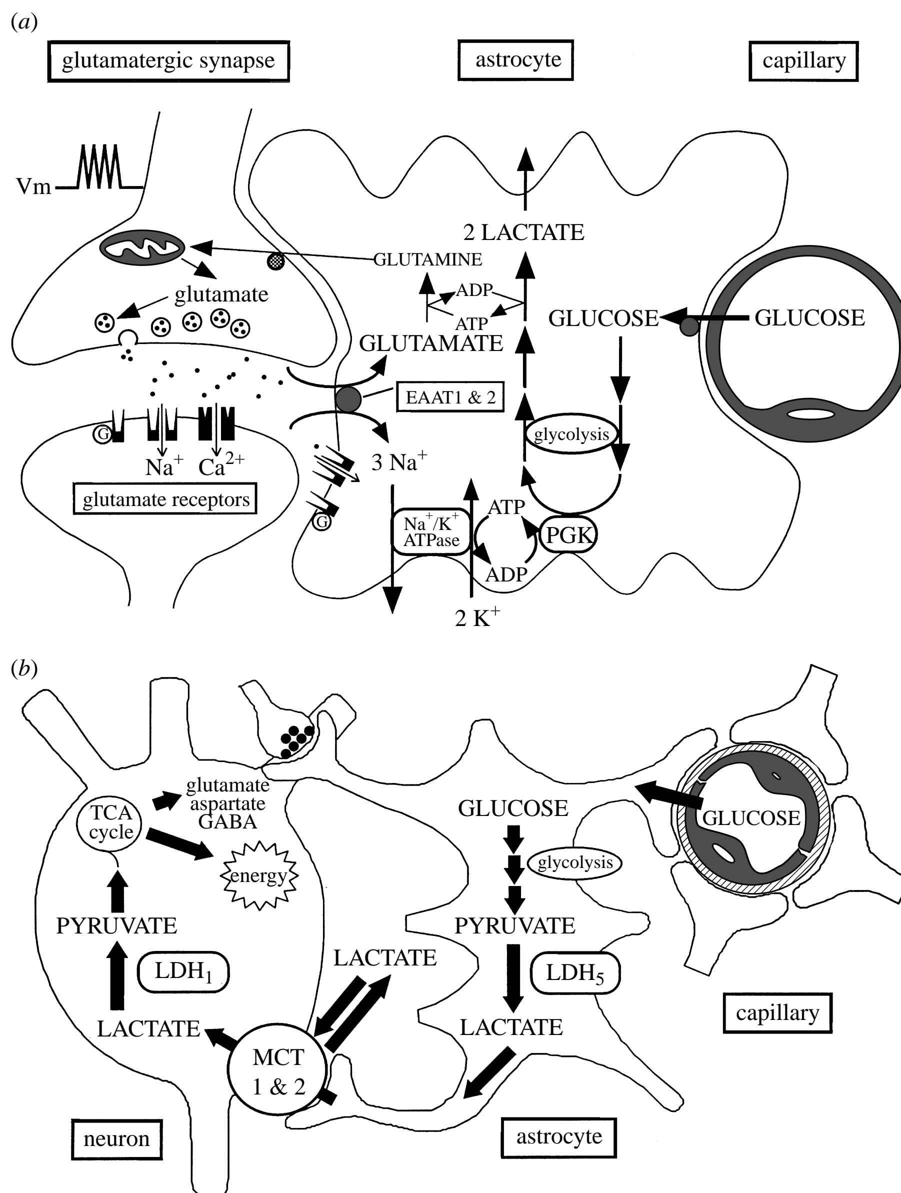

Figure 2. (*a*) Schematic representation of the mechanism for glutamate-induced glycolysis in astrocytes during physiological activation. At glutamatergic synapses, presynaptically released glutamate depolarizes postsynaptic neurons by acting at specific receptor subtypes. The action of glutamate is terminated by an efficient glutamate uptake system located primarily in astrocytes (see figure 1). Glutamate is co-transported with Na^+, resulting in an increase in the intra-astrocytic concentration of Na^+, leading to an activation of the astrocyte Na^+/K^+-ATPase. Activation of the Na^+/K^+-ATPase stimulates glycolysis, i.e. glucose use and lactate production. Lactate, once released by astrocytes, can be taken up by neurons and serves them 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 evidence indicating glutamate-induced glycolysis, is taken to reflect cellular and molecular events occurring during activation of a given cortical area. (*b*) Schematic representation of the proposed astrocyte–neuron lactate shuttle. Following neuronal activation and synaptic glutamate release, glutamate reuptake into astrocytes triggers increased glucose uptake from capillaries via activation of an isoform of the Na^+/K^+-ATPase which is highly sensitive to ouabain, possibly the α_2 isoform (Pellerin & Magistretti 1994, 1997). Glucose is then processed glycolytically to lactate by astrocytes which are enriched in the muscle form of LDH (LDH_5). The exchange of lactate between astrocytes and neurons is operated by monocarboxylate transporters (MCTs). Lactate is then converted to pyruvate since neurons contain the heart form of LDH (LDH_1). Pyruvate, via the formation of acetyl-CoA by pyruvate dehydrogenase (PDH), enters the TCA cycle thus generating 17 ATP molecules per lactate molecule.

The proposed stoichiometry of the molecular steps involved in the coupling between glutamate uptake and glucose use is the following: the uptake of one glutamate with three Na^+ ions triggers the entry of one glucose molecule, which through glycolysis produces two ATP molecules, one of which is consumed by the pump resulting in the extrusion of three Na^+ ions, while the other fuels the enzymatic conversion of glutamate to glutamine, an ATP-requiring, astrocyte-specific reaction catalysed by glutamine synthase (figure 2*a*); the glycolytic processing of glucose results in approximately two lactate molecules produced per glucose molecule, i.e. the expected

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stoichiometrical relationship between glucose and lactate (Magistretti & Pellerin 1997). The increase in lactate formation from extracellular glucose evoked by glutamate is expressed with an EC_{50} of approximately 80 μM (Magistretti & Pellerin 1997), a value which is very similar to the affinity of glutamate transport into astrocytes, and to the stimulation of glucose use and of the pump activity by glutamate (Pellerin & Magistretti 1994, 1997).

These data indicate that glutamate stimulates aerobic glycolysis (i.e. the transformation of glucose into lactate in the presence of sufficient oxygen) in astrocytes by a mechanism involving an activation of the Na^+/K^+-ATPase. 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 (Sokoloff 1991).

4. GLUTAMATE-STIMULATED LACTATE PRODUCTION BY ASTROCYTES PROVIDES AN ENERGY SOURCE FOR NEURONS

This view raises the question of the usefulness of lactate as an energy substrate for neurons. A vast array of experimental data has been accumulated over the years, indicating that *in vitro* lactate is an essential energy substrate for synaptic transmission and can adequately maintain synaptic activity even in the absence of glucose (Schurr *et al*. 1988, 1999; Izumi *et al*. 1997). *In vivo*, lactate is not an adequate substrate since it crosses only marginally the blood–brain barrier (Pardridge & Oldendorf 1977); however, if formed within the brain parenchyma through the mechanism described above (figure 2*b*), or if applied to *in vitro* preparations, lactate may in fact be consumed preferentially to glucose, particularly during periods of intense activity (Larrabee 1995).

Since lactate can be used as an energy fuel by neurons, we considered the possibility that a selective distribution of lactate dehydrogenase (LDH) isoenzymes could exist among lactate-producing and lactate-consuming cells. Indeed LDH is the enzyme that catalyses the interconversion of lactate and pyruvate. We have raised polyclonal antibodies against the LDH_1 (heart-type) and the LDH_5 (muscle-type) subunits and used them for immunohistochemistry of human hippocampus and visual cortex. The results show that the immunoreactivity against LDH_5 (the form enriched in lactate-producing tissues) is restricted to a population of astrocytes while neurons are stained only by an antibody directed against LDH_1 (the form enriched in lactate-consuming tissues) (Bittar *et al*. 1996). These data support the idea that some astrocytes would preferentially process glucose glycolytically into lactate, which, once released, could be transformed by neurons into pyruvate and enter the tricarboxylic acid (TCA) cycle to serve as an energy fuel (figure 2*b*). It should be stressed that one molecule of lactate entering the TCA cycle through the LDH-catalysed reaction, can yield, in normoxic conditions, 17 ATP molecules.

In summary, since glutamate release occurs following the modality-specific activation of a brain region, these data and the proposed model are consistent with the view that during activation, glutamate uptake into astrocytes leads to increased glucose use and lactate production, which can be subsequently used by neurons to meet their energy needs (Magistretti *et al*. 1999). Further support for this notion of an 'astrocyte–neuron lactate shuttle' in the brain (figure 2*b*), has been provided by the recent identification of two lactate transporters, MCT-1 and MCT-2, which are present in the central nervous system (Pellerin *et al*. 1998) and selectively expressed in astrocytes or neurons. Thus, MCT-1 is enriched in astrocytes in culture while MCT-2 is predominantly expressed in neurons (Bröer *et al*. 1997).

5. *IN VIVO* OBSERVATIONS ARE CONSISTENT WITH THE PROPOSED MODEL

Results obtained in a variety of *in vivo* paradigms, both in laboratory animals and in humans, support the existence of a transient lactate production during activation. Thus, microdialysis studies in rats indicate a marked increase in the concentration of extracellular lactate in striatum during physiological sensory stimulation (Fellows *et al*. 1993). This activity-linked lactate peak is completely inhibited when glutamate uptake inhibitors such as β-threohydroxyaspartate or pyrrolidine-2,4-dicarboxylate are present in the perfusate, thus providing further support for the existence of glutamate-stimulated glycolysis during activation (Fray *et al*. 1996; Demestre *et al*. 1997). In the hippocampus, electrical stimulation of the perforant pathway triggers an increase in lactate levels which occurs within 50–60 s, i.e the same time-frame as glutamate release and uptake (Hu & Wilson 1997). Moreover, it was demonstrated that following the initial sustained increase in lactate levels, repeated stimulations caused transient decline in lactate concentration in the extracellular space, consistent with increased lactate use during neuronal activation (Hu & Wilson 1997).

Magnetic resonance spectroscopy (MRS) in humans has also revealed that during physiological activation of the visual system, a transient lactate peak is observed in the primary visual cortex (Prichard *et al*. 1991). Thus, microdialysis and MRS data *in vivo* support the notion of a transient glycolytic processing of glucose during activation. In addition, recent data obtained *in vivo* by MRS indicate a tight coupling between glutamate-mediated synaptic activity and glucose use. The simultaneous measurements over a range of synaptic activity of the TCA cycle and the cycling of glutamate to glutamine (a process which occurs exclusively in astrocytes) using ^{13}C-glucose MRS has revealed a striking stoichiometric relationship of 1:1 between glutamate cycling (a reflection of synaptic activity) and glucose use (Sibson *et al*. 1998). According to these data, for each glutamate molecule released from active terminals and taken up by astrocytes one glucose molecule would be oxidized, a result fully consistent with the stoichiometry proposed from data obtained *in vitro* (Pellerin & Magistretti 1997).

6. FOOD FOR THOUGHT: IS GLUCOSE USE COUPLED TO OXYGEN CONSUMPTION DURING ACTIVATION?

Metabolic studies at the whole-brain level have demonstrated beyond doubt that virtually all glucose entering

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the brain parenchyma is oxidized to CO_2 and H_2O (Kety 1957). However, PET analyses pioneered by Raichle and Fox have indicated that local oxygen consumption does not increase commensurately with blood flow and glucose use in activated brain areas (Fox *et al*. 1988), suggesting the occurrence of an activity-dependent glycolytic processing of glucose. This issue has been the object of intense debate since other investigators have found that the degree of uncoupling between glucose use and oxygen consumption during activation may actually vary, and even may not occur, depending on the stimulations used (Marrett *et al*. 1995). In addition, using ^{13}C-glucose MRS, Shulman and colleagues have recently reported data consistent with a significant increase in oxygen use during activation (Hyder *et al*. 1996). At present, the critical question of how much oxygen is consumed during activation remains open: certainly a set of PET data is consistent with the notion that the brain resorts transiently to glycolysis to meet the increased energy demands during activation (Fox *et al*. 1988). Other data from PET and MRS (Marrett *et al*. 1995; Hyder *et al*. 1996), and from imaging of intrinsic optical reflectance signals in animals (Malonek & Grinvald 1996), would argue for a significant increase in oxygen consumption during functional activation. While the debate is still open, the model proposed on the basis of studies at the cellular level (figure 2*a*,*b*) would be consistent with an initial glycolytic processing of glucose occurring in astrocytes during activation, resulting in a transient lactate overproduction, followed by a recoupling phase during which lactate would be oxidized by neurons. The spatio-temporal 'window' during which a lactate peak could be detected by MRS would depend on the rapidity and degree of recoupling existing between astrocytic glycolysis and neuronal oxidative phosphorylation. Indeed, some evidence also obtained by MRS in man has suggested the occurrence of a dynamic uncoupling followed by recoupling of oxidative metabolism (Frahm *et al*. 1996), which would be consistent with such a scheme.

Recent observations made *in vivo* by electrochemical detection of glucose and lactate levels as well as of oxygen consumption in rat hippocampus have confirmed the existence of an activity-dependent glycolytic processing of glucose in register with glutamate release (Hu & Wilson 1997). These data also suggest the existence of a lactate pool in the extracellular space which would be readily available to activated neurons for oxidation. Upon activation, neurons would consume lactate from that pool while astrocytes would replenish it via the glutamate-stimulated glycolytic processing of glucose described in the proposed model (figure 2*b*).

7. BLOOD FLOW AND GLUCOSE USE ARE NOT ALWAYS COUPLED

The subject of the mechanisms coupling neuronal activity and CBF has received a great deal of attention over the years and is beyond the scope of this article. The reader is rather referred to excellent reviews which have appeared on the subject (Villringer & Dirnagl 1995; Welch *et al*. 1997). A rapid survey indicates that a variety of signals produced by active neurons have been proposed to mediate the coupling with CBF. These signals include metabolites such as lactate, K^+, H^+ or adenosine, and neurotransmitters such as vasoactive intestinal peptide, acetylcholine or noradrenaline. Nitric oxide (NO) is another messenger which appears to be involved in the activity-dependent increase of CBF (Akgören *et al*. 1994; Dalkara & Moskowitz 1997). A long-held view has been that the increased energy metabolism occurring at sites of neuronal activation is driven by the increased availability in energy substrates (e.g. glucose) caused by the increased CBF. Such a concatenation of events can be considered as an 'in-series' process whereby active neurons liberate signals that increase CBF, hence providing more glucose and oxygen to the active area and resulting in the overall stimulation of metabolism. If this were true, one would expect that the very same signals that cause increases in CBF should always lead to an increase in energy metabolism (glucose use).

Recent evidence indicates that this is not the case. Under certain circumstances, CBF and metabolism can be uncoupled. Thus, the muscarinic receptor antagonist scopolamine inhibits the increase in CBF in response to somatosensory stimulation in rats without affecting glucose use (Villringer & Dirnagl 1995). Conversely, electrical stimulation of the nucleus basalis, which projects acetylcholine-containing fibres to the cerebral cortex, produces marked increases in cortical blood flow without affecting glucose use (Vaucher *et al*. 1997). Bonvento and colleagues have shown that inhibition of the neuronal NO synthase by 7-nitroindazole prevents the CBF increase in the somatosensory cortex induced by stimulation of the vibrissae (Cholet *et al*. 1996). While the CBF increase was inhibited, the activity-dependent glucose use response was preserved (Cholet *et al*. 1997).

Uncoupling of CBF and energy metabolism suggests the existence of an 'in-parallel' regulation of activity-linked increase in CBF and metabolism. Thus, when glutamate is released from active synapses, a receptor-mediated NO formation occurs in neurons which may contribute to the activity-dependent increase in CBF; in parallel to this process, glutamate reuptake into astrocytes stimulates glucose use and lactate production by astrocytes (Magistretti 1997) (figure 3). This scheme implies that CBF and metabolism are linked under physiological conditions but can be uncoupled by selective pharmacological manipulations or under particular pathophysiological conditions.

8. PERSPECTIVES

The cellular resolution afforded by purified preparations (e.g. cultures of astrocytes and neurons) and by the localization of enzymes and transporters involved in cell-specific metabolic processes, has provided novel perspectives to interpret brain energy metabolism and the nature of the signals detected by functional brain imaging. One of the main novel perspectives is the evidence for a central role of astrocytes in coupling neuronal activity with energy metabolism (figure 4). To summarize, focal physiological activation of specific brain areas is accompanied by increases in glucose use; since glutamate is released from excitatory synapses when neuronal pathways subserving specific modalities are activated, the stimulation by glutamate of glucose use in astrocytes as described here

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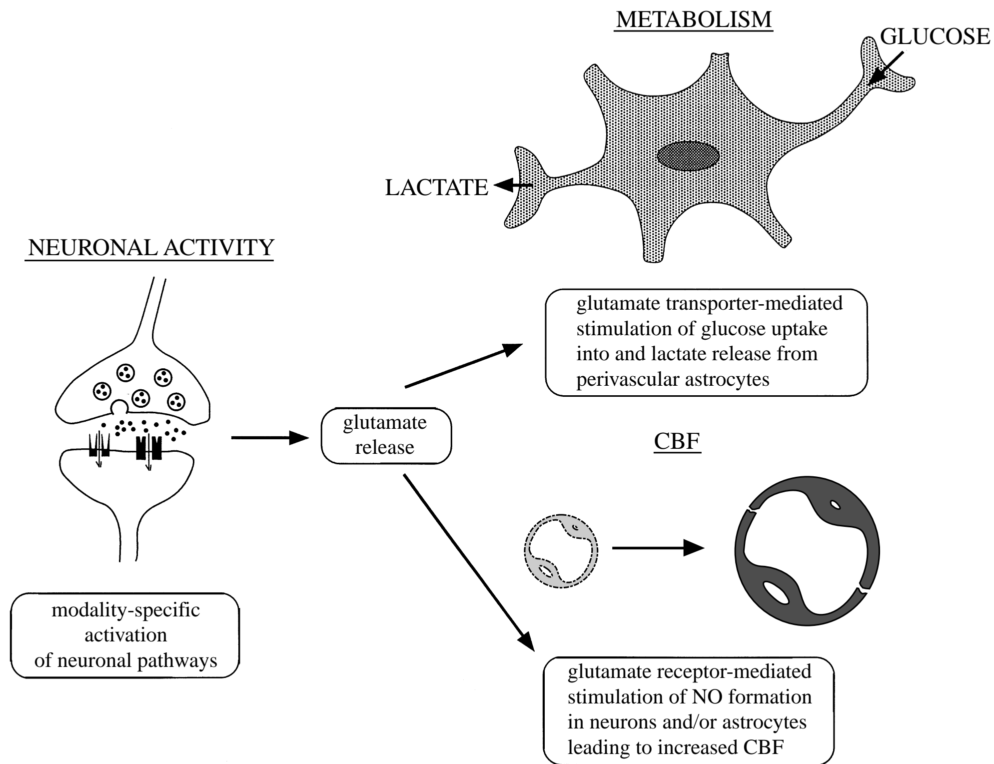

Figure 3. Coupling 'in-parallel' of neuronal activity with CBF and energy metabolism (e.g. glucose use). See text for discussion.

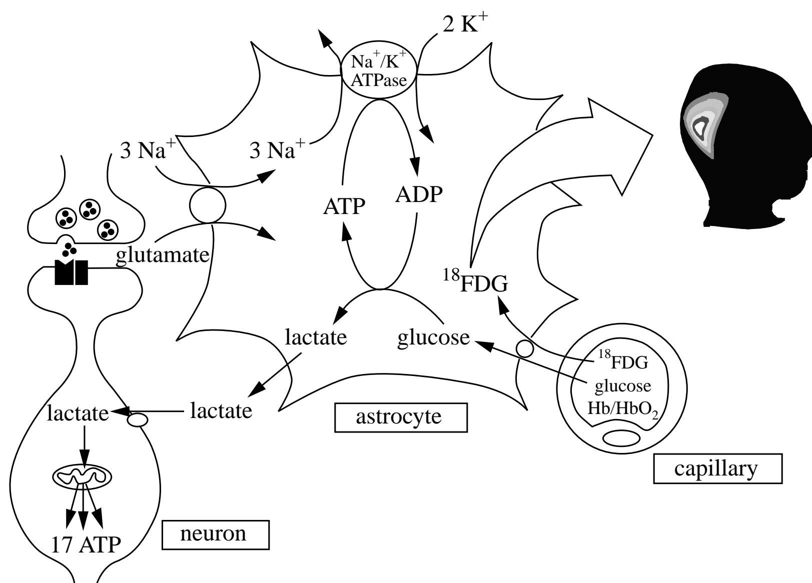

Figure 4. Relevance of metabolic trafficking between astrocytes and neurons for ^{18}FDG-PET. The proposed model of glutamate-induced glycolysis in astrocytes implies that the activity-linked uptake of ^{18}FDG monitored with PET, reflects primarily an astrocyte-based signal. Since neuronally released glutamate triggers the cascade of events that leads to glucose uptake, the ^{18}FDG-PET signal will faithfully reflect activation of neuronal circuits.

provides a direct mechanism for coupling neuronal activity to glucose use in the brain. The model is consistent with the variable degree of uncoupling between glucose uptake and oxygen consumption observed in PET studies. It is also consistent with the transient lactate peak measured *in vivo* in microdialysis and MRS experiments. In addition, these observations also strongly suggest that glucose use, as visualized during physiological activation in humans by PET using ^{18}FDG or in laboratory animals with a 2-DG autoradiography technique may reflect, at least in part, uptake of the tracer into astrocytes. This conclusion does not question the validity of DG-based techniques to map neuronal activity, rather it provides a cellular and molecular basis for these *in vivo* imaging procedures.

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