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Reviews

The cellular basis of neurovascular metabolic coupling

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Abstract

Functional brain imaging studies, such as positron emission tomography and functional nuclear magnetic resonance imaging, are based on the regional increases in cerebral blood flow, glucose consumption and oxygen consumption that are associated with regional increases in neuronal activity. The cellular basis of the signals that are measured is however incompletely understood at present. An increasingly important role for astrocytes in neurovascular metabolic coupling is being described, which was already suggested over 100 years ago as astrocytes are a structural link between endothelial cells of brain capillaries and neurons. Recent data have provided evidence for a role of the astrocytic intracellular sodium concentration in driving astrocytic glycolysis that may provide neurons with lactate as an energy substrate during activation. We have previously described intracellular sodium changes in astrocytes that are associated with astrocytic intercellular calcium waves. These intercellular calcium waves have been demonstrated both in astrocytes in culture as well as in brain slices. In this paper a new hypothesis concerning a role for astrocytic intercellular calcium waves in brain energy metabolism is formulated.

Key words : Astrocytes ; intercellular calcium waves ; brain energy metabolism ; neurovascular coupling ; traumatic brain injury.

Introduction

In the past few years powerful techniques have been developed that allow to see the brain "at work", such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (Hyder *et al*., 2001). Even though the application of these functional brain imaging techniques has increased, their cellular basis is incompletely understood. It is known that an increase in neuronal activity when a region is performing a task, is associated with an increase in regional cerebral blood flow (rCBF) and equivalent increases in local glucose consumption as well as with disproportional smaller increases in local oxygen consumption (Shulman *et al*., 2001). As the brain has very little energy reserve, a continuous supply of both glucose and oxygen is thus required (Swanson, 1992). The basis for the signal captured with the BOLD (blood oxygen level dependent) fMRI protocol is believed to be generated by the inherent magnetic susceptibility features of the iron atom in hemoglobin and to be dependent on the relative dissociation between blood flow/glucose consumption and oxygen consumption during activation (Magistretti and Pellerin, 1999). This will indeed lead to a decrease in the venous deoxygenated hemoglobin (deoxyHb) / oxygenated hemoglobin (O_2Hb) ratio and thus to changes in the BOLD fMRI signal as deoxyHb is paramagnetic whereas O_2Hb is diamagnetic. Using ^{15}O labelled oxygen, ^{15}O labelled water and ^{18}fluorodeoxyglucose (^{18}FDG) local oxygen consumption, rCBF and local glucose consumption can be measured respectively with PET (Frackowiak *et al*., 1980).

As neurons have very few direct contacts with blood vessels in the brain a role for astrocytes in neurovascular metabolic coupling was hypothesized over a 100 years ago. Astrocytes are a very abundant cell type in cerebral cortex with an astrocyte-to-neuron ratio of about 10 (Bignami *et al*., 1991). Given their smaller cellular volume as compared to neurons, astrocytes comprise about 50 % of the cerebral cortical volume (Kimelberg and Norenberg, 1989 ; O'Kusky and Colonnier, 1982). Astrocytes provide a structural link between neurons and capillaries as astrocytes cover almost 90 % of the abluminal membrane of endothelial cells of cerebral capillaries with their so-called endfeet and have numerous contacts with both neurons and synapses (Kacem *et al*., 1998 ; Peters *et al*., 1991). Furthermore, increasing numbers of receptors for neurotransmitters are being described that are present on the astrocyte plasma membrane (Vesce *et al*., 1999a ; Vesce *et al*., 1999b). As astrocytic processes are wrapped around synaptic contacts, they appear to be ideally positioned to sense synaptic activity and to couple it with energy metabolism (Derouiche and Frotscher, 1991). Several roles for astrocytes, including metabolic and trophic support to neurons, ion buffering (spatial potassium buffering) and clearance/recycling of neurotransmitters, have already been unravelled (Kimelberg and Norenberg, 1989). The hypothesis of a functional

role for astrocytes in neurovascular metabolic coupling will be further elaborated below based on relevant literature as well as on some personal data.

Astrocytes and glutamatergic neurotransmission

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system as 80 to 90 % of all cerebral cortical synapses are glutamatergic (Somogyi *et al*., 1998). Glutamate is released from the presynaptic terminal upon depolarisation and acts on a variety of glutamate receptors, both ionotropic and metabotropic that are present on neuronal as well as on astrocytic plasma membranes (Nedergaard *et al*., 1980 ; Seifert and Steinhauser, 2001 ; Schools and Kimelberg, 2001). Glutamate is cleared from the synaptic cleft by sodium (Na^+)/glutamate co-transporters, that are present both on neuronal and astrocytic membranes, to end the excitatory signal and to avoid excitotoxicity (Pellerin and Magistretti, 1994). The stoechiometry of the Na^+/glutamate co-transporter is such that 3 Na^+ ions are being shuttled intracellulary with one glutamate molecule using the electrochemical gradient for Na^+ (Pellerin and Magistretti, 1994). Glutamate in astrocytes is subsequently transformed into glutamine by means of glutamine synthetase activity, is released by astrocytes and taken up again by neurons which will resynthesize it into a glutamate molecule (glutamate recycling) (Sonnewald *et al*., 1997). Glutamatergic axon terminals can also synthesize glutamate from pyruvate as was recently shown (Hassel and Brathe, 2000). Na^+ influx by Na^+/glutamate co-transport, that could lead to a diminished electrochemical gradient for Na^+, is counterbalanced by several Na^+ regulatory mechanisms, including Na^+/potassium (K^+) ATP-ase activity, such that the astrocytic intracellular Na^+ concentration ($[Na^+]_i$) is maintained at about 20 mM (Silver and Erecinska, 1997).

Brain energy metabolism

Under basal physiological conditions the brain relies completely on glucose oxidation as ATP source (Vannucci *et al*., 1997). The different steps in glutamatergic neurotransmission, i.e. establishing a resting potential, firing action potentials, presynaptic calcium (Ca^{++}) influx prior to vesicle release, postsynaptic receptor activation and glutamate recycling, all require energy. Although the weight of the mature brain is only 2-3 % of the total body weight, the brain may require up to one fourth of the total glucose consumed by the body (Wiesinger *et al*., 1997). The major part of energy consumption serves to maintain/restore ionic gradients (Hawkins, 1995). The action of the Na^+/K^+ ATP-ase plays an important role in this process and is responsible for about 80 % of the total ATP consumption. It moves 2 K^+ ions inwards for 3 Na^+ ions outwards with the consumption of 1 ATP molecule. The brain has very little glucose reserve by means of a small amount of glycogen that is mainly stored in astrocytes (Walz and Mukerji, 1988). Glucose delivery to the brain neurons and glial cells across the blood-brain barrier is achieved by the action of the facilitative glucose transporter proteins or GLUTs (Vannucci *et al*., 1997). The primary isoform at the blood-brain barrier is the 55 kD GLUT1 protein. The less glycosylated 45 kD GLUT1 form is linked to glial cells, whereas neurons take up glucose by the GLUT3 isoform. Glucose, metabolized into glucose-6-phosphate by the action of hexokinase, may then enter glycolysis, the pentose phosphate pathway or glycogenogenesis in astrocytes (Wiesinger *et al*., 1997). Entering glycolysis, glucose-6-phosphate is first processed in the cytosol to pyruvate, which is then transported into the mitochondrion to enter the Krebs cycle and to undergo oxidative phosphorylation. Aerobic metabolism of one molecule of glucose thus yields 38 molecules of ATP (2 delivered by glycolysis, the remaining 36 by oxidative phosphorylation). Furthermore, an oxygen-to-glucose ratio of 6 is predicted, based on the stoechiometry of $C_6H_{12}O_6 + 6O_2 \leftrightarrow 6H_2O + 6CO_2$, and under basal physiological conditions the measured value for the oxygen-to-glucose ratio indeed approaches 6 (Shulman *et al*., 2001). However during activation the oxygen-to-glucose ratio decreases indicating that the brain partially relies on anaerobic glycolysis (Hedera *et al*., 1995). This notion is furthermore supported by magnetic resonance spectroscopy studies indicating an increased lactate peak during activation (Prichard, 1991 ; Sappey-Marinier *et al*., 1992) and by measurements of extracellular lactate concentrations with microdialysis (Magistretti and Pellerin, 1999). Increased lactate production has been shown from astrocytes under anaerobic conditions in vitro (Walz and Mukerji, 1988). Anaerobic glycolysis may furthermore explain why during activation local increases in rCBF and glucose consumption are equivalent, whereas increases in oxygen consumption are disproportional smaller (Frahm *et al*., 1997).

A role for astrocytic intracellular sodium concentration in glucose utilization during activation

It has been shown that the increase in $[Na^+]_i$, that is associated with the uptake of glutamate into astrocytes by the Na^+/glutamate co-transporter, stimulates glucose utilization and lactate release from astrocytes (Pellerin and Magistretti, 1994). It was proposed that Na^+ extrusion by the Na^+/K^+ ATP-ase, to maintain the electrochemical gradient for Na^+, drives glycolysis in astrocytes to maintain the astrocytic intracellular ATP concentration

leading to increased uptake of glucose and release of lactate. Lactate can freely cross the astrocytic plasma membrane into the extracellular space and thus become available to neurons (Dringen *et al*., 1993). Lactate may be taken up by neurons by the action of a monocarboxylate transporter to enter oxidative phosphorylation and be used as an energy source (Dienel and Hertz, 2001). Interestingly, astrocytes express the lactate dehydrogenase (LDH) 5 subtype similar to skeletal muscle tissue that is predominantly glycolytic whereas neurons express the LDH1 subtype similar to myocardium that uses lactate as a substrate (Pellerin and Magistretti, 1999). One consequence of this hypothesis is that the signal captured with ^{18}FDG PET would reflect, at least partly, uptake of tracer into astrocytes (Pellerin and Magistretti, 1994).

Astrocytic intercellular calcium waves are associated with intracellular sodium changes

The intracellular Ca^{++} concentration ($[Ca^{++}]_i$) in astrocytes is tightly controlled and acts as a signal influencing several cell processes (Verkhratsky *et al*., 1998). Increases of $[Ca^{++}]_i$ can be brought about either through Ca^{++} influx from the extracellular medium, Ca^{++} release from intracellular stores, reduction of Ca^{++} efflux, or through a combination of these mechanisms. The Ca^{++} signal is terminated by reuptake of Ca^{++} into the intracellular Ca^{++} stores, mainly the endoplasmic reticulum, by means of the sarco-endoplasmic reticulum Ca^{++} ATP-ase (SERCA) pump, and by shuttling excess Ca^{++} to the extracellular space by the action of the plasma membrane Ca^{++} ATP-ase and Na^+/Ca^{++} exchange activity. Intercellular propagating Ca^{++} changes can be induced in astrocytes by a variety of stimuli, including mechanical, electrical and chemical (ATP, glutamate) (Cornell-Bell *et al*., 1990 ; Paemeleire and Leybaert, 2000a). These so called Ca^{++} waves have been demonstrated both in brain slices as well as in astrocytes in culture (Charles *et al*., 1991 ; Basarsky *et al*., 1998). The mechanism of these waves is believed to be mediated by both an extracellular and an intercellular pathway. The extracellular messenger involved is perhaps glutamate or ATP, that may be released by an exocytotic mechanism (Guthrie *et al*., 1999 ; Vesce *et al*., 1999a ; Vesce *et al*., 1999b). The intercellular pathway most likely relies on the intercellular diffusion of the Ca^{++} mobilizing messenger inositoltrisphosphate (IP_3) through gap junctions (Boitano *et al*., 1992 ; Sanderson, 1995). Gap junctions are abundant in astrocytes and allow the intercellular transfer of molecules with an molecular weight up to 1 kD, such as IP_3 (Giaume *et al*., 1997). We have shown that astrocytic intercellular Ca^{++} waves, induced by mechanical stimulation, are associated with significant $[Na^+]_i$ changes using the Na^+-sensitive dye SBFI (Paemeleire and Leybaert, 2000b). These $[Na^+]_i$ changes appear to be secondary to the $[Ca^{++}]_i$ changes. Additional experiments have indicated that the measured changes are due to Na^+ influx from the extracellular compartment. Indirect evidence indicates that the $[Na^+]_i$ changes can at least partly be explained by Na^+/Ca^{++} exchange activity to restore the $[Ca^{++}]_i$ to its resting value.

A new hypothesis to be tested

Combining the data presented above one can hypothesize a role for astrocytic intercellular Ca^{++} waves in cerebral energy metabolism. With certain pathophysiological events conditions are created that can induce astrocytic intercellular Ca^{++} waves such as traumatic brain injury and brain ischemia, leading to either mechanical stimulation of astrocytes, extracellular glutamate release or ATP release (Doberstein *et al*., 1993). Local extracellular glutamate concentrations may be very high thereby overwhelming Na^+/glutamate cotransport and activating glutamate receptors on astrocytes and initiating astrocytic intercellular Ca^{++} waves (Norenberg *et al*., 1997). ATP, acting on astrocytic purinoceptors, and mechanical stimulation are also known to initiate astrocytic intercellular Ca^{++} waves (Charles *et al*., 1991 ; Paemeleire and Leybaert, 2000a). The increase in astrocytic $[Ca^{++}]_i$ may be associated with secondary increases in $[Na^+]_i$ that, according to the scheme presented by Pellerin and Magistretti (1994), may drive glycolysis in astrocytes and increase the release of lactate from these cells (Dringen *et al*., 1993). Lactate may then be used by neurons as the preferred substrate given its high energetic yield (Dienel and Hertz, 2001 ; Vicario *et al*., 1993). Astrocytes may thus function mainly with the small amount of 2 ATP molecules produced by glycolysis of one glucose molecule, while neurons may readily oxidize lactate by oxidative phosphorylation, with a high ATP yield of 36 ATP molecules per 2 molecules of lactate originating from one glucose molecule, to meet their energetic demands. Astrocytic gap junctions are permeable to lactate, which may contribute to its diffusion (Giaume *et al*., 1997 ; Tabernero *et al*., 1996). Furthermore the signal may be passed to more distant cells as astrocytic Ca^{++} waves travel a few 100 µm in culture. Thereby the changes of survival of compromised neuronal tissue may be increased. The hypothesis of lactate release from astrocytes by increased $[Ca^{++}]_i$ is currently being tested.

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