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ORIGINAL PAPER

The Response to Stimulation in Neurons and Astrocytes

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Abstract
The brain uses mainly glucose as fuel with an index of glucose to oxygen utilization close to 6, the maximal index if all glucose was completely oxidized. However, this high oxidative index, contrasts with the metabolic traits of the major cell types in the brain studied in culture, neurons and astrocytes, including the selective use of the malate-aspartate shuttle (MAS) in neurons and the glycerol-phosphate shuttle in astrocytes. Metabolic interactions among these cell types may partly explain the high oxidative index of the brain. In vivo, neuronal activation results in a decrease in the oxygen glucose index, which has been attributed to a stimulation of glycolysis and lactate production in astrocytes in response to glutamate uptake (astrocyte–neuron lactate shuttle, ANLS). Recent findings indicate that this is accompanied with a stimulation of pyruvate formation and astrocyte respiration, indicating that lactate formation is not the only astrocytic response to neuronal activation. ANLS proposes that neurons utilize lactate produced by neighboring astrocytes. Indeed, neurons can use lactate to support an increase in respiration with different workloads, and this depends on the Ca^{2+} activation of MAS. However, whether this activation operates in the brain, particularly at high stimulation conditions, remains to be established.

Keywords Mitochondria · Oxygen consumption · Calcium · Neuron · Astrocyte · Malate-aspartate shuttle

Introduction

The brain consumes mainly glucose and is the major glucose consumer organ of the body. In the resting state, most of this consumption is oxidative, as evidenced from measurements of the stoichiometry between oxygen ($CMRO_2$) and glucose (CMRglc) utilization, or oxygen-glucose index (OGI), which is close to 6:1, the theoretical maximum for the full oxidation of glucose (reviewed in [4, 22]). A submaximal OGI value, of around 5.5–6 is found in animal or human brain at rest, the difference generally attributed to lactate formation and efflux from the brain (reviewed in [22]). However, upon activation, the OGI in the activated brain region drops further. In this short review we will examine the properties of neurons and astrocytes in culture, especially those that have been described recently, relevant to whole brain variations in OGI.

Neurons and Astrocytes: Respiration Matters

The very high oxidative consumption of glucose contrasts with the metabolic properties of the two major cell types in the brain, neurons and astrocytes. Glucose metabolism in neurons is more oxidative than in astrocytes [2, 27, 41]. Glycolysis is less active in neurons and this correlates with lower PFK1 activity than astrocytes, with low levels of the glycolysis regulating enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoform 3 (PFKFB3), and lower

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levels of the glycolysis activator Fructose 2,6-bisphosphate [25, 27]. In addition, neurons have high levels of the two mitochondrial carriers involved in the malate-aspartate shuttle (MAS), the aspartate-glutamate carrier Aralar/AGC1/Slc25a12 (AGC1) and the oxoglutarate-malate carrier OGC/Slc25a11 [60], which allow for a vigorous oxidation of cytosolic NADH in mitochondria and the maintenance of high respiration rate during glucose metabolism [18, 36, 48, 58]. In contrast, glycolysis and lactate production are very active in astrocytes, while oxygen consumption in mitochondria from acutely isolated brain astrocytes is lower than in those isolated from neurons [41]. Although AGC1 protein is present in astrocytes in culture [33, 53] its levels in brain astrocyte mitochondria are much lower than those in neurons, both in brain astrocytes [49], and in Müller cells in the retina [35, 66]. Its loss does not result in any difference in glucose dependent OCR in cultured astrocytes [30], indicating that the malate-aspartate shuttle does not contribute to glucose oxidation in these glial cells. In contrast, astrocytes express the two components of the glycerol-phosphate shuttle (GPS) [30], with activity values as those reported for other tissues or cell types [16, 42]. Earlier work by Nguyen et al. [46] showed that the major isoform of cytosolic glycerophosphate dehydrogenase (GPDH) had very low levels in neurons and astrocytes, suggesting that GPS might be of little importance in these cells. However, the mRNA of a second cytosolic glycerol-P-dehydrogenase (GPD1-like), functionally identified in heart [40, 64], is expressed in neurons and astrocytes [12]. It is possible that the activity of cGPDH found in mouse astrocytes, which matches that of mitochondrial GPDH (45.30 ± 3.69 and 44.00 ± 2.26 µmol NADH/mg/min, respectively, Juaristi et al. [30]) corresponds to this novel isoform. These results suggest GPS in cultured mouse brain astrocytes as main NADH shuttle responsible for the transfer of redox equivalents from cytosolic NADH to mitochondria during glucose utilization and explains the lack of impact of ARALAR deficiency on lactate production and respiration rates in these cells. The finding of a glycerol-phosphate shuttle in astrocytes agrees with the mild but significant effects of inhibitors of this shuttle on the cytosolic NADH/NAD^+ ratios and lactate formation examined by imaging with fluorescent probes in astrocytes [32] and with the reports of McKenna et al. [45].

The use of these two different redox shuttles by neurons and astrocytes adds another layer of complexity to the known interrelations between these two cell types. Indeed, the mitochondrial component of the glycerol-phosphate shuttle, mitochondrial glycerophosphate dehydrogenase, is a powerful source of reactive oxygen species (ROS) in mitochondria, and is activated by calcium from the external face of the inner mitochondrial membrane [1, 63]. The presence of the glycerol-phosphate shuttle in astrocytes may be related to the high ROS production in astrocyte mitochondria as compared to neuronal mitochondria [41]. The high production of mitochondrial ROS has been shown to be associated with the existence of a large proportion of deactive complex I in astrocytes, and to a greater proportion of complex I assembled into supercomplexes in neurons [41, 65]. However, as an extra ROS source, glycerophosphate dehydrogenase may add to the large ROS output in astrocytes. In addition, astrocytes are equipped with a strong redox antioxidant system, mediated by the presence of active Nrf2, a transcription factor activated by ROS that controls the expression of antioxidant genes, whereas the Nrf2 pathway activity is lower in neurons [9, 11].

The very high oxidative consumption of glucose in the brain contrasts with the metabolic properties of pure primary cell cultures studied at low, physiological glucose levels (Table 1). In the case of neurons, the more oxidative cells in the brain, mouse cortical neurons in culture convert into lactate about half of the glucose utilized, the remaining half being used in cell respiration [30], a result at odds with the almost full oxidation of glucose in the brain in vivo. In the case of astrocytes in culture, which are known to be glycolytic cells, glucose consumption is much larger than for neurons and lactate production also accounts for about half of the consumed glucose. However, basal respiration is only one tenth of that expected if the remaining glucose

Table 1 Metabolic characterization of primary cultures of mouse cortical neurons and astrocytes

	Neurons	Astrocytes
Glucose consumed[a]	0.40	1.09
Lactate produced[a]	0.42	1.08
Basal OCR[a]	1.2	0.3
Predicted OCR if half of each glucose molecule was fully oxidized[a]	1.2	3.2
Increase in oligomycin-sensitive OCR upon glutamate/NMDA stimulation	2.3–2.4 (fold)	1.6–1.9 (fold)[b]

Primary cultures from mouse cortical neurons [56] and astrocytes [30, 31] were used after 9 DIV and 14 DIV, respectively for the assay of glucose consumption and lactate production when cultured with 5 mM glucose. Glucose consumption and lactate production were determined in parallel from the changes in glucose and lactate concentration in the cultures during a 12 h interval [30]. Oxygen consumption rate (OCR) in intact neurons and astrocytes incubated with 2.5 mM glucose was studied using Seahorse XF24 Extracellular Flux Analyzer. OCR was stimulated by the addition of 200 µM glutamate (astrocytes) and 100 µM NMDA (neurons). Oligomycin sensitive respiration was determined following 6 µM oligomycin addition and after substraction of non mitochondrial respiration

[a]Data were taken from Juaristi et al. [30, 31] and Rueda et al. [56], and expressed in $\mu moles \times mg^{-1} \times h^{-1}$

[b]The increase upon glutamate stimulation was 1.6 fold. Other stimulants (extracellular ATP) were able to stimulate oligomycin-sensitive OCR even further

was fully oxidized in mitochondria, again at odds with the brain OGI (Table 1).

It may be argued that some glucose utilization and lactate production in neuronal cultures is partially due to contaminating astrocytes present in the cultures, and that the actual OGI in cultured neurons is probably closer to 6. However, this argument cannot be applied to astrocyte cultures, in which respiration is ten times lower than that required for the full oxidation of glucose [30, 31]. A factor to be considered to explain the high lactate production of these brain cells is the large volume of extracellular medium in culture as compared to the brain, which would favor lactate release and dilution in the external medium, thereby enhancing glycolysis.

In the case of astrocytes, these cells in culture are thought to differ from those in brain by becoming more glycolytic, as supported by changes in mRNA levels in rapidly isolated brain cells with respect to cultures [12]. However, a proteomic study in cultured and acutely isolated brain astrocytes revealed that these differences were not so large, and involved mainly increases in proteins from the extracellular matrix in cultured astrocytes [60].

One of the mechanisms that would resolve this paradox is through interactions and exchange reactions between neurons and astrocytes which would allow a complete oxidation of brain glucose. The astrocyte to neuron lactate shuttle, ANLS [50, 51], proposes that neuronal activity results in glutamate release and uptake by astrocytes, which stimulates lactate production in these cells. Astrocytic lactate is then taken up and oxidized by neurons. Additionally, pyruvate produced by neurons may be taken up and oxidized by astrocytes [13]. This transcellular pathway of glucose oxidation would provide an almost complete oxidation of brain glucose and glycogen.

This shuttle is supported by the distribution of specific glucose transporters and plasma membrane pyruvate/lactate transporters in neurons and astrocytes [60] which favor lactate production and efflux from astrocytes and lactate uptake and oxidation to pyruvate in neurons (see [8]). In addition, the malate aspartate NADH shuttle, which is essential for lactate and glucose oxidation by neurons [38] does not play any major role in glucose oxidation in astrocytes, either in basal or in stimulated conditions [30, 31].

Notably, in vivo studies have shown that disruption of astrocytic or neuronal lactate transporters in hippocampus leads to amnesia, suggesting the requirement of ANLS components for memory formation [61]. However, the exact connection between the lactate transporters and the process of memory remains to be established.

On a cellular level, the astrocyte response to neuronal activation has been studied in mixed cultures of astrocytes and neurons each derived from a different species, a procedure allowing to follow the transcriptomic changes in astrocytes that take place after chemically induced neuronal activation [26]. Neuronal stimulation caused upregulation of the transporters involved in ANLS, glucose and glutamate transporters and lactate dehydrogenase (LdhA), suggesting an important influence of neuronal activity on the expression of ANLS. However, these transcriptomic changes are late events which took place after several hours of neuronal activation. Importantly, they were attributed to a transcriptional pathway involving cAMP–PKA–CREB rather than to the action of glutamate on astrocytes. In fact, the ANLS is still a matter of debate ([7, 8, 22]; and references therein).

Astrocyte and Neuronal Energy Metabolism Upon Neuronal Activation

Central to ANLS is the link between neuronal activity and lactate production by astrocytes. According to the prevailing hypothesis, glutamate capture by astrocytes results in an increase in cytosolic Na^+ which drives different ion pumps in the plasma membrane, to restore resting Na^+ levels. The ATP required for this Na^+-dependent workload is obtained by aerobic glycolysis from blood glucose or from endogenous glycogen. The other task carried out by brain astrocytes is the clearance of K^+ from the extracellular space, a process dependent on the activity of the sodium bicarbonate cotransporter NBCe1/Slc4a4 [57].

Glutamate stimulation of glucose consumption and lactate formation in astrocytes was initially reported by Pellerin, Magistretti and coworkers [14, 17, 50] and other groups [39] but not by other laboratories who found that glutamate uptake in astrocytes was not accompanied by increases in lactate production or glucose consumption [21, 28, 34, 52, 62]. The reasons for this discrepancy probably lie on the culture conditions, media and/or glucose concentrations [22]. Recent findings by a number of laboratories in cultured astrocytes, indicate that upon exposure to glutamate, both K^+ and glutamate stimulate glycolysis and lactate formation [10, 23], pyruvate production [31] and an increase in the NADH/NAD^+ ratio [32] as determined with genetically coded sensors.

Another important consideration is whether this response to glutamate is accompanied by an increase in OXPHOS or not. In the case of K^+, OXPHOS does not participate in the response as K^+ does not stimulate astrocyte respiration; this is due to a variant of the classical Crabtree effect [23]. The uptake of bicarbonate through NBCe1/Slc4a4 which follows astrocyte K^+-depolarization, causes cytosolic alkalinization and stimulation of glycolysis to increase ATP beyond its use by the Na^+ pump, explaining the lack of a respiratory response to K^+ [23]. However, in contrast to previous observations [5] OXPHOS does participate in the response to glutamate [31, 54]. The stimulation of astrocyte respiration by

glutamate (about 1.6 fold, Table 1) responds to the strictly Na^+-dependent workload caused by glutamate uptake, and does not depend on the Ca^{2+} signals elicited by glutamate in these cells [31]. It is accompanied by an increase in glycolytic pyruvate production, indicating that the response to glutamate is a stimulation of glycolysis with pyruvate production, and also oxidation in mitochondria. In addition, some of the glutamate taken up is also oxidized in astrocyte mitochondria [31] as proposed previously [29], contributing to the increase in astrocyte respiration. Interestingly, when faced to the double challenge of K^+ and glutamate removal, respiratory stimulation persists, suggesting that in the in vivo situation astrocytes possibly respond to neuronal stimuli by increasing respiration [31] and, depending on the stimulation conditions, with lactate production.

It may be argued that the fact that astrocytes produce lactate and also obtain ATP from OXPHOS upon neuronal stimulation is not an objection to ANLS. However, recent findings from the field of neuronal metabolism, particularly during strong activation conditions, question aspects of the ANLS hypothesis.

Neuronal activation is associated with the workloads involved in the return to the resting state, a mostly postsynaptic activity [3]. The source of energy to obtain the required ATP is largely OXPHOS, as neurons are able to upregulate their own respiration in order to match ATP consumption [36, 37, 55] (Table 1). Interestingly, work in cortical neurons in culture has shown that the upregulation of OXPHOS is Ca^{2+} dependent, not only because part of the workload is also Ca^{2+} dependent, but due to a prominent role of Ca^{2+} in boosting the respiratory response. In the case of neurons, Ca^{2+} activation of the malate-aspartate shuttle (MAS), plays an important role in stimulation of respiration, and the effect of Ca^{2+} is thought to be due to Ca^{2+} binding to EF-hand Ca^{2+} binding motifs of the mitochondrial aspartate/glutamate carrier, AGC1, which face the intermembrane space [44, 47]. Indeed, the AGC1 catalyses the exchange of glutamate plus a proton against aspartate, so that the overall reaction is electrogenic. This makes this step irreversible in polarized mitochondria, a condition favored for a controlling step, and drives the entire MAS in the direction of redox equivalent transfer into mitochondria.

An important consequence of Ca^{2+} activation of AGC1-MAS is the supply of pyruvate to mitochondria [24]. In fact, the supply of exogenous pyruvate fully reverts the limited stimulation of respiration in response to different workloads observed in AGC1-deficient neurons [36, 55], suggesting that pyruvate supply controls neuronal respiration either on glucose or lactate [38]. The affinity for Ca^{2+} of AGC1-MAS is about 300 nM [15, 24], a value lower than the apparent affinity for Ca^{2+} of the mitochondrial Ca^{2+} uniporter (MCU), another Ca^{2+} target in mitochondria, and probably the major one in AGC1-deficient neurons.

In sum, the strong stimulation of respiration in cultured neurons and their ability to use external lactate via MAS for that purpose support ANLS, at least under basal and mild activation conditions. However, recent findings by Yellen's lab have questioned the notion that in vivo activated neurons take up lactate from the extracellular medium [19, 20]. In fact, using biosensors expressed in neurons, it was shown that synaptic (electrical) stimulation of hippocampal dentate granule neurons or the whisker stimulation of neurons in layers II/III of the primary somatosensory barrel cortex of an awake mouse was accompanied with rapid increases in cytosolic NADH and, in dentate gyrus neurons, also with increases in cytosolic lactate. Importantly, the use of the specific MCT1 and MCT2 inhibitor AR-C155858 which prevented the uptake of lactate from the extracellular medium in hippocampal slices, did not prevent the stimulation-induced increase in lactate levels. This indicated that it arose from an increase in glycolysis and lactate formation in the stimulated neuron itself [19] although it was somewhat surprising that lactate over-accumulation in the presence of AR-C155858 was not observed. It would be interesting to know whether this presumably neuronal lactate production is maintained or varies depending on the stimulation conditions.

Regardless of that, as a standing lactate gradient exists, with higher lactate concentration in astrocytes than neurons [43], neuronal lactate may be extruded to nearby neurons with lower lactate concentrations or to the extracellular space. In fact, claims for the possible formation of lactate by neurons under strong stimulation conditions are not new. Indeed, studies in isolated brain mitochondria indicated that high calcium loads, below those required to induce the permeability transition, cause an inhibition of the malate aspartate shuttle which is expected to slow down glycolysis, and increase lactate and NADH formation [16]. This effect was attributed to an inhibition of the second transporter of the malate aspartate shuttle, the oxoglutarate-malate carrier (OGC/Slc25a11). Inhibition would be due to competition between oxoglutarate dehydrogenase and the OGC for the common substrate oxoglutarate (OG). Activation by matrix Ca^{2+} of oxoglutarate dehydrogenase lowers its K_m for OG with a drop in matrix OG concentration resulting in lower OG efflux along OGC and a resulting drop in MAS activity. An inhibition of MAS under strong stimulation conditions has also been proposed in cultured neurons [6, 59], but Díaz-García et al. [19] did not find evidence for an inhibition of the shuttle. Whether this mechanism of shuttle inhibition actually operates in vivo is still an open question.

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