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REVIEW

A metabolic switch in brain: glucose and lactate metabolism modulation by ascorbic acid

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Abstract

In this review, we discuss a novel function of ascorbic acid in brain energetics. It has been proposed that during glutamatergic synaptic activity neurons preferably consume lactate released from glia. The key to this energetic coupling is the metabolic activation that occurs in astrocytes by glutamate and an increase in extracellular [K^+]. Neurons are cells well equipped to consume glucose because they express glucose transporters and glycolytic and tricarboxylic acid cycle enzymes. Moreover, neuronal cells express monocarboxylate transporters and lactate dehydrogenase isoenzyme 1, which is inhibited by pyruvate. As glycolysis produces an increase in pyruvate concentration and a decrease in NAD^+/NADH, lactate and glucose consumption are not viable at the same time. In this context, we discuss ascorbic acid participation as a metabolic switch modulating neuronal metabolism between rest and activation periods. Ascorbic acid is highly concentrated in CNS. Glutamate stimulates ascorbic acid release from astrocytes. Ascorbic acid entry into neurons and within the cell can inhibit glucose consumption and stimulate lactate transport. For this switch to occur, an ascorbic acid flow is necessary between astrocytes and neurons, which is driven by neural activity and is part of vitamin C recycling. Here, we review the role of glucose and lactate as metabolic substrates and the modulation of neuronal metabolism by ascorbic acid.

Keywords: astrocyte–neuron lactate shuttle hypothesis, glucose transporter, monocarboxylate transporter, sodium–vitamin C transporter.

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Introduction: metabolic sources for brain energy metabolism

The mammalian brain is an expensive organ in energetic terms. While comprising only 2% of the body mass in an adult human, it uses 25% of the total (resting) corporal energy consumption (Siesjèo 1978). Most of the energy consumed in the brain is attributable to restoration of the membrane resting potential following neuronal depolarization. This is accomplished by the Na^+/K^+-ATPase (Attwell and Laughlin 2001). Neuronal activity accounts for 80% of brain energy consumption (Sibson *et al.* 1998; Rothman *et al.* 1999). Other energy-consuming processes are neurotransmitter recycling and axonal and dendritic transport (Ames 2000).

Glucose is an essential energy source for the adult human brain. Normal blood glucose levels are 5 mM. With slightly lower blood glucose levels, 2–3 mM, there is some cognitive impairment, and with levels lower than 1 mM, mental confusion is evident (Cox *et al.* 2002; Dwyer 2002). There are two barriers that protect the brain from sudden changes in the concentration of blood metabolites: the blood–brain

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Abbreviations used: 4-CIN, alpha-cyano-4-hydroxycinnamate; ANLSH, astrocyte–neuron lactate shuttle hypothesis; BBB, blood–brain barrier; DOG, deoxyglucose; EPSC, excitatory post-synaptic current; GLUT, glucose transporter; HK, hexokinase; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; OMG, *O*-methylglucose; PK, pyruvate kinase; PPP, pentose-phosphate pathway; SGLT, sodium–glucose transporter; SLC2, solute carrier 2; SMCT, sodium-coupled monocarboxylate transporter; SVCT, sodium–vitamin C transporter; TCA, tricarboxylic acid.

barrier (BBB; Huber *et al.* 2001) and the blood–CSF barrier (choroid plexus epithelium; Zheng and Chodobski 2005). Normally, glucose enters the brain through the BBB because these cells possess facilitative glucose transporters, GLUTs (see Glucose and glucose transporters in brain section; Agus *et al.* 1997; Boado and Pardridge 1994; Nualart *et al.* 1999). Glucose transport through the BBB is very stable. Neither acute hyperglycemia nor hyperinsulinemia affect glucose transport across the BBB. Nor is cerebral glucose consumption affected by these conditions (Hasselbalch *et al.* 1999, 2005). However, under starvation conditions, the BBB permeability may be altered and other metabolic substrates may enter the brain in substantial quantities. In this case, ketone bodies concentration increases in blood and there is also an increased expression of monocarboxylate transporters, MCT (that transport ketone bodies, lactate, and pyruvate) in cells forming the BBB (Hasselbalch *et al.* 1995).

The brain is a complex organ composed of a variety of cells, each having different metabolic needs. Neurons, astrocytes, oligodendrocytes, and capillary endothelial cells have a high rate of oxidative metabolism (Hertz 2008). Every brain cell can use glucose because all of them possess GLUTs and glycolytic and tricarboxylic acid (TCA) cycle enzymes (see Glucose and glucose transporters in brain section). Some glycolytic intermediaries are transported through astrocytic gap junctions (Giaume *et al.* 1997; Rouach *et al.* 2009). Moreover, astrocytes are able to synthesize glycogen from glucose. Glycogen can be used for energy production (glycogen degradation to glucose and glucose oxidation via glycolysis and TCA cycle) and for pyruvate carboxylation to oxalacetate catalyzed by pyruvate carboxylase (Ibrahim 1975; Hamprecht *et al.* 2005; Hertz 2008). Although the longstanding dogma of the brain as an organ relying solely on glucose for energy production is still largely valid to this day, a debate has begun regarding whether neuronal oxidative metabolism relies on astrocyte-derived lactate (the astrocyte–neuron lactate shuttle hypothesis; ANLSH) rather than on glucose during neurotransmission activity (Pellerin and Magistretti 1994). This hypothesis is controversial and has not yet been fully accepted. Our group has published some data suggesting that ascorbic acid can function as a neuronal metabolism modulator (Castro *et al.* 2007, 2008). Ascorbic acid, the reduced form of vitamin C, is very highly concentrated in CNS (Hornig 1975; Kratzing *et al.* 1982) and functions as an antioxidant, reducing reactive oxygen species to stable molecules (Padh 1990; Rose and Bode 1993; Sauberlich 1994). Glutamate is able to stimulate ascorbic acid release from astrocytes (Wilson *et al.* 2000; Castro *et al.* 2007) and neurons can take up ascorbic acid because they possess sodium–vitamin C transporters (SVCTs) (Castro *et al.* 2001). Intracellular ascorbic acid inhibits glucose use and stimulates lactate uptake by neurons (Castro *et al.* 2007, 2008). Therefore, ascorbic acid entry to neurons can change the metabolic substrates used in these cells.

In this review, we discuss the role of glucose- and lactate-like metabolic substrates and the modulation of neuronal metabolism by ascorbic acid. We review the expression and use of glucose and lactate for neurons and astrocytes. Further, we consider ascorbic acid transporters and ascorbic acid homeostasis in CNS. And finally, we discuss the idea of a metabolic switch driven by changes of ascorbic acid concentration in brain.

Glucose use and glucose transporters in brain

The transport of glucose and other hexoses into most mammalian cells is mediated by the solute carrier 2 (SLC2) family, which comprises 14 transport proteins of molecular sizes ranging from 45 to 55 kDa (Mueckler 1994; Doege *et al.* 2000a,b, 2001; Phay *et al.* 2000; Joost and Thorens 2001; Lisinski *et al.* 2001; McVie-Wylie *et al.* 2001; Uldry *et al.* 2001; Ibberson *et al.* 2000; Joost *et al.* 2002; Rogers *et al.* 2002; Wu and Freeze 2002). Glucose transport catalyzed by GLUTs is stereospecific for D-glucose, and it is passive and is driven by a glucose concentration gradient. GLUTs are expressed in mammalian cells, including brain cells (Table 1). GLUT1 is ubiquitously expressed and it is responsible for providing basal glucose to different tissues and cells. In brain, GLUT1 exists in two different molecular weight forms: 45 and 55 kDa. Both forms are similar in their protein structure and kinetic characteristics (Birnbaum *et al.* 1986). The different extent of glycosylation explains the difference in their relative molecular weights (Birnbaum *et al.* 1986). Expression of the 55 kDa GLUT1 is prominent in endothelial cells from BBB (Agus *et al.* 1997; Boado and Pardridge 1994; Bolz *et al.* 1996; Nualart *et al.* 1999). Expression of the 45 kDa GLUT1 occurs in epithelial cells from choroid plexus, ependymal cells, and glial cells, with high expression in astrocytic endfeet surrounding endothelial cells (Simpson *et al.* 1994; Nualart *et al.* 1999; Silva-Alvarez *et al.* 2005). The 45 kDa GLUT1 is poorly expressed in neurons *in vivo*, but under stress and *in vitro* conditions, its expression is increased (Lee and Bondy 1993; Maher 1995; Vannucci *et al.* 1998). GLUT2 is a low-affinity transporter and has very limited localization in brain. GLUT2 is present in the embryonic granule layer of cerebellum (Nualart *et al.* 1999) and in ependymal hypothalamic cells (García *et al.* 2003). GLUT3 is the predominant isoform expressed in neurons (Maher *et al.* 1992). It has a high apparent affinity and catalytic-center activity (‘turnover number’; Maher *et al.* 1996; Rauch *et al.* 2006). These characteristics could be an appropriate adaptation to meet the demands of neuronal metabolism at extracellular brain glucose concentrations (2–3 mM). GLUT4 is poorly expressed in hippocampus and cerebellum (Choeiri *et al.* 2002), while GLUT5, a fructose transporter, has been described in embryonic cerebellum (Nualart *et al.* 1999). The mRNA of GLUT6 has been localized in brain (Doege

Table 1 Glucose transporter expression in brain

Transporter	Tisular expression	Function	Reference
55 kDa GLUT1	Endothelial cells from BBB	Providing basal glucose	Agus *et al.* (1997); Boado and Pardridge (1994); Bolz *et al.*, (1996); Nualart *et al.* (1999)
45 kDa GLUT1	Epithelial cells from choroid plexus, ependymal cells, glial cells, and astrocytic endfeet surrounding endothelial cells	Providing basal glucose Entry of glucose to the brain	Nualart *et al.* (1999); Silva-Alvarez *et al.* (2005); Simpson *et al.* (1994)
GLUT2	Embryonic granule layer of cerebellum and in ependymal hypothalamic cells	Glucose *sensing*	Nualart *et al.* (1999); García *et al.* (2003)
GLUT3	Predominant isoform expressed in neurons	Neuronal support of glucose	Maher *et al.* (1992); Aller *et al.* (1997)
GLUT4	Poorly expressed in hippocampus and cerebellum		Choeiri *et al.*, (2002)
GLUT5	Embryonic cerebellum		Nualart *et al.* (1999)
GLUT6	Only mRNA of GLUT6 has been localized in brain		Doege *et al.* (2000b); Godoy *et al.* (2006)
GLUT8	Some neurons from hippocampus and other brain areas		Ibberson et al. (2000, 2002); Lisinski *et al.* (2001)

GLUT, glucose transporter; BBB, blood–brain barrier.

et al. 2000b), but not the protein (Godoy *et al.* 2006). GLUT8 is highly expressed in neurons from hippocampus and other brain areas. This isoform is predominantly localized intracellularly because it has a dileucine sequence similar to GLUT4 (Lisinski *et al.* 2001; Ibberson *et al.* 2002). However, in neurons, neither insulin nor depolarization treatments are able to stimulate GLUT8 translocation to plasma membrane (Shin *et al.* 2004). Finally, the expression product from gene SLC2A13 is localized in brain. However, the so-called H(+)-myo-inositol transporter has no affinity for glucose (Uldry *et al.* 2001).

There is another system for cellular glucose transport: the sodium–glucose co-transporters (SGLTs). There are five members in the SGLT family (SGLT1–5) and six related sequences are known (Kong *et al.* 1993; Mackenzie *et al.* 1994; Wright *et al.* 1994; You *et al.* 1995). Immunodetection of SGLT1 was demonstrated at the BBB and was shown to be up-regulated after brain ischemia and reperfusion (Elfeber *et al.* 2004). The mRNA encoding for SGLT2 was also shown to be present and enriched in isolated rat brain microvessels (Enerson and Drewes 2006) and functionality for SGLTs in BBB was demonstrated by Vemula *et al.* (2008). In addition, SGLT1, SGLT3a, and SGLT3b mRNAs were detected in hypothalamic cultured neurons (O'Malley *et al.* 2006). It has been suggested that these transporter types may have some relation to glucose sensing by hypothalamic glucose-excited neurons (O'Malley *et al.* 2006; González *et al.* 2009).

Metabolism of glucose is tightly regulated to generate ATP and to provide carbon for biosynthetic reactions in conjunction with local functional brain activities. Sensory, motor and cognitive activities all consume ATP. The glycolytic pathway and TCA cycle are present in all brain cells (Lowry and Passonneau 1964; Goldberg *et al.* 1966). Thus, to obtain ATP all brain cells metabolize glucose through glycolysis and the TCA cycle. Control of glycolytic flux is carried out by key enzymes: hexokinase (HK, EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11), and pyruvate kinase (PK, EC 2.7.1.40; Lowry and Passonneau 1964). Type-I HK is the predominant isoenzyme in brain (Siesjèo 1978). HK expression has been determined in neurons, astrocytes, choroid plexus cells, and endothelial cells from BBB (Wilkin and Wilson 1977; Wilson 1995). All three isotypes of phosphofructokinase (muscle [M], liver [L], and brain [B]) have been observed by immunohistochemical analysis in neurons and astrocytes. The three isoenzymes differ in their allosteric properties and in their distribution in different brain cells (Zeitschel *et al.* 1996). These differences might be of importance for regulation of brain glycolysis in the different cellular compartments of the brain. PK is expressed in neurons and astrocytes (Gali *et al.* 1981). But apparently, in brain, the reaction catalyzed by PK does not appear to be a major control point in glycolysis (Lowry and Passonneau 1964). Another metabolic pathway for glucose oxidation is the pentose-phosphate pathway (PPP). In post-mitotic cells such as neurons, most PPP-derived NADPH is mainly used as a cofactor for the regenerating pathway of antioxidant glutathione (GSH), which is reduced from its oxidized form (GSSG) through a reaction catalyzed by glutathione reductase (Kletzien *et al.* 1994). The antioxidant role of the PPP has been demonstrated in neurons (Ben-Yoseph *et al.* 1996) and in astrocytes (Kussmaul *et al.* 1999). In the last years, it has been demonstrated that nitric oxide activates PPP suggesting that nitric oxide exerts a fine control of neuronal energy metabolism by fine-tuning the balance of glucose-6-phosphate consumption between glycolysis and PPP (Bolaños *et al.* 2008). Finally, glucose can be stored in the form of glycogen. Most, if not all, of the brain glycogen is localized in astrocytes (Ibrahim 1975; Vilchez *et al.* 2007). Glycogen

is the brain's main energy reserve. However, this store may only sustain brain metabolic turnover for a few minutes at most (McKenna *et al.* 2006). Thus, a sole role for glycogen during hypoglycemia is unlikely.

Glucose metabolism in the brain is closely linked to neuronal activity. Deoxyglucose (DOG) autoradiographic methods, functional magnetic resonance imaging and positron emission tomography have demonstrated that an increase in local glucose consumption is related to an increase in functional activity (Sokoloff *et al.* 1977; Giove *et al.* 2003). However, the limitations on spatial resolution of these techniques do not allow identification of individual cells or those cell types responsible for glucose consumption.

Lactate use and lactate transporters in brain

Monocarboxylates play an important role in the metabolism of all cells. Among them, lactate is perhaps the most remarkable. Normally, when the glycolysis rate exceeds the rate of triose entry into the TCA cycle, pyruvate can be reduced to lactate by lactate dehydrogenase (LDH, EC 1.1.1.27). Lactate must be released because local lactate accumulation would otherwise become an opposing driving force that would influence many reversible NAD^+/NADH-coupled redox reactions. Some tissues, like heart, can use lactate as source of energy (Baker *et al.* 1998). In these tissues, lactate is transported into the cell and is oxidized to pyruvate in a reversible reaction catalyzed by LDH.

The transport of lactate is accomplished by the SLC16 family of MCTs. There are 14 members (MCT1–14) in this family. Data from kinetic analyses are known only for MCT1–4 (Bröer *et al.* 1997; Bröer *et al.* 1998; Grollman *et al.* 2000; Manning Fox *et al.* 2000). Several isoforms of MCTs have been described in brain (Table 2). MCT1 is highly expressed during early postnatal development in all cell types from brain (Leino *et al.* 1999; Hanu *et al.* 2000; Pierre *et al.* 2000; Pellerin *et al.* 2005). In adult brain, MCT1 expression decreases in neurons and it increases in astrocytes (Vannucci and Simpson 2003). Furthermore, MCT1 is expressed in endothelial cells from BBB, choroid plexus cells, and ependymal cells (Koehler-Stec *et al.* 1998). MCT2 is a transporter with greater affinity for lactate than MCT1, 3, and 4. MCT2 is mainly localized in neurons and their expression increases with neuronal maturity and synaptogenesis (Bergersen *et al.* 2005). Indeed, it has been observed that MCT2 expression in neuronal cells is increased in the presence of some neurotransmitters (e.g. norepinephrine; Chenal and Pellerin 2007). MCT3 expression is restricted to choroid plexus and retinal pigment epithelia (Philp *et al.* 2001). MCT4 has been extensively characterized in muscle and testis where it would be involved with lactate efflux (Wilson *et al.* 1998; Dimmer *et al.* 2000; Brauchi *et al.* 2005). In brain, MCT4 is localized only in astrocytes (Pellerin *et al.* 2005). Expression of MCT5–7, 11, and 14 have been described in brain but the affinity for lactate of these isoforms is not known (Halestrap and Meredith 2004). Finally, MCT8 is localized in brain, but this isoform is a thyroid hormone transporter (Friesema *et al.* 2003).

The sodium-coupled monocarboxylate transporters (SMCTs) are another recently identified monocarboxylate transport system (Li *et al.* 2003; Ganapathy *et al.* 2008). These transporters are from the SLC5 family (sodium co-transporters family), which is the same gene family as SGLTs (see above; Hediger 2004). Only two members of this family are SMCTs: SMCT1 (product of gene SLC5A8) and SMCT2 (product of gene SLC5A12; Ganapathy *et al.* 2008). Both SMCT isoforms are present in brain. Like MCT2, SMCT1 is a high-affinity transporter (Miyauchi *et al.* 2004) and is specifically expressed in neurons (Martin *et al.* 2006). In contrast, the low-affinity SMCT2 is expressed only in astrocytes (Martin *et al.* 2007). The physiological role of this transporter in astrocytes remains to be established.

Table 2 Monocarboxylate transporter expression in brain

Transporter	Tisular expression	Function	Reference
MCT1	BBB endothelial cells, choroid plexus cells, and ependymal cells. Highly expressed in every brain cell types during early postnatal development. In adult brain is highly expressed in astrocytes	Lactate entry into the brain	Koehler-Stec *et al.* (1998); Hanu *et al.* (2000); Leino *et al.* (1999); Pellerin *et al.* (2005); Pierre *et al.* (2000); Vannucci and Simpson (2003)
MCT2	Neurons, astrocytic endfeet	Lactate uptake in neurons	Bergersen *et al.* (2005); Bröer *et al.* (1997); Hanu *et al.* 2000; Pellerin *et al.* (2005); Pierre and Pellerin, (2005)
MCT3	Choroid plexus and retinal pigment epithelia	Lactate entry into the brain	Philp *et al.* (2001)
MCT4	Astrocytes	Lactate efflux	Pellerin *et al.* (2005)
SMCT1	Neurons		Martin *et al.* (2006)
SMCT2	Astrocytes		Martin *et al.* (2007)

MCT, monocarboxylate transporter; SMCT, sodium-coupled monocarboxylate transporter; BBB, blood–brain barrier.

Although BBB cells express MCT1, monocarboxylates cross the BBB with poor efficiency under physiological conditions. Only under extreme conditions, like starvation or prolonged exercise, could these compounds be important exogenous sources of metabolic fuel for the brain (Hasselbalch *et al.* 1995; Dalsgaard 2006; Quirstoff *et al.* 2008). Therefore, such substrates could replace glucose if produced locally. The interconversion of lactate and pyruvate and vice versa is catalyzed by the enzyme LDH. Two distinct subunits combine to form the five tetrameric isoenzymes of LDH. The LDH-5 subunit (muscle type, also named A or M subunit) has higher maximal velocity (V_{max}) and is present in glycolytic tissues, favoring the formation of lactate from pyruvate. While, the LDH-1 subunit (heart type, also named B or H subunit) favors the reaction towards the production of pyruvate. The different catalytic properties of the five isoenzymes of LDH (H4 or LDH-1, H3M or LDH-2, H2M2 or LDH-3, HM3 or LDH-4, and M4 or LDH-5) are in proportion to the ratio of LDH-5 to LDH-1 subunits (Cahn *et al.* 1962; Bishop *et al.* 1972; Markert 1984). It has been demonstrated that neurons contain predominantly LDH-1, while astrocytes expressed LDH-5 (Tholey *et al.* 1981; Bittar *et al.* 1996). This evidence supports the idea that energy metabolism differs between neurons and glia. Indeed, lactate synthesized within astrocytes and released into the interstitial space in brain may serve as energy fuel for neurons (Izumi *et al.* 1997; Waagepetersen *et al.* 1998). Astrocytes release lactate at a greater rate than neurons (Walz and Mukerji 1988) and lactate is preferentially metabolized in neural cells (Bouzier-Sore *et al.* 2003; Brown *et al.* 2001; Itoh *et al.* 2003; Izumi *et al.* 1997; Schurr 2006; Tekkok *et al.* 2005; Waagepetersen *et al.* 1998).

Lactate use during brain activation

For many years, glucose has been thought to be almost the sole metabolic fuel for brain cells. In the last 15 years, this view has changed. Attention has been focused on the role of monocarboxylates in supporting brain activity, with the ANLSH (Magistretti *et al.* 1999; Pellerin *et al.* 2007; Pellerin 2008) providing a new view of brain energetics. The ANLSH proposes that under resting conditions, neurons consume glucose, while during synaptic activity, they preferentially consume lactate.

Glutamate is the major excitatory neurotransmitter in the CNS. To ensure an adequate synaptic transmission and to prevent excitoxicity events it is necessary to maintain a low glutamate concentration in the extracellular space. Therefore, glutamate released from pre-synaptic terminals must be removed from the synaptic space. In a healthy brain, toxic concentrations of glutamate are prevented because of the presence of excitatory amino acid transporters that take up glutamate (Choi 1988). In astrocytes, glutamate is converted to glutamine via glutamine synthetase (EC 6.3.1.2). Glutamine is released into the extracellular space and is taken up by adjacent neurons which synthesize glutamate from glutamine in a reaction catalyzed by glutaminase (EC 3.5.1.2). However, it has been reported that some glutamine is used as energy substrate, being completely oxidatively degraded (Peng *et al.* 2007). This recycling of glutamate is named the glutamate–glutamine cycle (Sibson *et al.* 1997; Maciejewski and Rothman 2008) and it drives changes in neuronal metabolism between resting and activity periods and energetic coupling between neurons and astrocytes (Shen *et al.* 1999). Under glutamatergic synaptic activity, glucose provided by blood is taken up by astrocytes and oxidized predominantly to lactate. It has been reported that glutamate uptake by these cells stimulates GLUT1-mediated glucose transport (Pellerin and Magistretti 1994; Loaiza *et al.* 2003; Porras *et al.* 2008) and glycolysis followed by the release of lactate (Pellerin and Magistretti 1994; Demestre *et al.* 1997). It has also been reported that glutamate is able to stimulate lactate production from glucose uptake and coupled to neuronal activity via α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor activation in cerebellum (Caesar *et al.* 2008). Metabolic astrocyte activation is a key step in metabolic coupling between brain cells. Astrocytic energy demand is also stimulated by glutamate uptake and by extracellular $[K^+]$ increase and glutamate uptake stimulates Na^+,K^+-ATPase (EC 3.6.1.3) activity (Pellerin and Magistretti 1994; Gegelashvili *et al.* 2007). In the same way, elevated extracellular $[K^+]$ activates the Na^+,K^+-ATPase (Grisar *et al.* 1979; Hajek *et al.* 1996) and reduces ATP content (Schousboe *et al.* 1970) in astrocytes, but not in neurons. An increased extracellular $[K^+]$ stimulates DOG phosphorylation (Hof *et al.* 1988) and lactate formation and its release (Walz and Mukerji 1988) in cultured astrocytes. Lactate released from glia is taken up by neurons, where the carbon skeleton is degraded to CO_2 and water (Izumi *et al.* 1997; Schurr *et al.* 1999; Magistretti *et al.* 1999; Pellerin *et al.* 2007). The flow of lactate from astrocytes to neurons would be possible from the differential expression of MCTs isoforms and LDHs isoenzymes in neurons and astrocytes (see above, Bittar *et al.* 1996; Pellerin *et al.* 2005; Pierre *et al.* 2002; Tholey *et al.* 1981). Likewise, lactate flow between neuronal and glial cells can be explained because astrocytes have low expression of aspartate/glutamate carriers (Berkich *et al.* 2007). Because aspartate/glutamate carriers are required for malate/aspartate shuttle (and for cytosolic NADH reoxidation) it is possible that astrocytes mainly catalyze glycolytic conversion of glucose to lactate. However, the use of neuronal lactate has been strongly debated by studies that support the idea that glucose is the main metabolic substrate for these cells (Chih *et al.* 2001; Chih and Roberts 2003; Dienel and Hertz 2005; Dienel and Cruz 2004; Gjedde 2002; Hertz 2004; Hertz *et al.* 2007). Indeed, neuronal cells express the high-affinity transporter GLUT3 (Maher *et al.* 1992), which surface expression is mediated by

the activation of AMP-activated protein kinase (Weisova *et al.* 2009). Moreover, all brain cells express glycolytic enzymes and the K_m of cerebral type-I HK (0.4 mM; Lowry and Passonneau 1964) is one order of magnitude lower than cerebral glucose concentration (2–3 mM; Silver and Erecińska 1994). Increasing the glycolytic activity inhibits the oxidation of lactate to pyruvate. The activation of glycolysis produces an increase in the pyruvate and proton concentration accompanied by a decrease in the NAD^+/NADH ratio. In this way, neuronal LDH-1 should catalyze lactate formation from pyruvate, rather than oxidation of lactate to pyruvate. Therefore, the use of lactate by neurons would only be possible if the consumption of glucose, either at the transport or glycolysis stage, were inhibited.

To date, three possible mechanisms have been proposed for glucose consumption inhibition in neurons under glutamatergic synaptic activity. They are not in conflict with each other; on the contrary, they can coexist and be complementary. This point will be discussed in The ascorbic acid metabolic switch section.

Using single cell time laps confocal microscopy in neuron-astrocyte co-cultures, Barros and colleagues have demonstrated that glutamate is able to stimulate glucose transport in astrocytes (Loaiza *et al.* 2003; Porras *et al.* 2008) and to inhibit glucose transport in neurons (Porras *et al.* 2004). Porras *et al.* (2008) demonstrated that calcium and sodium signaling are necessary for metabolic astrocytic activation. This may explain why glucose metabolism is strongly activated by glutamate but not by GABA. During glutamatergic activity, calcium and sodium propagation (calcium and sodium waves) occurs between neighboring astrocytes (Bernardinelli *et al.* 2004; Charles 2005). These waves drive the metabolic activation in astrocytic syncytium (Bernardinelli *et al.* 2004; Porras *et al.* 2008). In fact, the traffic of metabolites across astrocytic gap junction (Rouach *et al.* 2009) or between cells from the molecular layer of cerebellum (Barros *et al.* 2009) occurs and it may be responsible to sustain synaptic activity in neighbor neurons (Rouach *et al.* 2009).

The second mechanism is explained by the redox switch/redox coupling hypothesis (Cerdán *et al.* 2006; Ramírez *et al.* 2007). This mechanism is based on the assumption of two different pools of pyruvate: one thought to operate in exchange with extracellular lactate or pyruvate and the other derived from glycolytic activity. This mechanism considers a lactate/pyruvate redox shuttle, which is able to transfer lactate from astrocytes to neurons. Then, high cytosolic lactate inhibits neuronal glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) step by competition with cytosolic NAD^+, favoring the oxidation of extracellular lactate.

Finally, in our laboratory, we have demonstrated that ascorbic acid, which is highly concentrated in the CNS (Kratzing *et al.* 1982; Rose and Bode 1993), inhibits glucose consumption in neurons during glutamatergic synaptic activity (Castro *et al.* 2007) and is also able to stimulate lactate uptake in neuronal cultures (Castro *et al.* 2008). Because brain extracellular concentration of glucose fluctuates according to synaptic activation (Basse-Tomusk and Rebec 1991; Ghasemzadeh *et al.* 1991; O'Neill *et al.* 1984; Yusa 2001), ascorbic acid may function as a metabolic switch, permitting lactate consumption and inhibiting glucose consumption under activity conditions. Later in this review, we will refer to the roles of ascorbic acid in CNS, their homeostasis, and transport between nervous cells and ascorbic acid properties as a brain metabolism modulator.

Ascorbic acid and ascorbic acid transporters in brain

Vitamin C is synthesized as ascorbic acid in plants and animals. In most mammals, ascorbic acid synthesis occurs in the liver, with the formation of intermediaries as acid D-glucuronic acid and L-gulono-γ-lactone. However, humans, other primates, and guinea pigs are unable to synthesize ascorbic acid, because these animals do not express L-gulono-γ-lactone oxidase (EC 1.1.3.8). L-Gulono-γ-lactone oxidase catalyses the oxidation of L-gulono-γ-lactone to L-ascorbic acid (Kiuchi *et al.* 1982; Grünewald 1993). In these species, the expression of a transport system in small intestine is very important because it permits absorption of ascorbic acid from the diet (Siliprandi *et al.* 1979). In aqueous solutions, ascorbic acid is a powerful reductant and is oxidized to dehydroascorbic acid. The regeneration of ascorbic acid from dehydroascorbic acid is not spontaneous. This reduction is an enzymatically catalyzed reaction, which may be glutathione-dependent reaction (Park and Levine 1996; Himmelreich *et al.* 1998; Ishikawa *et al.* 1998) or -independent (Guaiquil *et al.* 1997; May *et al.* 1998; Savini *et al.* 2000). Ascorbic acid is an important enzyme cofactor in the biosynthesis of collagen, carnitine, and catecholamines (Park and Levine 1996; Himmelreich *et al.* 1998; Ishikawa *et al.* 1998). Because it is a powerful antioxidant agent ascorbic acid is an important neuroprotective agent (Padh 1990). Perhaps, the most dramatic acute oxidant stress in the CNS is the ischemia-reperfusion injury. Ascorbic acid administration decreased infarct size in animals models of middle cerebral artery ischemia-reperfusion (Ranjan *et al.* 1993). In CNS, ascorbic acid works as neuromodulator modifying dopaminergic and glutamatergic neurotransmission (Grünewald 1993; Girbe *et al.* 1994; Rebec and Pierce 1994). Moreover, it has been involved in the synthesis of noradrenaline (Diliberto *et al.* 1987) and some neuroendocrine peptides (Glembotski 1987; Grünewald 1993). Ascorbic acid is necessary for norepinephrine and acetylcholine release from synaptic vesicles (Kuo *et al.* 1979) and it is also able to promote myelin formation in Schwann cells (Eldridge *et al.* 1987).

Ascorbic acid can affect synaptic neurotransmission because it is able to prevent the binding of neurotransmitters to receptors (Todd and Bauer 1988; Majewska and Bell 1990; Majewska *et al.* 1990), to modulate release and reuptake of neurotransmitters (Levine *et al.* 1985a), and to function as a cofactor in neurotransmitter synthesis (Levine *et al.* 1985b; Paterson and Hertz 1989). The modulation of neurotransmitter binding to receptors seems complex because it depends on the brain area and levels of ascorbic acid concentration and behavorial activity (Kiyatkin and Rebec 1998; Rebec *et al.* 2005; Sandstrom and Rebec 2007). The mechanism by which ascorbic acid affects neural transmission has not been established. This modulation could be related with redox modulation on receptors by ascorbic acid itself (Ciani *et al.* 1996; Atlante *et al.* 1997) or by direct scavenging of reactive oxygen species generated by receptor activation (Majewska and Bell 1990; Majewska *et al.* 1990). Because synaptic activity is not constant over time, it is possible that local ascorbic acid concentration in brain changes according to neural activity environment. Indeed, extracellular ascorbic acid concentration increases in response to brain activity (O'Neill *et al.* 1984; Boutelle *et al.* 1989). It has been demonstrated that ascorbic acid efflux in CNS is strongly related to glutamatergic activity (O'Neill *et al.* 1984; Basse-Tomusk and Rebec 1991; Ghasemzadeh *et al.* 1991). Astrocytes play a fundamental role in this efflux, releasing ascorbic acid in response to glutamate (Portugal *et al.* 2009; Wilson *et al.* 2000).

Ascorbic acid is concentrated in adrenal gland, pituitary, liver, spleen, and brain (O'Neill *et al.* 1984; Rose and Bode 1993). These organs have the greatest retention capacities. Under conditions of ascorbic acid deficiency, however, brain ascorbate content is retained tenaciously, with decreases of less than 2% per day (Rice 2000). In brain, ascorbic acid accumulates mainly in cortex, nucleus accumbens, hypothalamus, hippocampus, and choroid plexus (Grünewald 1993; Milby *et al.* 1982; Spector and Lorenzo, 1973). Ascorbate enters the brain through the blood–CSF barrier (Spector and Lorenzo, 1973) and probably through the BBB too (Lam and Daniel 1986). In rat brain, ascorbic acid accumulates in CSF to a concentration of about 500 μM (Stamford *et al.* 1984). Extracellular ascorbic acid concentration in brain is 200–400 μM (Miele and Fillenz 1996; Schenk *et al.* 1982; Stamford *et al.* 1984), while in plasma is 60 μM (Spector and Lorenzo 1973). Ascorbic acid uptake by cells occurs via a specific transport system, stereospecific and driven by a sodium gradient: SVCTs (Table 3). To date, two isoforms have been cloned, SVCT1 and SVCT2, from cDNA libraries of kidney rat, mouse, and human (Faaland *et al.* 1998; Daruwala *et al.* 1999; Tsukaguchi *et al.* 1999). In brain, SVCT2 is exclusively expressed in neurons (Faaland *et al.* 1998; Tsukaguchi *et al.* 1999; Castro *et al.* 2001), in hypothalamus glial cells (García *et al.* 2005), and in choroid plexus epithelial cells (Tsukaguchi *et al.* 1999). SVCT2 is only expressed in cultured astrocytes but it is not detected in astrocytes *in situ* (Berger and Hediger 2000). Although endothelial cells from BBB are able to transport ascorbic acid *in vivo* (Agus *et al.* 1997) and *in vitro* (Ziylan *et al.* 2006), it has not been possible to detect the presence of protein or mRNA corresponding to SVCT2 or SVCT1 in these cells (García *et al.* 2005). Qiao and May (2008) demonstrated that capillary endothelial cells do not express SVCT2, but this isoform can be induced in culture. Oxidized vitamin C, dehydroascorbic acid, is transported by GLUTs (Table 1). GLUT1, GLUT2, GLUT3, and GLUT4 are able to transport dehydroascorbic acid bidirectionally with low affinity (K_m 1–4 mM; Angulo *et al.* 2008; Vera *et al.* 1993, 1995; Rumsey *et al.* 1997). In this way, an alternative mechanism for ascorbic acid entry across the BBB is the transport of dehydroascorbic acid by GLUT1, with the subsequent reduction of dehydroascorbate to ascorbate once it is in the brain (Agus *et al.* 1997). Another important detail to consider is that in order for GLUTs-mediated vitamin C transport to occur, it is necessary for ascorbic acid to be oxidized locally (Taverna and Langdon 1973; Himmelreich *et al.* 1998; Agus *et al.* 1999; Nualart *et al.* 2003) because

Table 3 Sodium–vitamin C transporters expression in brain

System	Tisular expression	Function	Reference
SVCT2	Neurons, glial cells from hypothalamus and epithelial cells from choroid plexus	Ascorbic acid uptake	Castro *et al.* (2001); Tsukaguchi *et al.* (1999); García *et al.* (2005); Tsukaguchi *et al.* (1999)
Glutamate/ascorbate heteroexchanger		Ascorbic acid efflux	Cammack *et al.* (1991)
Volume-sensitive organic anion channels		Ascorbic acid efflux	Siushansian *et al.*, (1996); Wilson *et al.* (2000)
Hemichannels		Ascorbic acid efflux	Ahmad and Evans (2002)
GLUTs (1,2,3 and 4)	See Table 1	Dehydroascorbic acid bidirectional transport	See Table 1

GLUT, glucose transporter; SVCT, sodium–vitamin C transporter.

almost 100% of vitamin C in the body is present as ascorbic acid (Schorah *et al.* 1996).

Within the brain, ascorbic acid is recycled as it is used, so that antioxidant protection is maintained. It has been proposed that astrocytes are involved in vitamin C recycling (Astuya *et al.* 2005; Harrison and May 2009). During synaptic activity, ascorbic acid is released from intracellular reservoirs (O'Neill *et al.* 1984; Ghasemzadeh *et al.* 1991; Yusa 2001). The molecular basis of ascorbic acid efflux is not yet well known. It has been proposed that ascorbate could be released through several mechanisms: glutamate/ascorbate heteroexchanger (Cammack *et al.* 1991); volume-sensitive organic anion channels (Siushansian *et al.* 1996; Wilson *et al.* 2000); exocytosis (Von Zastrow *et al.* 1986); or hemichannels (Ahmad and Evans 2002; Table 3). A possible role for SVCT2 in ascorbic acid efflux has recently been described (Portugal *et al.* 2009). This proposition is based on the fact that sulfinpyrazone, or the absence of sodium, is able to inhibit ascorbic acid efflux. However, both are non-specific SVCTs inhibitors. Neurons can take up ascorbic acid efficiently because they express SVCT2 (Castro *et al.* 2001). Ascorbic acid is oxidized within neurons because during synaptic activity many oxidant species are generated. Neuronal dehydroascorbic acid can be released through GLUT1 or GLUT3 (Korcok *et al.* 2000; Hediger 2002; Takanaga *et al.* 2004) and astrocytes should take it up through GLUT1 (Patterson and Mastin 1951; Rose *et al.* 1992; Siushansian *et al.* 1997; Rice 2000). Within astrocytes, dehydroascorbic acid should be reduced to ascorbic acid because these cells have a greater concentration of glutathione than neurons (Rice and Russo-Menna 1998). Therefore, ascorbic acid is highly concentrated in CNS because of the expression and differential distribution of specific mechanisms of transport in this tissue. Presence of these transport systems allows ascorbic acid to move between brain cells (Astuya *et al.* 2005; Harrison and May 2009).

The ascorbic acid metabolic switch

According to our data, ascorbic acid may work as a metabolic switch inhibiting glucose consumption during episodes of glutamatergic synaptic activity (Castro *et al.* 2007) and stimulating lactate uptake in neuronal cultures (Figs 1 and 2; Castro *et al.* 2008). In studies of rat hippocampus slices, we showed that ascorbic acid is able to modulate the ability of glucose to serve as an energetic fuel sustaining glutamatergic synaptic activity (Castro *et al.* 2007) (Fig. 1). The synaptic activity can be measured as recordings from pyramidal neurons from hippocampal CA1 region, using the whole-cell configuration of the voltage-clamp technique. Excitatory post-synaptic currents (EPSCs) are evoked by stimulating the Schaffer collateral pathway. Glucose deprivation produced a slowly developing depression of synaptic responses, with almost complete elimination of synaptic transmission (Schurr *et al.* 1988; Fowler 1993; Izumi *et al.* 1997; Castro *et al.* 2007). Glucose deprivation produces a slow depression of synaptic responses. After deprivation, glucose is not able to restore EPSCs in the presence of external 200 μM alpha-cyano-4-hydroxycinnamate (4-CIN, Fig. 1a; Castro *et al.* 2007; Izumi *et al.* 1997), a classical MCT inhibitor. Therefore, the inhibition of putative monocarboxylate export (Fig. 1a, schematic representation) from glia to neurons produces synaptic activity ablation even in the presence of glucose. These experiments strongly support the ANLSH. The use of 4-CIN has been criticized for its possible inhibition of pyruvate entry into mitochondria, a process mediated by MCT1 (Chih *et al.* 2001). The 4-CIN K_i for MCT1 is 425 μM (Bröer *et al.* 1997), while for MCT2 is 24 μM (Bröer *et al.* 1999). Therefore, in the presence of 200 μM 4-CIN, MCT1 function is partially inhibited, while MCT2 is almost completely inhibited. Moreover, Erilchman *et al.* (2008) demonstrated monocarboxylate flux from astrocytes to neurons with 100 μM 4-CIN under *in vivo* experimental conditions. Thus, glucose is not able to sustain neural function when monocarboxylate flux is interrupted. However, when ascorbic acid uptake through SVCT2 is inhibited, glucose is able to sustain EPSCs in the presence of 200 μM 4-CIN (Fig. 1b). This effect is reverted when ascorbic acid is introduced directly into the cell (Fig. 1c). In conclusion, intracellular ascorbic acid inhibits the ability of glucose to sustain EPSCs when lactate flux is inhibited. In contrast, when ascorbic acid uptake is inhibited in neurons, glucose serves as a metabolic substrate capable of sustaining glutamatergic transmission.

The 'ascorbic acid effect' in slice experiments could have an explanation in the knowledge that intracellular ascorbic acid inhibits glucose analog (DOG) consumption in cultured cortical and hippocampal neurons (Castro *et al.* 2007). But, intracellular ascorbic acid does not inhibit DOG uptake in astrocytes (Castro *et al.* 2007). Probably GLUT3, the predominant GLUT in neurons and which is absent in astrocytes (Aller *et al.* 1997; Leino *et al.* 1997; Vannucci *et al.* 1998; Nualart *et al.* 1999), may be directly associated with the selective ascorbic acid effect in these cells. Indeed, intracellular ascorbic acid inhibits DOG transport in non-neuronal cells expressing endogenous GLUT3 (Castro *et al.* 2008). However, intracellular ascorbic acid does not inhibit *O*-methylglucose (OMG) transport even in cells expressing GLUT3 (Castro *et al.* 2008). Because DOG is a HK-phosphorylable glucose analog and OMG is not, it is possible that HK could be important in the mechanism of glucose transport inhibition, or rather in the inhibition of glucose use. These results are consistent with *in vitro* HK activity inhibition by dehydroascorbic acid, the oxidized form of ascorbic acid (Fiorani *et al.* 2000). If glucose use is inhibited the transport of other substrates may be stimulated. Intracellular ascorbic acid is indeed able to stimulate lactate in cells such cultured neurons and human embryonic kidney

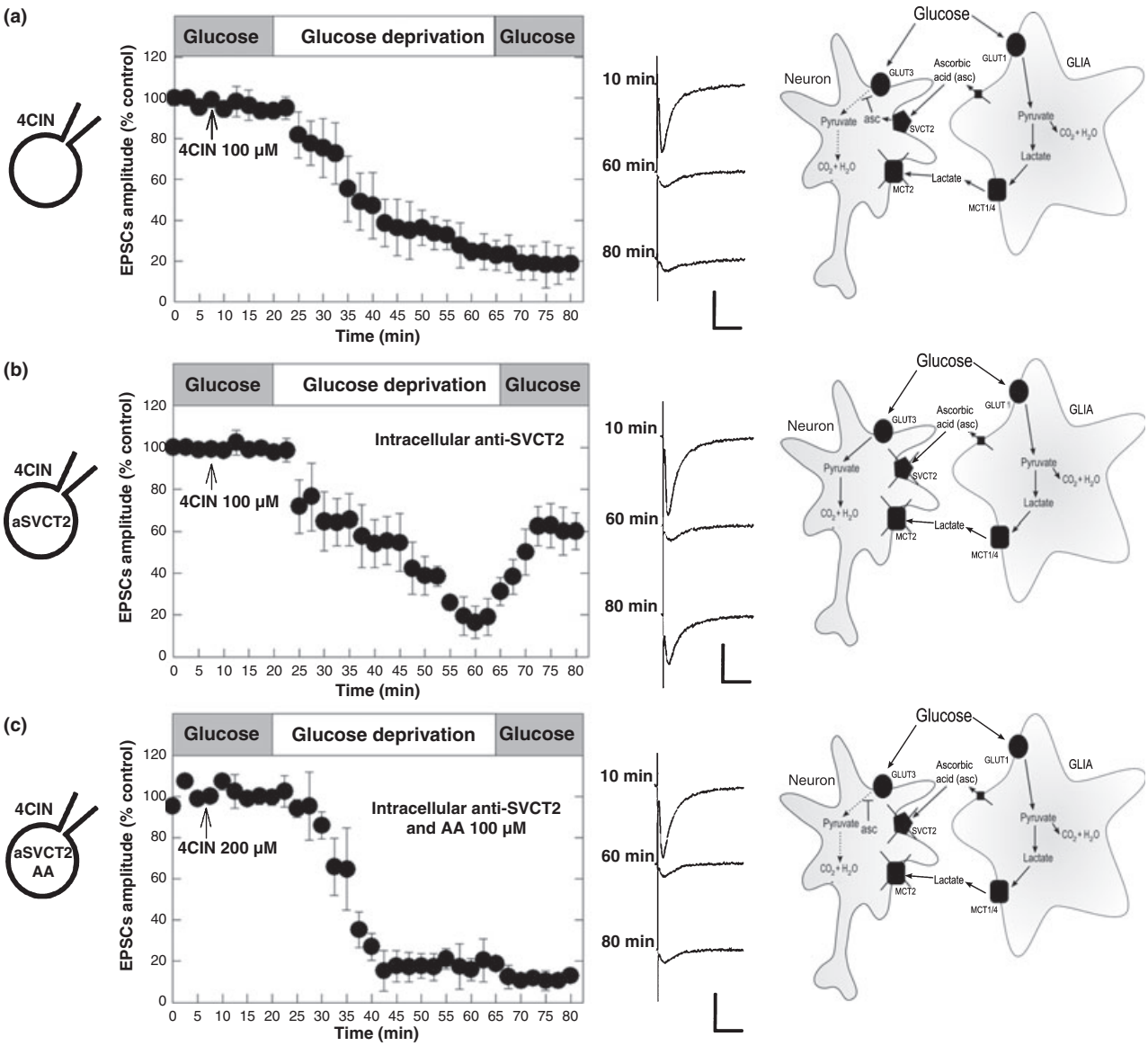

Fig. 1 Effect of intracellular ascorbic acid on the restoration of glucose supported excitatory post-synaptic currents (EPSCs). (a, b, and c) Left side: schematic representation of experiments using voltage-clamp technique in whole-cell configuration. Graphs: time course of EPSCs amplitude. Traces at the center are representative field EPSCs for a glucose-deprived slice. Calibration bar: 100 pA, 20 ms. Right side: schematic representation of glia–neuronal interaction after glucose deprivation at different experimental conditions analyzed. (a) EPSCs after glucose deprivation in the presence of 200 μM 4-CIN. (b) EPSCs recovery after glucose deprivation in the presence of 200 μM 4-CIN and intracellular anti-SVCT2 (1 : 2000 into intracellular solution). (c) EPSCs after glucose deprivation in the presence of 200 μM 4-CIN, intracellular anti-SVCT2 (1 : 2000 into intracellular solution) and 100 μM ascorbic acid. Data represent mean ± SD (n = 5–7). The antibody and 4-CIN were incorporated after 7 min of glucose incubation and maintained for ~75 min. Adapted from Castro *et al.* (2007) with permission of the publisher.

293 cells (Castro *et al.* 2008) that express endogenous GLUT3. Therefore, ascorbic acid seems to work as a metabolic switch, switching on lactate consumption and switching off glucose use.

During glutamatergic synaptic activity there is an increase in extracellular ascorbic acid in the brain (O'Neill *et al.* 1984; Basse-Tomusk and Rebec 1991; Ghasemzadeh *et al.* 1991; Yusa 2001). This ascorbic acid can be taken up by neurons because they express SVCT2 (Tsukaguchi *et al.* 1999; Castro *et al.* 2001). Astrocytes release ascorbic acid in response to glutamate (Wilson *et al.* 2000; Castro *et al.* 2007) and ascorbic acid is taken up by neurons (Fig. 2a and c;

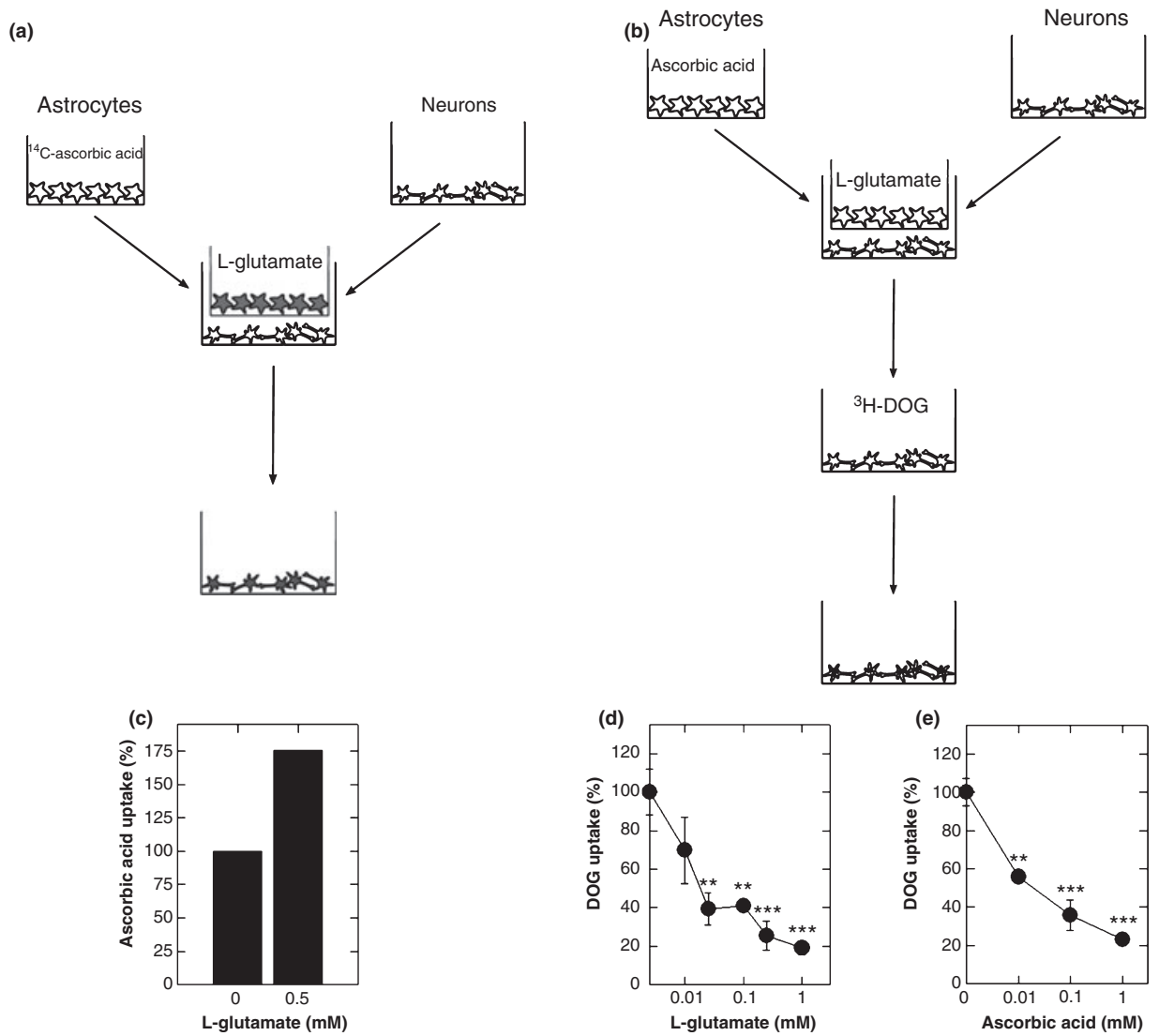

Fig. 2 Effect of astrocytic ascorbic acid on neuronal deoxyglucose (DOG) uptake. Cortical astrocytes were plated in transwell inserts and co-cultured with cortical neurons during 48 h. (a and c) Determination of flow glutamate-dependent of ascorbic acid between astrocytes and neurons. Previous to the experiment, only astrocytes were pre-loaded with 1 mM ^{14}C-ascorbic acid for 60 min. Loaded astrocytes and neurons were co-incubated in presence of 0.25 mM L-glutamate during 60 min and measurements of ^{14}C-ascorbic acid content were assed in neurons. In (c) bars plot for the stimulation of ascorbic acid flow by L-glutamate is shown. (b, d, and e) Study of astrocytic ascorbic acid on neuronal DOG uptake. Previous to the experiment, only astrocytes were pre-loaded with ascorbic acid for 60 min. Loaded astrocytes and neurons were co-incubated in presence of L-glutamate during 60 min and measurements of ^{3}H-DOG uptake (0.5 mM DOG, 15 s uptake assay, 20°C) were assed in neurons. In (d), semi-log plot of 2-DOG uptake inhibition in cells treated with different concentrations of L-glutamate. In (e) semi-log plot of 2-DOG uptake inhibition in cells treated with different concentrations of ascorbic acid. For better understanding, the radioactive tracers were marked in gray in the upper schemes (a and b). The data represent the mean ± SD of three experiments; $^{**}p < 0.01$ and $^{***}p < 0.001$. Unpublished data, experimental procedure was performed as described in Castro *et al.* (2007).

Castro *et al.* 2007). When ascorbic acid is within the neurons, DOG uptake is inhibited (Fig. 2b, d, and e). Glutamate stimulates ascorbic acid flux between astrocytes and neurons. DOG inhibition is dependent on glutamate stimulation (Fig. 2d) and ascorbic acid presence (Fig. 2e). Therefore, the DOG uptake inhibition is dependent on ascorbic acid flux.

In conclusion, fluctuations in ascorbic acid concentration are related to brain activity (O'Neill *et al.* 1984; Boutelle *et al.* 1989), but are also related to brain energetics (Fig. 3).

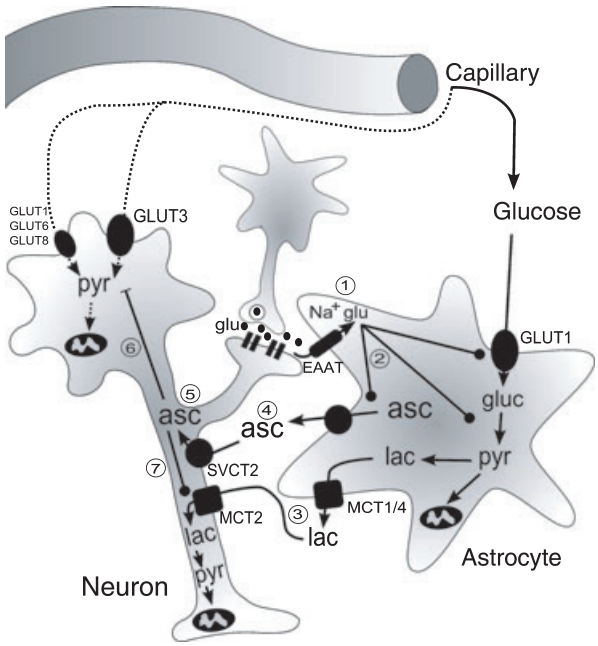

Fig. 3 Ascorbic acid metabolic switch on astrocyte–neuron lactate shuttle hypothesis. During glutamatergic synaptic activity, astrocytes take up glutamate from the synaptic cleft (1). The glutamate and Na^+ entry into astrocytes stimulate glucose transport, glycolysis, and lactate efflux (2). Lactate is taken up by neurons to support their energetic needs (3). Glutamate uptake also stimulates ascorbic acid release from astrocytes (4). Ascorbic acid enters neurons through SVCT2 (5). Intracellular ascorbic acid inhibits glucose consumption (6) and stimulates lactate uptake in neurons (7). The abbreviations used in this figure were: asc, ascorbic acid; glu, glutamate; lac, lactate; pyr, pyruvate.

During glutamatergic synaptic activity, astrocytes remove glutamate from the synaptic cleft to ensure the success of synaptic transmission (Choi 1988). Glutamate uptake in astrocytes stimulates glucose transport (Pellerin and Magistretti 1994; Loaiza *et al.* 2003; Porras *et al.* 2008), glycolysis and lactate efflux (Pellerin and Magistretti 1994; Demestre *et al.* 1997). Glutamate uptake (Wilson *et al.* 2000) or activation of glutamate ionotropic receptors (Portugal *et al.* 2009) stimulates ascorbic acid release from astrocytes. Ascorbic acid enters neurons because these cells express SVCT2 (Tsukaguchi *et al.* 1999; Castro *et al.* 2001). Intracellular ascorbic acid inhibits glucose consumption (Castro *et al.* 2007) and stimulates lactate uptake in neurons (Castro *et al.* 2008). Lactate is metabolized in neurons to CO_2 and water to sustain neural function (Castro *et al.* 2007; Fowler 1993; Izumi *et al.* 1997; Schurr *et al.* 1988).

The mechanism of ascorbic acid effect remains to be understood. The differences in ascorbic acid effect on DOG and OMG uptake are consistent with HK inhibition by ascorbic acid *in vitro* (Fiorani *et al.* 2000). Thus, we can speculate that lactate stimulation is a consequence of glucose phosphorylation inhibition. However, HK inhibition has been demonstrated only in assays using purified HK and not in intact cells. Further, ascorbic acid does not inhibit DOG uptake in astrocytes which express HK. So, further experiments are required to clarify this idea. However, because SVCT2 co-transports sodium and ascorbate, it is possible that a Na^+ increase affects glucose consumption in a reverse manner to that in astrocytes (Bernardinelli *et al.* 2004; Porras *et al.* 2008). Neuronal activity stimulates the increase in astrocyte intracellular calcium concentration, which is propagated through neighboring astrocytes (Charles 2005). Calcium propagation (calcium wave) is accompanied by the release of glutamate. Sodium-dependent glutamate uptake leads to a secondary astrocytic sodium wave, which stimulates glucose uptake and metabolism in these cells (Bernardinelli *et al.* 2004; Porras *et al.* 2008). Finally, it has been proposed that glutamate directly inhibits hexose transport in neurons co-cultivated with astrocytes (Porras *et al.* 2004). However, glutamate does not affect DOG uptake in pure cultured neurons (Castro *et al.* 2007). Porras *et al.* (2004) described that glutamate inhibits glucose uptake via α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor-mediated sodium entry. Another explanation is that the phenomenon observed actually occurs subsequent to glutamate-activated release of ascorbic acid from the astrocytes present in co-cultured cells. In cultured astrocytes and neurons using transwells system, neuronal DOG transport was not inhibited in the presence of glutamate and unloaded astrocytes (Fig. 2e). However, cell culture is a model with some limitations. Although it has been demonstrated that the expression of transporters and enzymes involved in the hypothesis of this review are similar to that observed *in situ* (Castro *et al.* 2007, 2008; Gali *et al.* 1981; Loaiza *et al.* 2003; Maher *et al.* 1992; Maher 1995; Wilkin and Wilson 1977; Wilson 1995; Zeitschel *et al.* 1996; and other studies) the architecture of intercellular interactions is not the same as *in vivo*. This situation is different in brain slices where tissue architecture is preserved. Electrophysiological experiments performed on acute slices showed that ascorbic acid alone is able to inhibit EPSCs recovery (Fig. 2b and c). Therefore, for the case of hippocampal slices it is more likely that ascorbic acid rather glutamate is the messenger that contribute to the observed results.

Concluding remarks

Brain energy metabolism was one of the first areas of brain biochemistry studied (Lowry and Passonneau 1964; Siesjèo 1978). For many years, knowledge of brain energy metabolism focused on neural energy needs; postulating that glucose was the exclusive energy substrate for these cells. The emergence of astrocytes onto this scene has been invaluable for the elaboration of new testable hypotheses. Astrocytes do indeed play a central role in the regulation of brain metabolic responses to activity. One such mechanism is

the ANLSH. Over the last 15 years, evidence has mounted to reinforce the idea that astrocytes support neurons metabolically through lactate production (Caesar *et al.* 2008; Izumi *et al.* 1997; Rouach *et al.* 2009; Schurr *et al.* 1999). However, much of this work does not explain what happens to neuronal glucose consumption. Indeed, activation of glycolysis produces an increase in pyruvate and proton concentration and a decrease in the NAD^+/NADH ratio. In this situation, neuronal LDH-1 could not catalyze lactate oxidation. Therefore, neuronal lactate consumption would only be possible if glucose uptake or metabolism were inhibited. In previous work from our laboratory, we demonstrated that intracellular ascorbic acid inhibits of glucose transport in cortical and hippocampal neurons (Castro *et al.* 2007). Ascorbic acid is highly concentrated in brain (Hornig 1975) and extracellular ascorbic acid concentration fluctuates according to brain activity (Basse-Tomusk and Rebec 1991; O'Neill *et al.* 1984; Yusa 2001). The expression of different vitamin C transport systems permits ascorbic acid flow between brain cells (Astuya *et al.* 2005). Synaptic activity stimulates ascorbic acid release from intracellular reservoirs (O'Neill *et al.* 1984; Ghasemzadeh *et al.* 1991; Yusa 2001). Glutamate is able to stimulate ascorbic acid efflux from astrocytes (Portugal *et al.* 2009; Wilson *et al.* 2000) and neurons express SVCT2. Thus, ascorbic acid is taken up by neurons to scavenge oxidant species generated by neurotransmission. Neuronal dehydroascorbic acid is released through GLUTs (Korcok *et al.* 2000; Hediger 2002; Takanaga *et al.* 2004) and astrocytes take it up through GLUT1 (Patterson and Mastin 1951; Rose *et al.* 1992; Siushansian *et al.* 1997; Rice 2000). This flow also permits that metabolic switch to function. Within the neurons ascorbic acid can inhibit glucose consumption. Therefore, pyruvate and proton concentrations are decreased, accompanied by an increase in the NAD^+/NADH ratio. In this scheme, LDH-1 can catalyze lactate oxidation. Indeed, intracellular ascorbic acid stimulates lactate uptake in cortical neurons (Castro *et al.* 2008). The mechanism of ascorbic acid effect must be clarified. It is possible that GLUT3 is a key factor in this phenomenon. But we cannot rule out the possible involvement by Na^+ or direct inhibition of HK. In conclusion, the metabolic switch of ascorbic acid is consistent with the ANLSH and it gives an explanation of how neurons consume lactate when expressing GLUTs and glycolytic enzymes. Moreover, although ascorbic acid is concentrated in many other tissues (Hornig 1975; Kratzing *et al.* 1982), so, ascorbic acid concentration variations in these tissues could drive metabolic changes in cells of different tissues.

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