

# Aspects of astrocyte energy metabolism, amino acid neurotransmitter homeostasis and metabolic compartmentation

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## ABSTRACT

Astrocytes are key players in brain function; they are intimately involved in neuronal signalling processes and their metabolism is tightly coupled to that of neurons. In the present review, we will be concerned with a discussion of aspects of astrocyte metabolism, including energy-generating pathways and amino acid homeostasis. A discussion of the impact that uptake of neurotransmitter glutamate may have on these pathways is included along with a section on metabolic compartmentation.

Key words: amino acid, astrocyte, compartmentation, energy, metabolism.

## ASTROCYTE ENERGY METABOLISM

### Glucose entry, phosphorylation and generation of glycogen in astrocytes

Astrocytes are ideally positioned to sense synaptic activity in the brain (Kacem et al., 1998), they control blood flow (Gordon et al., 2008; Allen and Barres, 2009), interact with neurons and endothelial cells (Vesce et al., 1999) and likely act as signalling integrators at different temporal and spatial domains (Parpura and Zorec, 2010; Parpura et al., 2010). Astrocytic endfeet lie between all brain capillaries and neuronal terminals

(Tsacopoulos and Magistretti, 1996; Mathiesen et al., 2010) and during neuronal activity astrocytes may exhibit an increased glucose uptake and possibly have a key role in coupling synaptic activity to glucose utilization (Magistretti, 2006) and provision of glucose for neuronal metabolism (DiNuzzo et al., 2010, 2011). Sodium-coupled uptake of glutamate by astrocytes and the ensuing activation of the Na<sup>+</sup>/K<sup>+</sup>-ATPase may trigger glucose uptake and glycolytic processing (Fox et al., 1988, Magistretti, 2006), the latter process providing energy in the form of ATP to fuel glutamate uptake (Schousboe et al., 2011).

The [glc]<sub>i</sub> (intracellular glucose concentration) is dependent on the [glc]<sub>e</sub> (external glucose concentration), but is limited to a maximal value of ~0.4 mM (Bittner et al., 2010; Prebil et al., 2011a). This upper limit is likely due to the plasma membrane permeability for glucose, which is in balance with the cytosolic glucose utilization. The rate of glucose flux through GLUT1 (glucose transporter 1), the major GLUT in astrocytes (Loaiza et al., 2003), is thus in balance with the rate of utilization by hexokinase activity. Interestingly, the level of [glc]<sub>i</sub> in astrocytes (0.4 mM) is much lower than that measured in 3T3-L1 fibroblasts (10.2 mM) and preadipocytes (2.6 mM), but comparable with adipocytes (0.6 mM) (Kovacic et al., 2011), determined at similar extracellular glucose levels.

In astrocytes, the cytosolic glucose concentration declines to a new lower steady-state value in approximately 20 s, when extracellular glucose changes from 0.5 to 0.0 mM. At higher initial extracellular glucose level (i.e. 5 mM; higher load for the metabolism), the decline in cytosolic glucose concentration is slower (60 s), which is likely due to the rate-limited cytosolic consumption of glucose (Prebil et al., 2011a).

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**Abbreviations:**  $\alpha$ -KG,  $\alpha$ -ketoglutarate; AAT, aspartate aminotransferase; CFP, cyan fluorescence protein; DAB, diaminobenzidine; FRET, fluorescence resonance energy transfer; [glc]<sub>i</sub>, intracellular glucose concentration; GABA,  $\gamma$ -aminobutyric acid; GABA-T, GABA aminotransferase; GDH, glutamate dehydrogenase; GLUT, glucose transporter; GP, glycogen phosphorylase; GS, glutamine synthetase; GSK3, GS kinase 3; PAG, phosphate-activated glutaminase; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; TCA, tricarboxylic acid; YFP, yellow fluorescence protein.

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GLUT1 (45-kDa isoform) is located in the astrocytic endfeet around blood vessels (Morgello et al., 1995) and in astrocytic cell bodies and processes (Leino et al., 1997). The GLUT2 was also found in astrocytes (Leloup et al., 1994; Leino et al., 1997; Arluisson et al., 2004). On the other hand, the 55-kDa isoform of GLUT1 is located in endothelial cells which form the blood–brain barrier. Glucose enters neurons *trans*-cellularly through astrocytes via the 45-kDa isoform of GLUT1 or directly via GLUT3, a neuronal GLUT (Maher et al., 1994). It may be noted that GLUT1 is stimulated by glutamate *in vitro* and by neuronal activity *in vivo* (Loaiza et al., 2003; Porras et al., 2008; Chuquet et al., 2010).

After glucose entry, glucose is phosphorylated by type I hexokinase (Needels and Wilson, 1983; Griffin et al., 1992). In astrocytes, most of the type I hexokinase is associated with mitochondria (Lynch et al., 1991), and the activity of hexokinase bound to mitochondria is greater than the cytosolic hexokinase (Nagamatsu et al., 1996). However, inhibition of gap junctions promotes the translocation of type I hexokinase from mitochondria towards microtubules, and induces a significant expression of type II hexokinase and GLUT3, which are normally not present in astrocytes; all these changes aid to sustain a higher rate of cell proliferation (Sánchez-Alvarez et al., 2004). It was shown that levels of glucose-1,6-bisphosphate higher than 0.2 mM inhibit astroglial hexokinase in a concentration-dependent manner; at 1.2 mM, the hexokinase activity is almost completely inhibited (Lai et al., 1999). Endothelin-1, a vasoconstricting agent (Nie and Olsson, 1996), stimulates the translocation and up-regulation of both GLUT1 transporter and type I hexokinase in astrocytes (Sánchez-Alvarez et al., 2004). Type II hexokinase is induced by deprivation of glucose (Niitsu et al., 1999). It was recently shown that ischaemic stress increases the expression of GLUT3, which is responsible for the enhanced storage of intracellular glucose during reperfusion (Iwabuchi and Kawahara, 2011).

After glucose phosphorylation, glucose-6-phosphate is the intermediate from which either glycolysis, the pentose-phosphate pathway or the generation of glycogen may start (Figure 1). The amount of glucose metabolized in the pentose-phosphate pathway is less than one-tenth compared with glycolysis in cultured astrocytes (Leo et al., 1993; Ben-Yoseph et al., 1994), where lactate is a major metabolic product (Wiesinger et al., 1997). Several toxic agents, such as chlorinated acetates, fluoxetine, ethanol and copper, were shown to disturb glucose uptake or metabolism in astrocytes (Abdul Muneer et al., 2011; Allaman et al., 2011; Scheiber and Dringen, 2011; Schmidt et al., 2011).

It was reported that in rat brain the extracellular concentration of glucose, measured by a microdialysis technique, is as low as 0.47 mM (Fellows et al., 1992). Moreover, in ischaemic conditions, the extracellular glucose concentration, measured with micro-electrodes, is less than 0.05 mM, and in hypoglycaemia less than 0.19 mM (Silver and Erecińska, 1994), which may be the milieu where glucose may leak out of the astrocyte, since the resting cytosolic

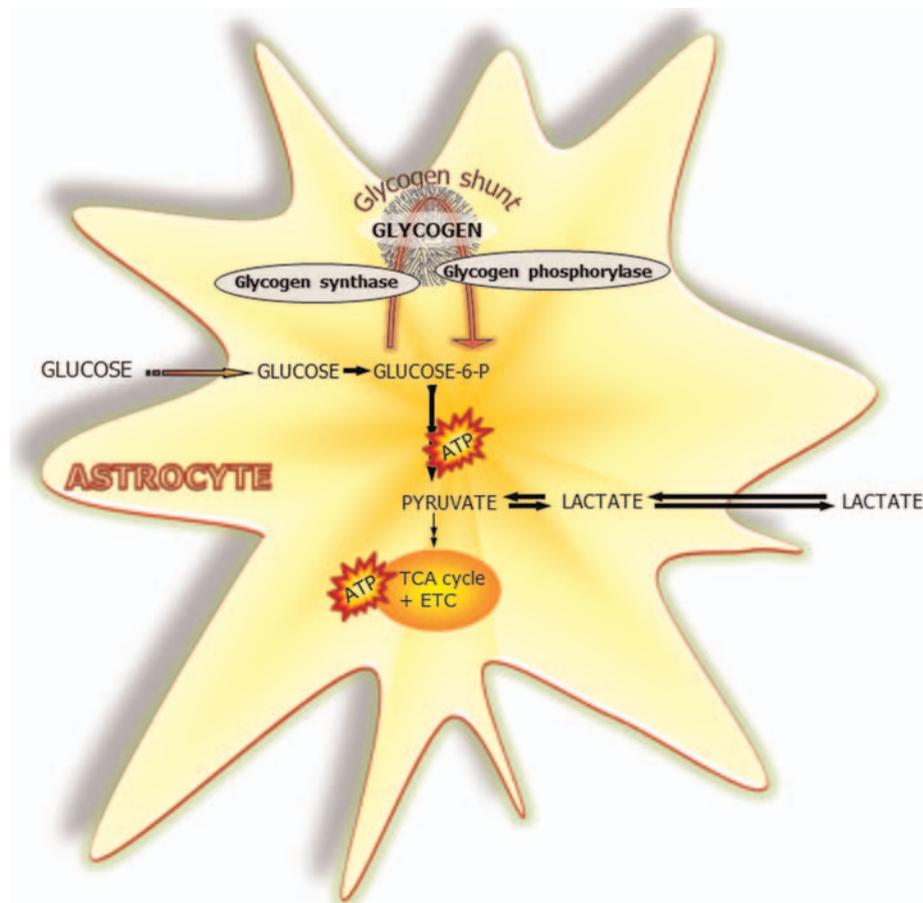
glucose concentration was measured to be 0.4 mM, especially if noradrenalin is present (Prebil et al., 2011a).

Emerging evidence suggests that metabolic substrates other than glucose (e.g. glycogen, lactate and glutamate) provide significant amounts of energy in the brain (Brown et al., 2001; Dienel and Cruz, 2006). In the brain, glycogen (Cataldo and Broadwell, 1986; Wender et al., 2000) as well as GP (glycogen phosphorylase) predominantly reside in astrocytes (Ibrahim, 1975). The term *glycogen shunt* (Figure 1) represents the fraction of metabolized glucose that passes through glycogen molecules, prior to entering the glycolytic pathway, and may be as large as 40% of overall glucose metabolism (Walls et al., 2009). The glycogen reservoir can provide fuel for energy production during hypoglycaemia (Swanson and Choi, 1993; Brown and Ransom, 2007), as well as during normal brain metabolism (Swanson, 1992; Fillenz et al., 1999; Walls et al., 2009). Glycogen content appears to be dependent on insulin signalling in astrocytes (Heni et al., 2011).

Glycolysis and glycogenolysis appear to provide most of the energy required during an abrupt energy demand (Hertz et al., 2007). Glycogen might serve as a source of lactate which may be transferred to neurons (Wender et al., 2000; DiNuzzo et al., 2011), or converted to pyruvate, which enters the TCA (tricarboxylic acid) cycle (Sickmann et al., 2005). Lactate and ketone bodies have been shown to fuel a substantial portion of brain-energy metabolism in prolonged starvation, diabetes and under hypoglycaemia (reviewed by Pellerin and Magistretti, 2004). In addition, lactate may act as a signalling molecule (reviewed by Barros and Deitmer, 2010). On the other hand, astrocytic networks can also effectively remove lactate from activated glycolytic domains, and the lactate can be dispersed throughout the syncytium to the endfeet for release to blood (Gandhi et al., 2009). Finally, channelling of blood-borne glucose to the extracellular space for use in neurons has been suggested by DiNuzzo et al. (2010, 2011) based on the idea that breakdown of glycogen inhibits phosphorylation by hexokinase.

### Effects of glutamate entry on energy metabolism

A FRET (fluorescence resonance energy transfer)-based approach employing nanosensors (Fehr et al., 2003) may be reliably used to monitor dynamic activity-induced changes in cytosolic glucose levels in astrocytes (Bittner et al., 2010, 2011; Prebil et al., 2011a, 2011b). In the first generation of such nanosensors, such as FLIPglu-600 $\mu$ , a decrease in the FRET signal was observed upon glucose binding (Takanaga et al., 2008). The second generation of nanosensors, such as FLII2PGLU-700 $\mu$  $\Delta$ 6, have an improved signal-to-noise ratio and a higher dynamic measuring range *in vivo*, from 0.05 to 9.6 mM (Takanaga et al., 2008). Phosphorylated sugars have no effect on the FRET ratio (Fehr et al., 2003). To dynamically monitor the cytosolic glucose concentration ([glc]<sub>i</sub>), the CFP (cyan fluorescence protein) fluorescence is excited and the fluorescence of CFP and YFP (yellow fluorescence protein) is



**Figure 1** Glucose and glycogen metabolism in astrocytes

Simplified schematic representation of glucose metabolism via glycolysis or via the 'glycogen shunt' illustrating how glucose units may be metabolized via incorporation into and subsequent hydrolysis from the branched glycogen molecule preceding metabolism to pyruvate and lactate, i.e. glycogenolysis. Glucose-6-P, glucose-6-phosphate; ETC, electron transport chain.

monitored. The ratio between YFP and CFP is calculated over a defined region of the imaged cell. Cells superfused with extracellular medium, containing a high glucose concentration, display high intensity of CFP fluorescence and low intensity of YFP fluorescence. Thus, the high YFP/CFP ratio ( $\Delta R$ ) indicates an elevated cytosolic glucose concentration. The exchange of glucose-rich external solution with the one devoid of glucose results in the FRET ratio decline, indicating glucose depletion from the cell (Takanaga et al., 2008).

The sensor may be calibrated *in situ* by measuring the difference between the FRET ratio during superfusion with the increasing extracellular glucose concentration and intermittent superfusion with a solution devoid of glucose ( $\Delta R$ ). To calibrate the sensor, the plasma membrane should be permeabilized (e.g. by using  $\beta$ -escin) to allow fast and unhindered access of glucose to the sensor in the cell interior. The saturation level of the sensor is first calculated ( $S = \Delta R / \Delta R_{\max}$ ), and then by using the binding constant  $K_d$  of the sensor the intracellular concentration of glucose is estimated ( $[glc]_i = \{K_d \times S\} / \{1 - S\}$ ; Prebil et al., 2011a). It is important

to note that since the sensor measures the level of unphosphorylated glucose, it is assumed that a decrease in  $[glc]_i$  reflects increased utilization of glucose, i.e. increased uptake and glycolytic breakdown of extracellular glucose.

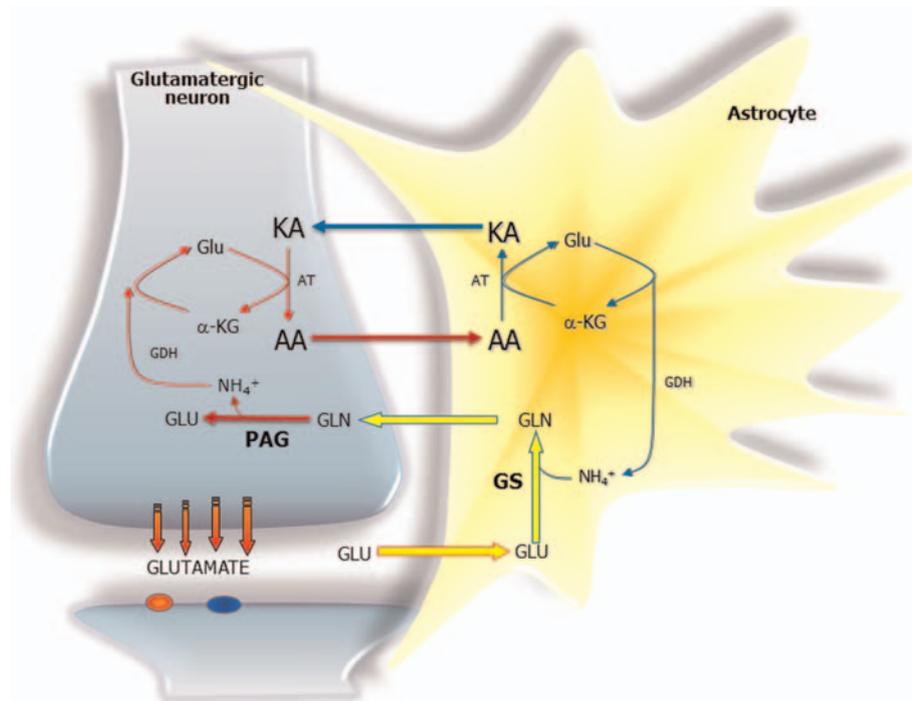
The effect of glutamate as a neurotransmitter in the synapse is strongly dependent on astrocytic metabolism (Hertz, 2006). Since glutamate does not readily cross the blood-brain barrier, glucose serves as a precursor for glutamate synthesis (Hertz and Dienel, 2002). Neurons lack the enzyme pyruvate carboxylase (Yu et al., 1983) and therefore depend on astrocytes for *de novo* synthesis of glutamate (Danbolt, 2001; Hertz and Zielke, 2004). Rapid glutamate uptake by glial transporters located near release sites (Chaudhry et al., 1995; Bergles et al., 1997; Clark and Barbour, 1997; Dzubay and Jahr, 1999) removes the transmitter and thus terminates the excitatory postsynaptic potential. In physiological conditions, glutamate uptake into astrocytes is driven by the electrochemical gradient of sodium (O'Kane et al., 1999) and mediated mainly through two glial glutamate transporters: GLAST (EAAT1) and GLT1 (EAAT2) (Rothstein et al., 1994; Danbolt, 2001).

Trafficking of glutamate transporters to the plasma membrane has been studied (Robinson, 2002; Fournier et al., 2004; Stenovec et al., 2008) and it is likely that glutamate transporters get incorporated into the plasma membrane by exocytosis (Cheng et al., 2002; Stenovec et al., 2008). Consistent with the presence of a regulated exocytotic pathway in astrocytes (Kreft et al., 2004; Pangrsic et al., 2006, 2007; Parpura and Zorec, 2010), a calcium-dependent increase in cumulative exocytosis increases the glutamate transporter density (Stenovec et al., 2008), which is important for maintaining a low extracellular glutamate concentration, essential for the prevention of chronic glutamate neurotoxicity (Rothstein et al., 1996).

After glutamate uptake into astrocytes, it is either converted to glutamine (Figure 2) by the astrocyte-specific GS (glutamine synthetase; Derouiche, 2004) or at high glutamate concentration is oxidatively degraded after conversion to  $\alpha$ -KG ( $\alpha$ -ketoglutarate; Yu et al., 1982; McKenna et al., 1996, 2000). The latter pathway (Figure 3) clearly shows that besides being an excitatory transmitter, glutamate is an important metabolic fuel, which is oxidatively metabolized in astrocytes (Miller et al., 1975; Hertz et al., 1988; Swanson et al., 1990; Zielke et al., 1998; Diel and

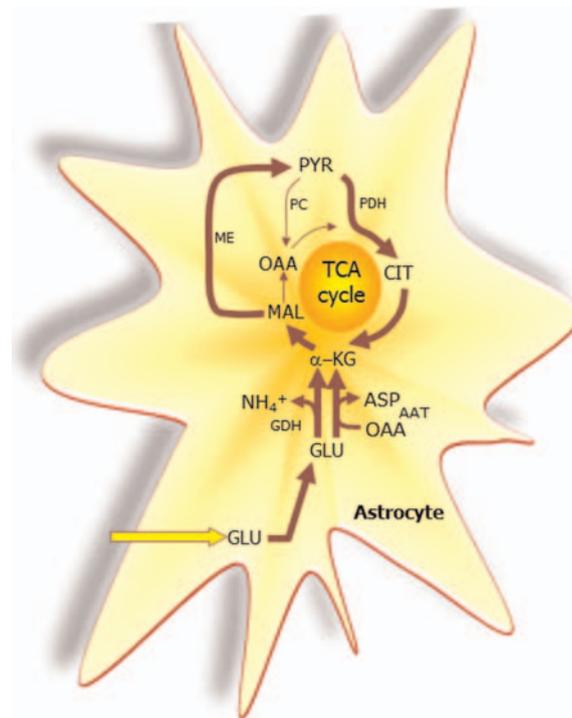
Cruz, 2006; Hawkins, 2009). This aspect is discussed in further detail in a subsequent section.

Astrocytes respond to glutamate by enhancing both glucose utilization and lactate production and release (Pellerin and Magistretti, 1994, 2003; Fray et al., 1996), which has been suggested to lead to the increase in extracellular lactate that follows cortical activation (Hu and Wilson, 1997); however, the exact sources and sinks of extracellular lactate during activation are still elusive, as discussed recently by Kasischke (2011), and neurons might indeed contribute to extracellular lactate (Caesar et al., 2008; Bak et al., 2009; Contreras and Satrustegui, 2009) as well as consume at least half of the available extracellular glucose (Zielke et al., 2007). Glutamate may induce glycolysis in astrocytes (Pellerin et al., 2007) which is mediated by the activation of a  $\text{Na}^+$ -dependent uptake system rather than the activation of extracellular glutamate receptors (Pellerin and Magistretti, 1994); however, others have not been able to show this coupling between glutamate uptake and stimulation of glycolysis, probably indicating astrocytic diversity (Hertz et al., 1998, 2007). Using FRET nanosensors for glucose, it was recently confirmed that glutamate stimulates glycolysis in cultured astrocytes, but only after a lag of several



**Figure 2** Schematic representations of the proposed amino acid (AA) shuttles at the glutamatergic synapse involved in the return of ammonia generated in neurons when the glutamate–glutamine cycle is running

In the lactate–alanine shuttle the amino acid (AA) is alanine and the ammonia produced in neurons is fixed into  $\alpha$ -KG by the GDH reaction to form glutamate, then transaminated by alanine aminotransferase into the KA (keto-acid) pyruvate to form alanine which is exported to astrocytes. In the astrocytes this process is then reversed, and pyruvate is transported in the other direction (it is likely that pyruvate may be converted to lactate for the transfer process to occur). In the branched-chain AA shuttle the AA is a branched-chain AA such as leucine. Here, the ammonia fixed in the GDH reaction in the neurons is transaminated into the KA (keto-acid)  $\alpha$ -ketoisocaproate to form leucine, which is exported to astrocytes. In the astrocytes, the process is reversed, i.e.  $\alpha$ -ketoisocaproate is transported in the other direction. Abbreviations: Glc, glucose; Gln, glutamine; Glu, glutamate.



**Figure 3** The extent to which glutamate (GLU) is oxidized in astrocytes seems to increase particularly during higher GLU concentrations. A net synthesis of TCA cycle intermediates occurs when the initial reaction is catalysed by GDH which paves the way for the complete oxidation of the carbon skeleton of GLU. This requires pyruvate recycling via the concerted action of malic enzyme (ME) and pyruvate dehydrogenase (PDH) converting malate into acetyl-CoA producing NAD(P)H. Acetyl-CoA is oxidized completely in one turn of the TCA cycle. A partial oxidation of GLU is acquired when pyruvate (PYR) is reduced to lactate (LAC) instead of being oxidized to acetyl-CoA. The redox state of the cell is likely important in the regulation of the destiny of the GLU molecule. Alternative to the activity of GDH, AAT facilitates the formation of  $\alpha$ -KG from GLU at the expense of OAA (oxaloacetate); thus no net synthesis of TCA cycle intermediates is obtained. In contrast to the complete oxidation initiated by the activity of GDH, AAT enables the truncated TCA cycle which refers to the net synthesis of aspartate from GLU, a pathway shown to accelerate during hypoglycaemic conditions. PC, pyruvate carboxylase; CIT, citrate.

minutes (Bittner et al., 2011). On the other hand, a prolonged application of glutamate results in a switch of the astrocytic metabolism from glycolytic to oxidative, which is manifested as a stimulation of mitochondrial activity, decreased glucose uptake and decreased glycolytic lactate production (Dienel, 2004; Liao and Chen, 2003). This may be related to the fact that glutamate is an energy substrate in astrocytes (Hertz et al., 2007). It was recently shown that glutamate added to the extracellular solution containing 3 mM of glucose results in a significant decrease in cytosolic glucose concentration in astrocytes (Prebil et al., 2011a). The high glutamate concentration may interfere with the intermediates of the TCA cycle (Yu et al., 1982). This suggests that elevated glutamate may be used by astrocytes as an energy source and that glucose may be directed towards glycogen synthesis, hence a decrease in cytosolic glucose concentration. The time-course of the glucose concentration decrease has a time-constant of approximately 50 s with a delay to onset of the change of 24 s after stimulation, comparable with the delay of glutamate-stimulated hexose uptake (Loaiza et al., 2003). The glutamate together with  $K^+$  triggers an increase in

the rate of glycolysis in astrocytes (Bittner et al., 2010). On the other hand, when glutamate is added to a glucose-free extracellular medium, a slow increase in the cytosolic glucose concentration was detected (Prebil et al., 2011a). In this case the glutamate is likely to be used as an energy source (see Hertz et al., 2007) and it enabled glucose to be spared from immediate use. This mechanism was confirmed by the inhibition of glycogen mobilization using a GPa inhibitor DAB (diaminobenzidine), where such an increase in intracellular glucose after glutamate addition was attenuated (Prebil et al., 2011a). This confirms that glutamate may be used as an alternative source of energy (Swanson et al., 1990) and that the glycogen-derived glucose may be preserved in hypoglycaemic conditions. The increase in intracellular glucose in glucose-free extracellular medium is in agreement with the view that astrocytes may provide an endogenous source of brain glucose, since they express glucose-6-phosphatase- $\beta$  (Forsyth et al., 1993; Ghosh et al., 2005); however, the role of astrocytes as glucose-releasing cells by this mechanism is controversial and has to be further investigated (Dringen and Hamprecht, 1993; Forsyth, 1996).

## Modulation of glucose metabolism in astrocytes by noradrenaline, adrenaline and ATP

Receptors for multiple neurotransmitters co-exist on astrocytes and can regulate energy metabolism (Magistretti, 1988). In astrocytes, noradrenaline activates both  $\alpha$ - and  $\beta$ -adrenergic receptors (Northam et al., 1989; Hertz et al., 2010), which induces glycogen breakdown (Subbarao and Hertz, 1990a, 1991; Pellerin and Magistretti, 1994; Fray et al., 1996; Gibbs et al., 2008; Walls et al., 2009; Obel et al., 2012). In addition to rapid glycogen breakdown, noradrenaline stimulates long-term glycogen re-synthesis (Pellerin et al., 1997). The increase in glycogen turnover was found to be dependent on the activation of  $\alpha 2$  adrenergic receptors involving the  $G_i/o$ -PI3K (phosphoinositide 3-kinase) pathway in chick astrocytes (Hutchinson et al., 2011). Although  $\beta 1$ -adrenergic receptors are the predominant  $\beta$ -adrenergic receptors in mouse astrocytes, the activation of  $\beta 2$ - and  $\beta 3$ -adrenergic receptors was found to increase glucose uptake in mouse astrocytes (Catus et al., 2011). Noradrenaline-induced pyruvate decarboxylation was found to result from an increase in intramitochondrial concentration of  $Ca^{2+}$  in astrocytes (Chen and Hertz, 1999). This significantly stimulates the TCA cycle in astrocytes (Subbarao and Hertz, 1990b, 1991). The inhibition of  $\beta 2$ -adrenergic stimulation of glycogen synthesis is associated with cognitive impairment (Hertz and Gibbs, 2009).

An application of adrenaline or noradrenaline results in increased cytosolic glucose concentration from 0.3 to 0.5 mM, with the initial rates of [glc]; rising at 1.6  $\mu$ M/s (Prebil et al., 2011a). This is similar to the total glycolytic rate (1.8  $\mu$ M/s) measured in astrocytes bathed in the GLUT inhibitor cytochalasin B (Bittner et al., 2010). The  $\beta$ -adrenergic stimulation of astrocytes modulates cytosolic glucose via changes in cytosolic cAMP levels (Pellerin et al., 1997). In cells stimulated with noradrenaline and preincubated with DAB, a GPa inhibitor, cells displayed only one-third of the [glc]; increase compared with noradrenaline-stimulated cells without DAB preincubation (Prebil et al., 2011a).  $\alpha$ - and  $\beta$ -adrenergic receptors induce glycogen breakdown in astrocytes (Subbarao and Hertz, 1990a, 1991; Pellerin and Magistretti, 1994; Fray et al., 1996; Gibbs et al., 2008) and the increased availability of cytosolic glucose after adrenaline or noradrenaline stimulation suggests that the stimulated glycogen breakdown exceeds the cytosolic glucose utilization in astrocytes (Prebil et al., 2011a). On the other hand, noradrenaline-stimulated glucose uptake was also demonstrated (Yu et al., 1993).

Using the FRET-based glucose nanosensor protein FLIPglu-600  $\mu$  in 3T3-L1 fibroblasts and adipocytes revealed that the changes in cytosolic glucose concentration were only detected in 56% of 3T3-L1 fibroblasts and in 14% of 3T3-L1 adipocytes, where insulin increased cytosolic glucose concentration by a factor of 4. On the other hand, adrenaline increased cytosolic glucose concentration in fibroblasts but not in adipocytes (Kovacic et al., 2011). Similarly, adrenaline inhibits glycogen synthase and activates GP in muscle (Villa-Moruzzi et al., 1979). In astrocytes, glycogen is continuously

synthesized and degraded (Shulman et al., 2001), and lactate originating from glycogen is compartmentalized from that derived from glucose (Sickmann et al., 2005).

ATP is a major factor mediating intercellular communication and triggers a variety of different biological effects (Brake and Julius, 1996) and is considered to be the dominant extracellular messenger for astrocyte-to-astrocyte communication (Cotrina et al., 1998; Guthrie et al., 1999; Wang et al., 2000; Pangrsic et al., 2007; Parpura and Zorec, 2010). It is released from astrocytes upon mechanical stimulation (Guthrie et al., 1999) or glutamatergic receptor activation (Queiroz et al., 1997; Pangrsic et al., 2007). Astrocytes respond to ATP with a propagating wave of intracellular calcium increases (Guthrie et al., 1999), a process that is thought to serve as a long-range signalling system in the CNS (central nervous system; Cornell-Bell et al., 1990; Koizumi et al., 2005). ATP stimulation promotes exocytosis in astrocytes (Pangrsic et al., 2006) and ATP released from astrocytes as a result of neuronal activity modulates synaptic transmission (Zhang et al., 2003). Furthermore, astrocytes are capable of ATP-induced ATP release (Anderson et al., 2004). It should be noted that there is some controversy regarding release of ATP during hypoxic conditions (Martín et al., 2007; Fujita et al., 2012).

ATP is an agonist for P2Y and P2X receptors (Ralevic and Burnstock, 1998). Primary rat cortical astrocytes express ligand-gated P2X ion channels (i.e. P2X1–5 and P2X7) and G-protein-coupled P2Y receptors (i.e. P2Y1, P2Y2, P2Y4, P2Y6 and P2Y12) (Fumagalli et al., 2003). Up-regulation of receptors in astrocytes after injury has been found (Franke et al., 2001, 2004). The P2X7 subtype acts also as a permeabilization pore that can induce cell death under prolonged activation by ATP (Innocenti et al., 2004). Astrocyte-released ATP mediates a paracrine activation of microglial P2X7 receptors that triggers a perturbation of calcium homeostasis and finally leads to microglial cell death (Verderio and Matteoli, 2001).

Stimulation of isolated astrocytes with ATP decreases cytosolic glucose concentration with a time constant of approximately 150 s (Prebil et al., 2011b). The mechanism of ATP-dependent glucose concentration decrease is not yet fully understood, and may potentially affect glucose transport or metabolism. In astrocytes, purinergic receptors, particularly the P2X7 subtype, are coupled to the PI3K/Akt [also known as PKB (protein kinase B)] pathway (Jacques-Silva et al., 2004). On the other hand, in renal mesangial cells ATP was reported to activate Akt via the P2Y receptor (Huwiler et al., 2002). In astrocytes, ATP stimulates Akt phosphorylation, where calcium, PI3K and a Src family kinase are involved (Jacques-Silva et al., 2004), which may result in several cellular downstream effects related to the Akt pathway, such as glycogen synthesis (Brazil and Hemmings, 2001). Activated (phosphorylated) Akt phosphorylates and inactivates GSK3 (GS kinase 3), which is a major protein kinase involved in the regulation of glucose metabolism in muscle (Medina and Castro, 2008). Inactivated GSK3 results in less phosphorylated and thus more active glycogen synthase (Cross et al., 1995; Lawrence and Roach, 1997;

McManus et al., 2005), which may lead to a decline in cytosolic glucose levels as observed in ATP-stimulated astrocytes (Prebil et al., 2011b). On the other hand, stimulation of P2X7 receptors is associated with the activation of PKC (protein kinase C) and phospholipase D in astrocytes (Sun et al., 1999), which may represent an alternative pathway of glycogen synthase activation. ATP stimulation of P2 receptors of rat cortical astrocytes was shown to result in inhibition of GSK3 activity by a PKC-dependent pathway that is independent of Akt (Neary and Kang, 2006).

## AMINO ACID METABOLISM

Astrocytes are obviously involved in metabolism of all amino acids but this review will be focused on the role of astrocytes in the metabolic homeostasis of the two major neuroactive amino acids, glutamate and GABA ( $\gamma$ -aminobutyric acid) mediating the vast majority of excitatory and inhibitory neurotransmitter signalling respectively. The key enzymes involved in metabolic reactions pertinent to the turnover of the neurotransmitters glutamate and GABA as well as their prevailing cellular localization has recently been reviewed by Waagepetersen et al. (2009). It should be noted that GS is exclusively expressed in astrocytes (Norenberg and Martinez-Hernandez, 1979) and glutamate decarboxylase is only present in GABAergic neurons and not in astrocytes (Hertz et al., 1992). In addition, it is of functional importance that the activity of PAG (phosphate-activated glutaminase) is higher in neurons than in astrocytes (Schousboe et al., 1979; Drejer et al., 1985; Larsson et al., 1985; Lovatt et al. 2007). This difference in the expression level of GS and PAG in astrocytes forms the basis for the glutamate-glutamine cycle which was originally proposed on the basis of studies of glutamate and glutamine metabolism in brain tissue preparations which indicated different cellular compartments of these amino acids with different turnover rates (Bert et al., 1961, 1962; Van den Berg and Garfinkel, 1971). The glutamate-glutamine cycle is in short the clearance of glutamate from the synaptic cleft by uptake into astrocytes and the subsequent amidation of glutamate into glutamine which is transferred into neurons for re-synthesis of glutamate (Figure 2). Hence the cycle leads to a net transfer of nitrogen from the astrocytic to the neuronal compartment. In order to maintain nitrogen homeostasis in the 'tripartite' micro-environment, i.e. the pre- and post-synaptic neuron and the surrounding astrocyte, this nitrogen must be transferred back to the astrocyte (Figure 2). This may be accomplished by transfer of an amino acid, e.g. alanine, which is thought to be transaminated forming glutamate from which the amino group may be liberated by the action of GDH (glutamate dehydrogenase; Waagepetersen et al. 2000; Schousboe et al., 2003; Bak et al., 2006). The amino group may subsequently

take part in the GS reaction. For the shuttle to operate stoichiometrically, the GDH reaction has to operate in both directions, i.e. reductive amination in neurons and oxidative deamination in astrocytes. The high content of ammonia in the glutamatergic neurons may overcome the problem that deamination seems to be favoured (Plaitakis and Zaganas, 2001). Additionally, the branched chain amino acids have been proposed to provide the amino nitrogen for *de novo* synthesis of glutamate via pyruvate carboxylation in astrocytes followed by amidation by GS and transfer of glutamine to the neurons (Lieth et al., 2001). In order for the glutamate-glutamine cycle to operate stoichiometrically, all glutamate taken up by astrocytes via high affinity glutamate transporters (Danbolt, 2001) must be converted to glutamine in the GS-catalysed reaction (Cotman et al., 1981). However, numerous metabolic studies have demonstrated considerable oxidative metabolism of glutamate via the TCA cycle (Yu et al., 1982; McKenna et al., 1996; Sonnewald et al., 1997). The conversion of the carbon skeleton of glutamate to  $\alpha$ -KG can take place by two different enzymatic pathways, i.e. via the GDH-catalysed oxidative deamination or by transamination (Figure 3). The latter process may be catalysed by any aminotransferase, but since AAT (aspartate aminotransferase) is the member of this family of enzymes having by far the highest activity in the brain (Erecinska and Silver, 1990), this is the most likely enzyme to catalyse this reaction. It is probable that oxidative deamination catalysed by GDH plays a prominent role, since the aminotransferase inhibitor AOAA (aminooxyacetic acid) in several studies has been shown to inhibit oxidation of glutamate in the TCA cycle (e.g. Yu et al., 1982; Westergaard et al., 1996). The conclusion from the above-mentioned considerations is that a substantial fraction of the glutamate taken up into astrocytes during glutamatergic activity is oxidatively metabolized in the TCA cycle (see Westergaard et al., 1995) and hence, the glutamate-glutamine cycle is not operating stoichiometrically (McKenna et al., 2012). This imposes a need for *de novo* synthesis of the glutamate carbon skeleton which is dependent on the pyruvate carboxylase reaction that, like GS, is confined to astrocytes (Yu et al., 1983). It should also be pointed out that oxidation of the carbon skeleton of glutamate, i.e.  $\alpha$ -KG, requires pyruvate recycling (Figure 3), a process that has been shown to occur in astrocytes (Sonnewald et al., 1996; Waagepetersen et al., 2002). In this pathway, malate originating from the TCA cycle is converted to pyruvate by malic enzyme and subsequently decarboxylated by pyruvate dehydrogenase and oxidized in the TCA cycle (Bak et al., 2007; Obel et al., 2012).

The demonstration of a significant albeit low activity of PAG in cultured astrocytes (Schousboe et al., 1979) is compatible with the observation that glutamine can be oxidatively metabolized in astrocytes after conversion to first glutamate and then  $\alpha$ -KG (Yu and Hertz., 1983; Hertz et al., 1988). In line with this, the use of  $^{13}\text{C}$ -labelled glutamine and MR spectroscopy has demonstrated substantial metabolism of glutamine in astrocytes, a process

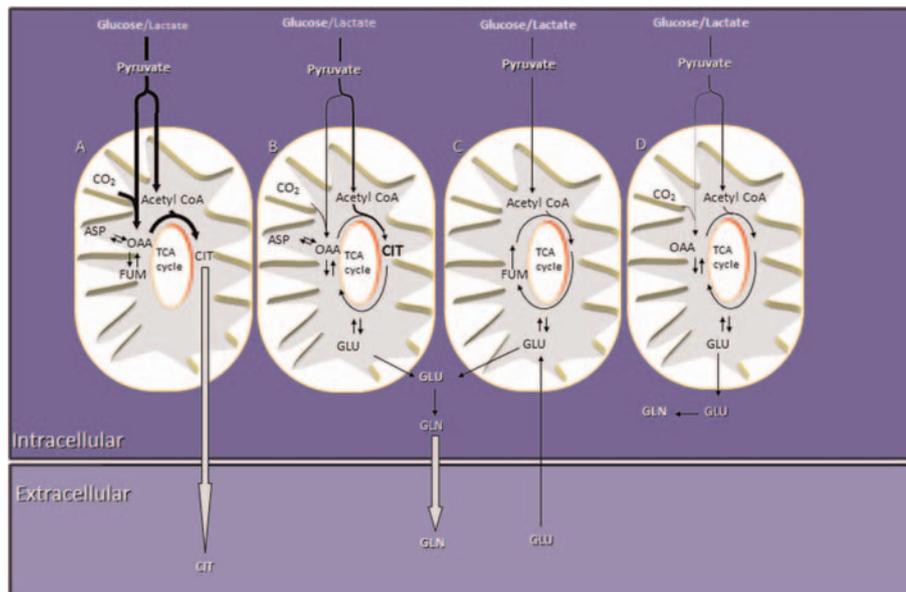
coupled to pyruvate recycling (Sonnwald et al., 1996). Metabolism of glutamine via PAG leads to production of not only glutamate but also ammonia and in the case of glutamate is oxidatively metabolized by GDH and an additional molecule of ammonia is produced. This ammonia must eventually be disposed of which likely occurs predominantly by conversion of glutamate to glutamine by GS. The fact that the PAG- and GS-catalysed reactions are intracellularly separated taking place in the mitochondrial (PAG) and the cytoplasmic (GS) compartments respectively, allows regulatory control. Nevertheless, exposure of astrocytes to elevated glutamine concentrations leads to adverse effects on mitochondria caused by ammonia liberated in the PAG reaction as demonstrated by Jayakumar et al. (2004).

Astrocytic uptake and metabolism of GABA appears to be of importance for the functional capacity of GABAergic neurotransmission, since inhibitors of astrocytic GABA transporters as well as GABA-T (GABA-aminotransferase) act as anticonvulsants (Sarup et al., 2003; Schousboe et al. 2010). GABA will be metabolized into succinic semi-aldehyde in the astrocytic mitochondria that contain appreciable activity of GABA-T (Waagepetersen et al., 2009). Succinic semi-aldehyde dehydrogenase catalyses the subsequent oxidation of succinic semi-aldehyde to succinate which may be used for glutamate and glutamine synthesis via conversion to  $\alpha$ -KG using acetyl-CoA from glucose metabolism

(Waagepetersen and Schousboe, 2007) or oxidized to CO<sub>2</sub> via pyruvate recycling.

## METABOLIC COMPARTMENTATION OF ENERGY METABOLISM

Metabolic compartmentation at the level of the single cell is defined as the presence of multiple, distinct intracellular pools of identical metabolites that are not in equilibrium. The energy metabolism of astrocytes in culture has been shown numerous times to be compartmentalized (Schousboe et al., 1993; Sonnwald et al., 1993; Bouzier et al., 1998; Qu et al., 1999; Cruz et al., 2001; Waagepetersen et al., 2001, 2006; Zwingmann et al., 2001). As mentioned above, the pyruvate pool seems to be highly compartmentalized (Figure 4) since lactate released from cultured astrocytes is derived from the metabolism of extracellular glucose but not breakdown of glycogen (Sickmann et al., 2005). This may be difficult to understand since metabolites are seemingly diffusing freely inside the aqueous environment of the cell. There are several possible answers to this; importantly, the intracellular compartment of an astrocyte, or indeed any cell, cannot be regarded as being analogous to a glass of water with all metabolites existing in a thermodynamically



**Figure 4** Schematic presentation of multiple compartments in astrocytes

Synthesis of a large amount of releasable citrate via pyruvate carboxylase occurs in compartment A, a compartment for preferential glucose metabolism. Releasable glutamine is synthesized from glutamate originating from compartments B and C. Glucose is the main oxidative substrate for compartment B, whereas lactate and glucose are metabolized to the same extent in compartments C and D. The main intracellular pool of glutamine is synthesized from glutamate originating from compartment D. The size of the arrows provide an estimate of the relative magnitudes of the respective fluxes. GLN, glutamine; GLU, glutamate; CIT, citrate; OAA, oxaloacetate; ASP, aspartate; FUM, fumarate.

ideal mixture. Cytoplasm is a very heterogeneous environment containing high concentrations of small metabolites, macromolecules, ions and multiple membrane-bound boundaries between organelles. These aspects are discussed in an almost 30-year-old review by Clegg (1984). Interestingly, recent mathematical modelling of the diffusion of metabolites inside cells shows that cytosolic compartmentation of high-concentration metabolites (e.g. glucose, lactate, pyruvate and ATP) due to molecular sinks such as enzymes and transporters should not be possible and that such compartmentation is restricted to signalling molecules such as cytosolic  $\text{Ca}^{2+}$  (Martinez et al., 2010; Barros and Martinez, 2007). Thus, metabolic compartmentation may not arise due to localized consumption of e.g. ATP or glucose. However, a number of issues complicate matters somewhat. First, the astrocyte (as well as the neuron) has a complex morphology and may be divided into functional domains (Kimmelberg and Nedergaard, 2010). Secondly, as implied above, the astrocyte may be regarded as a crowded place; metabolites and organelles are moving around in a morphologically and functionally complex manner and diverse cell in a semi-aqueous cytosolic environment with internal physical barriers (i.e. organellar membranes) to isotropic diffusion. Previous work in cardiomyocytes has shown that diffusion of ATP is anisotropic and 2–3 times slower than in dilute solution (Vendelin and Birkedal, 2008); these authors suggested that the anisotropy was caused by intracellular membranes hindering diffusion. Whether a 2–3 times slower rate of diffusion is sufficient to contribute to intracellular compartmentation of metabolism remains to be established. Thirdly, the existence of functionally and metabolically heterogeneous pools of mitochondria has been suggested and indeed mitochondria are very dynamic and heterogeneous organelles (Hollenbeck and Saxton, 2005; Waagepetersen et al., 2001). Lastly, some (astrocytic) biochemical processes, such as ATP-consuming membrane pumps, depend on ATP produced by substrate-level phosphorylation in the glycolytic pathway rather than the bulk pool of ATP; this might be explained by the formation of supramolecular complexes of enzymes and transporters such that at least some glycolytic ATP never enters the bulk cytosol (Schousboe et al., 2011). Solid evidence at the molecular level that mitochondria are indeed metabolically heterogeneous came from an immunogold-labelling experiment in cultured astrocytes conducted by Waagepetersen et al. (2006). Electron microscopic investigation of immunogold-labelled  $\alpha$ -KG dehydrogenase, a key TCA cycle enzyme, showed heterogeneous distribution among mitochondria within the same cell, indicating differential capacity for mitochondria to perform oxidative metabolism. This implies that some mitochondria may be tuned to produce energy in the form of ATP, whereas others may be tuned to perform a different task, e.g. anaplerotic reactions for synthesis of glutamine for export to neurons as a precursor for neurotransmitter glutamate and GABA synthesis (Figure 4 and Waagepetersen et al., 2001). Indeed, metabolic compartmentation of astrocytic (energy)

metabolism is complex and much is still left to be learned about this subject.

## CONCLUDING REMARKS

Brown and Prior (2006) noted that over the last few decades the methods to study brain-energy metabolism have evolved from inflating a pneumatic cuff in order to occlude the carotid arteries in 'volunteer' prisoners, to MRI (magnetic resonance imaging). They also note that there is a "desire to measure energy metabolism in a single cell (or even different regions of a single cell) in the brain in real-time" (Brown and Prior, 2006). However, some years ago this was not possible due to a limited spatial and temporal resolution of monitoring techniques. New implications of FRET-based nanosensors may exemplify such a high spatial and high temporal resolution technique to address brain energy metabolism at the subcellular level.

Pertinent research questions to be asked are many when it comes to subcellular compartmentation of (energy) metabolism, such as why are seemingly identical metabolic pathways such as glycolysis starting from extracellular glucose segregated from glycolysis starting from glucose-6-phosphate derived from breakdown of glycogen? And, are metabolically heterogeneous mitochondria localized to different cellular compartments? Heterogeneous populations of mitochondria between organs have been shown and might be therapeutically targeted (Jayakumar et al., 2007); in the same way, it might in the future be both desirable and possible to target specific cellular populations of mitochondria, the latter e.g. based on differences in their membrane potential.

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