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Role of Astrocytes in Glutamate Homeostasis: Implications for Excitotoxicity

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Glutamate homeostasis in the brain is maintained by its well balanced release, uptake and metabolism. It appears that astrocytes play a prominent role in this context since they possess a very powerful battery of glutamate transporters. Thus, malfunction of astrocytic glutamate transporters will lead to an excessively high extracellular glutamate concentration which may result in neurodegeneration caused by the excitotoxic action of glutamate.

Keywords: Astrocytes; Glutamate; Excitotoxicity; Neurotoxicity

INTRODUCTION

Glutamatergic neurotransmission involving packaging of glutamate into vesicles, exocytotic release and subsequent uptake into mainly astrocytes, and metabolism to glutamine which may be transferred back to the neuronal compartment is a costly process that may account for 3-5% of the total ATP cost related to a mean action potential (Attwell and Laughlin, 2001). It should, however, be kept in mind that glutamate in the glial compartment can be utilized as an energy substrate as it can enter the tricarboxylic acid (TCA) cycle after conversion to α-ketoglutarate (α-KG) by oxidative deamination (glutamate dehydrogenase, GDH) or transamination (primarily aspartate aminotransferase, AAT). The former enzymatic process appears to be prevalent under most conditions (see Schousboe and Waagepetersen, 2004).

METABOLISM OF GLUTAMATE

Biosynthesis

Neurotransmitter glutamate is mainly synthesized by conversion of glutamine to glutamate by phosphate activated glutaminase (Kvamme *et al.*, 2001) or by conversion of α-KG to glutamate by transamination (AAT) or reductive amination (GDH). The latter pathway involving GDH may not be quantitatively important since the GDH catalyzed reaction only rarely proceeds in direction of glutamate formation (Cooper *et al.*, 1979) due to the high NAD^+/NADH ratio in brain and the high K_m value for NH_3 of this enzyme (Zaganas *et al.*, 2001). It should be noted that AAT may also be indirectly involved in conversion of glutamine to transmitter glutamate as it has been demonstrated that inhibition of the malate-aspartate shuttle decreases availability of transmitter glutamate (Palaiologos *et al.*, 1988). This is in agreement with the recent observation that glutamate produced in brain mitochondria from glutamine *via* the action of glutaminase appears in the mitochondrial matrix (Zieminska *et al.*, 2004).

Regardless of the biosynthetic route, glutamine can only be made available in the glutamatergic neurons by surrounding astrocytes (Schousboe and Waagepetersen, 2004). This is due to the fact that the biosynthetic enzyme for glutamine, glutamine synthetase (GS) is an astrocyte specific enzyme (Norenberg and Martinez-Hernandez, 1979) and the enzymatic machinery necessary for *de novo* synthesis of TCA cycle intermediates by carboxylation involves primarily pyruvate carboxylase (PC) which is also an astrocyte specific enzyme (Yu *et al.*, 1983; Shank *et al.*, 1985).

Degradation of Glutamate

Glutamate is oxidatively metabolized in the TCA cycle after conversion to α-KG both in neurons and astrocytes (Hertz *et al.*, 1999). Complete oxidation to CO_2 requires, however, that the C-4 unit generated in the first round of the TCA cycle leaves the cycle and returns as acetylCoA after conversion to pyruvate and oxidation *via* the concerted action of malic enzyme and

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pyruvate dehydrogenase (recycling). Interestingly, this recycling process appears only to operate in astrocytes with little or no activity in neurons (Waagepetersen *et al.*, 2002). In spite of the capacity of astrocytes to oxidatively metabolize glutamate, the cellular glutamate content of these cells is around 5 mM (Erecinska and Silver, 1990).

GLUTAMATE TRANSPORT

Five transporters for glutamate have been cloned and they are named EAAT1-5 (Gegelashvili and Schousboe, 1998; Danbolt, 2001). Among these, EAAT1 (GLAST) and EAAT2 (GLT-1) are the most prominent and interestingly they are expressed preferentially if not exclusively on the astrocytic plasma membrane (Danbolt, 2001; Levy, 2002) providing these cells with an enormous capacity for glutamate uptake as already observed decades ago (Schousboe *et al.*, 1977; Hertz *et al.*, 1978). With regard to the actual process of inactivation, it has been proposed that binding of glutamate to the carriers rather than the translocation process *per se* may be instrumental as the cycling time of the carriers is rather long (Wadiche *et al.*, 1995). Nevertheless, ultimately the transport of glutamate into astrocytes for metabolism is necessary to maintain a low extracellular concentration of glutamate. As discussed below this is a prerequisite to avoid an excitotoxic action of glutamate (Choi and Rothman, 1990; Schousboe and Frandsen, 1995).

GLUTAMATE RELEASE

Physiological Conditions

Glutamate release may occur either from vesicular stores or by reversal of glutamate transporters. Vesicular release is triggered by a depolarization signal which activates voltage- and/or receptor-operated Ca^{++} channels (McMahon and Nicholls, 1991). Classically this phenomenon was thought to be a characteristic of glutamatergic neurons but it is now experimentally well supported that a similar release mechanism exists in astrocytes (Volterra and Meldolesi, 2004). The functional importance of the astrocytic release is, however, so far only poorly understood but very likely astrocytes could play a modulatory role in glutamatergic neurotransmission.

Release of glutamate mediated by reversal of the glutamate transporters may, due to the dependency of inward transport on an intact Na^{+}-gradient, be stimulated by a depolarization signal (Szatkowski *et al.*, 1990; Jensen *et al.*, 2000; Bak *et al.*, 2003). Contrary to vesicular release, release mediated by reversal of transporters primarily involves the cytoplasmic glutamate pool which very likely represents the metabolic pool (Waagepetersen *et al.*, 2001).

Energy Failure

Due to the fact that vesicular glutamate transporters are ATP dependent, vesicular release will be decreased during energy failure (Nicholls and Attwell, 1990). Contrary to this, energy failure will lead to release of glutamate *via* reversal of the plasma membrane transporters since the inward transport process is energy dependent (Rossi *et al.*, 2000; Danbolt, 2001). This latter release is reflected in the large increase in the extracellular glutamate concentration seen during ischemia and hypoglycemia (Benveniste *et al.*, 1984; Hagberg *et al.*, 1985; Sandberg *et al.*, 1986; Phillis *et al.*, 2000). Due to the exceedingly high density of glutamate transporters on astroglial cells (Levy, 2002) it is likely that these cells contribute significantly to the above mentioned overflow of glutamate during energy failure. The direct involvement of the transporters in this process is underlined by the repeated demonstration that glutamate release from brain cell or tissue preparations caused by lack of glucose and oxygen can be substantially reduced by application of the non-transportable glutamate transport blocker threo-ß-benzyloxyaspartate (TBOA) (Phillis *et al.*, 2000; Bonde *et al.*, 2003a). These aspects are illustrated schematically in fig. 1. It may be of considerable interest in this context that using organotypic hippocampal slice cultures which allow monitoring of a possible role of glutamate overflow by quantification of neuronal damage and cell death, it has been directly demonstrated that glutamate transporters are involved in neuronal death caused by energy failure (Bonde *et al.*, 2003a). Thus, in these cultures neuronal death induced by oxygen and glucose deprivation could be almost completely ameliorated by TBOA. On the contrary, under physiological conditions TBOA dose-dependently induced neuronal death by preventing glutamate removal by the transporters. That neuronal death was indeed induced by excess glutamate was demonstrated by the finding that it could be blocked by glutamate receptor antagonists (Bonde *et al.*, 2003a). These observations are in agreement with previous studies demonstrating that down-regulation of glutamate transporters and in particular GLT-1 is associated with neuronal degeneration (Rothstein *et al.*, 1996; Rao *et al.*, 1998). Moreover, neuronal degeneration following transient global ischemia is preceded by down-regulation of GLT-1 which may be related to the neurodegeneration process since low extracellular levels of glutamate cannot be maintained even during the reperfusion period (Torp *et al.*, 1995; Fujita *et al.*,

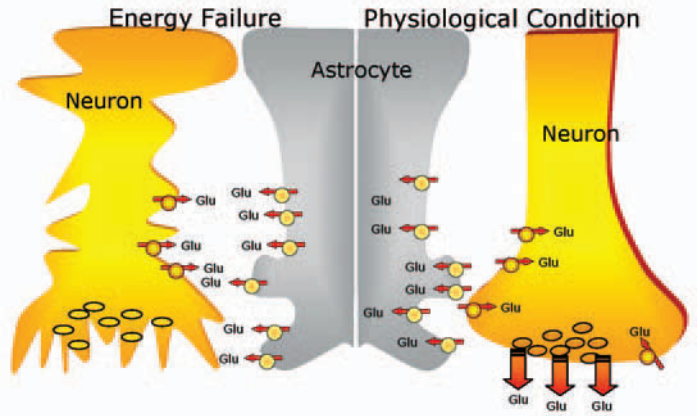

FIGURE 1 Schematic representation illustrating the changes in glutamate release and uptake processes induced by energy failure. Vesicular release is abolished and glutamate transporters are reversed causing a massive increase in the extracellular glutamate concentration. The neuron to the left is undergoing excitotoxic degeneration.

1999; Rao *et al.*, 2000).

Another finding which underlines the importance of the glutamate transporters for neurodegeneration induced by energy failure also originates from studies on organotypic hippocampal slice cultures. It was found that treatment of such cultures with glial cell-line derived neurotrophic factor (GDNF) up-regulated the expression of the glial glutamate transporters GLAST and GLT-1 (Bonde *et al.*, 2003b). In spite of the previous observation that GDNF acutely protects neurons in these cultures from glutamate induced neurotoxicity (Bonde *et al.*, 2000), it was found that the pre-treatment with GDNF resulting in up-regulation of the glutamate transporters greatly increased the neurodegeneration induced by oxygen and glucose deprivation (Bonde *et al.*, 2003b). A high expression level of the transporters accordingly can become detrimental under conditions of energy failure leading to release of glutamate *via* reversal of the transporters. This should be kept in mind when developing strategies aiming at enhancing the capacity for glutamate uptake. An alternative explanation for the neuronal degeneration observed in these cultures might be that it occurred subsequent to a GDNF induced up-regulation of NMDA receptor expression. This was, however, found not to be the case and therefore the cultures are unlikely to have changed their endogenous sensitivity to glutamate mediated neurotoxicity *via* activation of NMDA receptors (Bonde *et al.*, 2003b).

Other Adverse Conditions

In addition to the condition of energy failure which impairs uptake and enhances release of glutamate, it has been reported that the neurodegenerative disease amyotropic lateral sclerosis (ALS) is associated with an impairment of glutamate transporter activity (Rothstein *et al.*, 1992; 1995; Ferrarese *et al.*, 2001). It may be of interest in this context that it was recently reported that a neuroblastoma cell line expressing superoxide dismutase characteristic of familial ALS exhibits a decreased capacity for glutamate uptake (Sala *et al.*, 2004). This might be related to sensitivity of glutamate transporters to reactive oxygen species generated due to a lower ability to reduce the level of ROS in the cells expressing the mutant dismutase (Sala *et al.*, 2004). If this is indeed the case this finding underlines the importance of an intact machinery for glutamate uptake.

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