Neurochemistry International xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Review

Role of glutamate transporters in redox homeostasis of the brain

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Redox
Glutathione (GSH)
System x_c^- (SXC)
Excitatory Amino Acid Transporter (EAAT)
Reactive oxygen species (ROS)
Glioma

ABSTRACT

Redox homeostasis is especially important in the brain where high oxygen consumption produces an abundance of harmful oxidative by-products. Glutathione (GSH) is a tripeptide non-protein thiol. It is the central nervous system's most abundant antioxidant and the master controller of brain redox homeostasis. The glutamate transporters, System x_c^- (SXC) and the Excitatory Amino Acid Transporters (EAAT), play important, synergistic roles in the synthesis of GSH. In glial cells, SXC mediates the uptake of cystine, which after intracellular reduction to cysteine, reacts with glutamate during the rate-limiting step of GSH synthesis. EAAT3 mediates direct cysteine uptake for neuronal GSH synthesis. SXC and EAAT work in concert in glial cells to provide two intracellular substrates for GSH synthesis, cystine and glutamate. Their cyclical basal function also prevents a buildup of extracellular glutamate, which SXC releases extracellularly in exchange for cystine uptake. Maintaining extracellular glutamate homeostasis is critical to prevent neuronal toxicity, as well as glutamate-mediated SXC inhibition, which could lead to a depletion of intracellular GSH and loss of cellular redox control. Many neurological diseases show evidence of GSH dysfunction, and increased GSH has been widely associated with chemotherapy and radiotherapy resistance of gliomas. We present evidence suggesting that gliomas expressing elevated levels of SXC are more reliant on GSH for growth and survival. They have an increased inherent radiation resistance, however, inhibition of SXC can increase tumor sensitivity at low radiation doses. GSH depletion through SXC inhibition may be a viable mechanism to enhance current glioma treatment strategies and make tumors more sensitive to radiation and chemotherapy protocols.

1. Introduction

Many important biological processes involve redox reactions, and as a result, produce potentially dangerous byproducts. Oxidative phosphorylation, or oxidative metabolism, provides brain cells with most of their energy requirements. In fact, human brain cells use approximately 20% of the total oxygen consumed by the body, although the brain only makes up 2% of body weight (Clarke and Sokoloff, 1999). Reactive oxygen species (ROS) are continuously generated as a result of this large usage of oxygen, and therefore mechanisms are required to regulate the redox state of the brain. An unbalanced redox state and buildup of reactive oxygen species results in cell death and detrimental consequences for the brain.

Oxidative stress occurs if an imbalance exists between oxidant production and neutralization. ROS, as free radical species, are highly reactive compounds. If they are not neutralized by cellular antioxidants, they create DNA damage and protein/enzyme oxidation, which leads to cellular dysfunction and death (Brooker, 2011).

A number of characteristics of the brain make it more vulnerable to oxidative stress. In addition to the amount of ROS produced by its high oxygen consumption (Clarke and Sokoloff, 1999), some areas of the brain contain a high content of iron (Gerlach et al., 1994), which catalyzes ROS generation (Dringen, 2000), and the large amount of unsaturated fatty acid lipids found in the brain are targets for lipid peroxidation (Porter, 1984; Halliwell, 1992). Surprisingly, the levels of activity of common enzymes that catalyze the neutralization of free radicals, specifically superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), are lower in the brain than in other organs such as the liver and kidney (Ho et al., 1997).

Common pathologies including neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS) involve ROS-mediated oxidative stress as part of their etiology (Dringen, 2000; Surmeier et al., 2011; Dalle-Donne et al., 2008; Dringen and Hirrlinger, 2003; Uttara et al., 2009; Zundorf and Reiser, 2011). Even normal aging may be, at least in part, due to ROS. The "free radical theory of aging" hypothesizes that free radical induced cellular damage builds up over time, ultimately resulting in aging and death [see Richman and Meister, 1975 for more on this topic]. Many mechanisms exist to regulate redox homeostasis. Here, we focus on the main

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http://dx.doi.org/10.1016/j.neuint.2014.01.001

mechanisms of redox control in the brain. We review the role of glutamate transporters in the production of the intracellular antioxidant glutathione (GSH) and its importance in redox homeostasis. We also discuss the role of glutamate transporter mediated redox imbalance in disease, and present new evidence suggesting their involvement in the radiation resistance of gliomas.

2. Redox regulation in the brain

Reactive oxygen species are generated in cells by the mitochondrial respiratory chain, which occurs on the inner mitochondrial membrane (Murphy, 2009; Jensen, 1966; Dickinson and Chang, 2011). Interestingly, although most cancer cells are thought to primarily rely on glycolysis for energy production (Gatenby and Gillies, 2004), they produce an even higher level of ROS than normal cells (Szatrowski and Nathan, 1991). Different theories exist as to how cancer cells produce ROS, including enhanced metabolism (Hlavata et al., 2003), mitochondrial mutations and malfunction (Carew et al., 2003), and chronic inflammation (Hussain et al., 2003). Regardless, the production of ROS in both non-malignant and malignant cells necessitates the production of intracellular antioxidants to neutralize these free radicals to prevent cell death.

2.1. Glutathione

Glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine) is an important non-protein thiol that is found in all organs including the brain (Sagara et al., 1993; Watanabe and Bannai, 1987; Deneke and Fanburg, 1989; Pastore et al., 2003). It is the most abundant antioxidant in the central nervous system (CNS), and is found in the brain at concentrations around 1–3 mM (Dringen, 2000). In addition to its antioxidant role, GSH has many non-antioxidant functions. Some of these roles include storage and transport of cysteine (Meister and Anderson, 1983), regulation of apoptosis (van den Dobbelsteen et al., 1996; Ghibelli et al., 1998; Hall, 1999), cell proliferation (Poot et al., 1995), signal transduction (Janaky et al., 1999), and immune response (Paolicchi et al., 2002). As a thiol, it consists of three amino acids: L-cysteine (Cys), L-glutamate (Glu), and L-glycine (Gly). γ-glutamylcysteine synthetase mediates the rate-limiting step of GSH synthesis, combining Glu and Cys to form γ-glutamylcysteine (γGluCys). Glutathione synthetase then incorporates Gly to form GSH. Adenosine triphosphate (ATP) is required for the activity of both of these enzymes (Dringen and Hirrlinger, 2003; Meister and Anderson, 1983; Misra and Griffith, 1998). Regulation of GSH synthesis is accomplished through a feedback mechanism, where GSH regulates the synthesis of γ-glutamylcysteine synthetase (Richman and Meister, 1975), and, in turn maintains balance of GSH synthesis and consumption.

Intracellularly, GSH undergoes both enzymatic and non-enzymatic reactions during the detoxification of ROS. Non-enzymatically, GSH interacts directly with superoxide anions, hydroxyl radicals, and nitric oxide (Dringen, 2000). Enzymatically, GSH detoxifies peroxidases in a reaction mediated by glutathione peroxidase (Fig. 1). During this reaction GSH is oxidized to glutathione disulfide (GSSG). Glutathione reductase catalyzes the regeneration of GSH, forming a glutathione redox cycle (Chance et al., 1979). In turn, glutathione reductase relies on nicotinamide adenine dinucleotide phosphate (NADPH) to reduce GSSG back to GSH. The redox status of cells depends on the ratio of reduced and oxidized glutathione (GSH/GSSG). Under normal conditions the reduced form exceeds the oxidized form by a ratio of nearly 1:100, however, under oxidative stress conditions, this ratio can be reduced to values as low as 1:1 (Pastore et al., 2003). As oxidative stress is neutralized, GSSG is regenerated to GSH, and the homeostatic ratio is re-established.

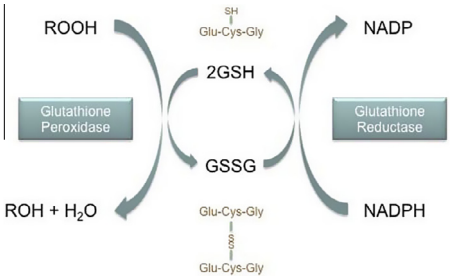

Fig. 1. Intracellular GSH/GSSG redox cycle. Glutathione (GSH) mediates the detoxification of reactive oxygen species (represented by the generic peroxide, ROOH) through an enzymatic reaction involving glutathione peroxidase. The sulfhydryl, or thiol, group (SH) serves as the proton donor in these reactions. GSH donates a reducing equivalent (H^+ and e^-) and in turn is converted to its oxidized form, glutathione disulfide (GSSG), which is created by the formation of a disulfide bond between two GSH molecules. GSSG is then reduced back to GSH by the enzyme glutathione reductase, which relies on NAPDH as an electron donor. The GSH:GSSG ratio indicates the redox state of cells. In healthy cells, greater than 90% of the glutathione is in the reduced GSH form. Oxidative stress can be indicated by a decrease in this ratio.

GSH is a critical molecule in the CNS that protects against oxidative stress induced cellular damage. Its loss is associated with brain cell death (Cooper and Kristal, 1997). Glutathione is found in neurons and glia (Dringen, 2000; Pileblad et al., 1991; Amara et al., 1994; Hjelle et al., 1994), including astrocytes (Dringen, 2000), oligodendrocytes (Wang et al., 2004) and microglia (Hota et al., 2008). Evidence suggests neurons and glial cells are all vulnerable to ROS damage and that they all rely upon glutathione-mediated detoxification for protection [see (Murphy, 2009) for a review on the role of glutathione in different brain cells]. Astrocytes are thought to contain the highest concentrations of glutathione in the brain (Rice and Russo-Menna, 1998), and are therefore considered to play the largest role in ROS detoxification (Peuchen et al., 1997; Juurlink, 1997; Wilson, 1997). In culture, astrocytes protect neurons and oligodendrocytes against many toxic compounds (Noble et al., 1994; Hochman et al., 1998; Noel and Tofilon, 1998; Desagher et al., 1996), further supporting the idea that astrocytes are the main regulators of oxidative stress in the brain.

Astrocytes extend their protection to neuronal cells through the export of GSH into the extracellular space. Neurons rely on extracellular cysteine for their synthesis of GSH (Sagara et al., 1993; Kranich et al., 1996; Dringen and Hamprecht, 1999) and this cysteine is provided by cysteine-precursors from astrocytic glutathione release (Dringen et al., 1999). The export of GSH is mediated by a family of multidrug resistance proteins (MRPs) (Borst et al., 1999; Leslie et al., 2001; Paulusma et al., 1999). Once released, astroglial ectoenzyme γ-glutamyl transpeptidase (γGT) acts on GSH, creating a CysGly compound(Dringen et al., 1997). Aminopeptidase N (ApN) then hydrolyzes CysGly to individual amino acids Cys and Gly (Dringen et al., 2001), which are both then available for import into neurons.

2.2. Glutamate transporters

2.2.1. System x_c^-

The GSH/GSSG redox cycle uses and recycles intracellular GSH. However, since some intracellular reactions consume GSH and it is released from cells for extracellular functions, intracellular GSH levels would become depleted if not regularly synthesized (Dringen, 2000). Intracellular GSH synthesis requires availability of its amino acid precursors, with the incorporation of cysteine being rate limiting. Cystine (Cys_2) is a dimeric amino acid that is formed by a disulfide bond between two cysteine molecules. Cysteine is not stable extracellularly due to oxidizing conditions that favor

Please cite this article in press as: Robert, S.M., et al. Role of glutamate transporters in redox homeostasis of the brain. Neurochem. Int. (2014), http://dx.doi.org/10.1016/j.neuint.2014.01.001