Analytical Biochemistry 529 (2017) 127–143

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Glutathione in the human brain: Review of its roles and measurement by magnetic resonance spectroscopy

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ARTICLE INFO

Article history:
Received 18 July 2016
Received in revised form
21 December 2016
Accepted 23 December 2016
Available online 26 December 2016

Keywords:
Glutathione
Magnetic resonance spectroscopy
Brain
Glyoxalase
Psychiatric disease
Neurological disease

ABSTRACT

We review the transport, synthesis and catabolism of glutathione in the brain as well as its compartmentation and biochemistry in different brain cells. The major reactions involving glutathione are reviewed and the factors limiting its availability in brain cells are discussed. We also describe and critique current methods for measuring glutathione in the human brain using magnetic resonance spectroscopy, and review the literature on glutathione measurements in healthy brains and in neurological, psychiatric, neurodegenerative and neurodevelopmental conditions In summary: Healthy human brain glutathione concentration is ~1–2 mM, but it varies by brain region, with evidence of gender differences and age effects; in neurological disease glutathione appears reduced in multiple sclerosis, motor neurone disease and epilepsy, while being increased in meningiomas; in psychiatric disease the picture is complex and confounded by methodological differences, regional effects, length of disease and drug-treatment. Both increases and decreases in glutathione have been reported in depression and schizophrenia. In Alzheimer's disease and mild cognitive impairment there is evidence for a decrease in glutathione compared to age-matched healthy controls. Improved methods to measure glutathione *in vivo* will provide better precision in glutathione determination and help resolve the complex biochemistry of this molecule in health and disease.

1. Biology and biochemistry of glutathione

1.1. Introduction

Glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine: (2*S*)-2-amino-4-{[(1*R*)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl] carbamoyl}butanoic acid) is a tripeptide thiol found in virtually all cells. Essential for cellular function, it plays roles in oxidation-reduction reactions, acts as a cofactor in enzyme reactions, protects against reactive oxygen species and potentially toxic xenobiotics, provides storage for cysteine and regulates cellular events including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation [1].

Two of the most challenging chemical species to deal with in biological systems are reactive oxygen species produced as a consequence of aerobic metabolism and an aerobic environment, and the production of oxoaldehydes, which are formed as a function of the oxidation of molecules such as glucose. The glutathione

Abbreviations: GSH, glutathione; GSSG, oxidized glutathione; NMDA, *N*-methyl-D-aspartate; GCL, glutamate-cysteine ligase; GCLC, glutamate-cysteine ligase subunit with catalytic activity; GCLM, glutamate-cysteine ligase subunit with modifier activity; MRS, magnetic resonance spectroscopy; PRESS, Point Resolved SpectroScopy; MEGA-PRESS, Mescher-Garwood Point resolved spectroscopy; BSO, L-buthionine-*S*,*R*-sulfoximine; MSO, methionine sulfoximine; ASC1, neutral amino acid transporter 1; EAAT3, excitatory amino acid transporter 3; ROS, reactive oxygen species; MRP, multidrug resistance pump; GST, glutathione-S-transferase; NAA, *N*-acetyl aspartate; STEAM, stimulated echo acquisition mode; 2D-COSY, 2-dimensional chemical shift correlated spectroscopy; TR, repetition time; TE, echo time; CRLB, Cramer-Rao Lower Bounds; BOLD, blood oxygenation level dependent; fMRI, functional magnetic resonance imaging; SPECIAL, Spin-echo full intensity acquired localized.

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http://dx.doi.org/10.1016/j.ab.2016.12.022
0003-2697/

system was developed early in evolution as a solution to deal with both of these chemical challenges [2]. Since both oxygen and glucose are key ingredients in brain function, there is therefore considerable interest in this molecule and in the measurement of it *in vivo*. Here, we review what is known about glutathione biosynthesis, metabolism and roles in the brain and also review best practice measurement of glutathione *in vivo* using ^{1}H magnetic resonance spectroscopy (MRS). There are a number of excellent, older reviews on glutathione in the central nervous system which retain much relevance if further reading is sought [3,4].

1.2. Where is glutathione found in the brain?

Studies using staining for sulfhydryl compounds have shown extensive brain staining for GSH with the common theme of relatively little reactivity in neuronal somata. Staining for GSH appears confined mostly to the neuropil and white matter tracts with the exception of some neurons in the cerebellum, such as cerebellar granule cells and Purkinje cells [5,6].

A study using two-photon imaging of monochlorobimane fluorescence [7], a selective enzyme-mediated marker for reduced GSH, reported high levels in lateral ventricle ependymal cells (2.73 ± 0.56 mM) and meningeal cells (1.45 ± 0.09 mM), with lower levels in astroglial cells (0.9 ± 0.08 mM) and relatively low levels in cortical neurons (0.21 ± 0.02 mM). Levels of GSH were up to threefold higher in developing neurons (e.g. in the subgranular zone of the dentate gyrus) than in older neurons in the neighbouring granular layer [8]. Earlier work, which measured GSH content then calculated GSH values for glia and neurons based on relative volumes, reported glial GSH as 3.8 mM vs 2.5 mM for neurons but these calculations could have been biased by higher concentrations of GSH in other cell types which were not estimated, such as ependymal cells [9]. Values in oligodendrocytes at various stages of development have been reported as less than half the levels found in astrocytes (i.e. ~12 vs 28 nmol/mg protein [10]. Total values reported in biopsies of fresh human cerebral cortex range from 0 to 0.77 μmol/g wet weight for GSH and 0.24–1.22 μmol/g wet weight for glutathione disulfide (GSSG) [11]. This contrasts with values recorded from cells in culture with levels in human neurons reported at 93 ± 13 μmol/g protein and 177 ± 10 μmol/g protein in astrocytes [12].

Careful study of the relative amounts of reduced vs oxidized glutathione has shown levels of GSSG in rat and monkey brain to be 1.2% [13], or less [14], of total glutathione indicating that previous reports of levels as high as 40% GSSG in brain are likely erroneous. Induction of ischemia in rat brain has been shown to decrease GSH without any concomitant increase in GSSG [15], while induction of oxidative stress by lipid peroxidation did result in accumulation of GSSG [14] showing different mechanisms at work in these conditions. While it is plain that the ratio of GSH/GSSG is altered in various disease states (e.g. Ref. [16]) and that the levels of total glutathione may also be reduced under pathological conditions (Table 1) it is also apparent that care must be taken when measuring GSH and GSSG.

The concentration of glutathione in extracellular fluid is relatively low. It has been reported in the rat cortex via microdialysis measurement at 2.10 ± 1.78 μM (N = 18 [17]; and in the caudate nucleus (2.0 ± 0.1), rising in the latter case under potassium depolarization to 3.0 ± 0.9 μM (N = 3; [18]. Glutathione efflux is also increased by application of *N*-methyl-D-aspartate (NMDA) and kainate and by imposition of ischaemia or hypoxic conditions [19].

In summary, the location of glutathione in the brain is highly tissue dependent and the majority of it is located in non-neuronal cells.

1.3. Synthesis of glutathione

Glutamate, the major excitatory neurotransmitter in the brain, is a precursor for GSH synthesis. It is combined with cysteine, an amino acid with a free sulfhydryl group, in an ATP-dependent reaction catalyzed by γ-glutamylcysteine synthetase (more properly glutamate-cysteine ligase; GCL; E.C. 6.3.2.2) to form γ-glutamylcysteine (Fig. 1). This dipeptide is then further combined with glycine, another potential neurotransmitter, in another ATP-dependent reaction catalyzed by glutathione synthase (E.C. 6.3.2.3).

The enzyme GCL is often referred to as “rate limiting” although in neurons, for example, the enzyme has been shown to have ample capacity if given sufficient substrate, particularly cysteine, whose levels are highly controlled in neurons [7]. The enzyme is known to be composed of two dissociable subunits; a heavy (73 kDa) chain which possesses catalytic activity (GCLC) and a light (27.7 kDa) chain which is a modifier (GCLM) [20]. Although the catalytic subunit GCLC can produce γ-glutamylcysteine, the presence of the modifier increases enzyme activity; *gclm* $^{-/-}$ mice show tissue levels of GSH between 9 and 40% of those of the wild-type [21]. The presence of GCLM lowers the K_M for glutamate and ATP and increases the catalytic rate of the enzyme complex [22].

These two subunits are encoded in mice and men by separate genes located on different chromosomes [23]. The ability of GCLM to modify GCL enzyme activity has made the *gclm* locus the subject of investigation across a range of condition. Alterations in GCLM have been associated with disorders such as schizophrenia [24] but others have indicated that the association does not hold in independent patient populations [25,26] nor in self-reported depression [27].

The apparent K_M of GCL for glutamate (1.8 mM) is well below the cellular concentration of glutamate (~8–12 mM), as estimated from numerous ^{1}H MRS studies *in vivo*. There are few reliable reports of glutamate concentrations within neurons in situ, with values reported from as high as 5.77 mM in rat cerebellar parallel fibre terminals [28]) while the apparent K_M for cysteine is considerably lower at 0.1–0.3 mM, more proximate to the concentration of cysteine within the cell. The activity of the enzyme is regulated by non-allosteric feedback competitive inhibition by GSH (Ki = 2.3 mM) thus controlling intracellular levels of both GSH and cysteine [29].

GLC can be irreversibly and rapidly inhibited by L-buthionine-*S*,*R*-sulfoximine (BSO) with limiting pseudo-first order rate constant of 3.7 min ($t_{1/2} \approx 11$ s) [30]as well as by methionine sulfoximine (MSO) [31], a commonly used inhibitor of glutamine synthetase [32]. Judicial use of lower concentrations of MSO and relatively short incubation times, however, mean that MSO can be used to inhibit glutamine synthetase without significant effects on glutathione concentrations [33]. Depletion of brain glutathione in rats with BSO administration for 10 days resulted in decreased complex IV and citrate synthase activity as well as decreased brain *N*-acetylaspartate levels, suggesting mitochondrial and neuronal damage [34], in agreement with an earlier study showing gross mitochondrial enlargement and degeneration [35].

The final step in GSH synthesis, catalyzed by glutathione synthetase, is generally considered to have less metabolic control over the rate of glutathione synthesis. It is a homo-dimer and its absence results in the most common of the inborn errors of GSH metabolism with around 25% of sufferers dying early, such as during the neonatal period [36]. There is some suggestion that supplementation with vitamin C from an early age may improve long term outcomes [37]; this may boost neuronal antioxidant capacity in the absence of glutathione.

The time constant for GSH turnover in rat brain has been reported as 13 ± 2 h or about 0.05% of the overall metabolic rate of