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OVERVIEW

Glutathione-Dependent Detoxification Processes in Astrocytes

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Abstract Astrocytes have a pivotal role in brain as partners of neurons in homeostatic and metabolic processes. Astrocytes also protect other types of brain cells against the toxicity of reactive oxygen species and are considered as first line of defence against the toxic potential of xenobiotics. A key component in many of the astrocytic detoxification processes is the tripeptide glutathione (GSH) which serves as electron donor in the GSH peroxidase-catalyzed reduction of peroxides. In addition, GSH is substrate in the detoxification of xenobiotics and endogenous compounds by GSH-S-transferases which generate GSH conjugates that are efficiently exported from the cells by multidrug resistance proteins. Moreover, GSH reacts with the reactive endogenous carbonyls methylglyoxal and formaldehyde to intermediates which are substrates of detoxifying enzymes. In this article we will review the current knowledge on the GSH metabolism of astrocytes with a special emphasis on GSH-dependent detoxification processes.

Special Issue: In honor of Gerald Dienel.

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Introduction

Glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine) is a tripeptide which is present in millimolar concentrations in most cell types including astrocytes [1]. GSH has essential cellular functions in many detoxification processes. In addition, to its chemical reaction with radicals and electrophiles, GSH serves as substrate or cofactor of a large number of detoxifying cellular enzymes [2]. Due to the dependence of many cellular processes on GSH it is not surprising that an impaired GSH metabolism has been connected with human diseases [3–6]. This appears to be especially the case for neurological disorders as ample evidence connects disturbances in the GSH metabolism of the brain with the progression of neurological disorders [7, 8], including Parkinson's disease [9], Alzheimer's disease [10], multiple sclerosis [11] as well as schizophrenia and bipolar disorder [12]. Also alterations of cognitive functions with ageing have been correlated with a decline in GSH content in brain and with impaired GSH-dependent functions [13].

Astrocytes have been reviewed to have important functions in synapse formation and modulation [14, 15], in brain metabolism [16–21] in the defence against oxidative stress [22–24], as well as in the homeostasis of essential metals [24–27]. Astrocytes are also considered to play an important role in the GSH metabolism of the brain [7, 16, 23, 28]. The high cellular GSH content and the strong capacity of astrocytes for GSH-dependent detoxification processes helps to protect these cells but also their neighbours against the toxic potential of oxidants and toxins

[28–30]. In addition, astrocytes supply essential precursors for GSH synthesis to neighbouring neurons. In co-cultures, the presence of astrocytes maintains [31] or increases GSH levels in neurons [32, 33]. Especially the availability of cysteine determines the level of neuronal GSH. The supply by astrocytes of the cysteine required for neuronal GSH synthesis includes the export of GSH from astrocytes, extracellular processing of the exported GSH by ectoenzymes, and uptake of the GSH precursor amino acids into neurons [8, 23, 28].

As astrocytes cover with their end feet almost completely the brain capillaries, astrocytes are the first parenchymal brain cells which come into contact with energy substrates and amino acids that are delivered via the blood to the brain [34]. However, astrocytes will also encounter as first parenchymal brain cells potentially toxic compounds which have crossed the blood brain barrier. Thus, the strategically important localization of astrocytes as well as their strong detoxification potential defines astrocytes as first line of defence against toxins and xenobiotics that enter the brain. The metabolism of GSH in brain and the pivotal role of astrocytes in the GSH homeostasis of the brain have previously been extensively reviewed [23, 28, 35–37]. Here we summarize the current knowledge on the synthesis and metabolism of GSH in astrocytes with a focus on the GSH-dependent detoxification processes in these cells.

GSH Metabolism of Astrocytes

GSH Synthesis and Consumption

GSH is synthesized in astrocytes by two ATP-consuming cytosolic enzymes (Fig. 1), as in other cell types. In the first reaction, glutamate cysteine ligase (GCL) catalyzes the formation of the dipeptide γ-glutamylcysteine (γGluCys) from the amino acids glutamate and cysteine. This reaction is the rate limiting and regulated step in cellular GSH synthesis. The current knowledge on structure, function and regulation of GCL in GSH synthesis has recently been summarized [38]. The second reaction involved in GSH synthesis is catalyzed by GSH synthetase (GSH-Syn) which adds glycine in an ATP-driven reaction to γGluCys to generate the tripeptide GSH (Fig. 1). The activity of both GCL and GSH-Syn has been determined in cultured astrocytes [39–41]. Continuous synthesis of GSH is required in astrocytes to compensate for a continuous consumption of GSH in these cells. This has been demonstrated by exposure of cultured astrocytes to the GCL inhibitor buthionine sulfoximine (BSO), which lowers the astrocytic GSH content with a half-time of around 5 h [42].

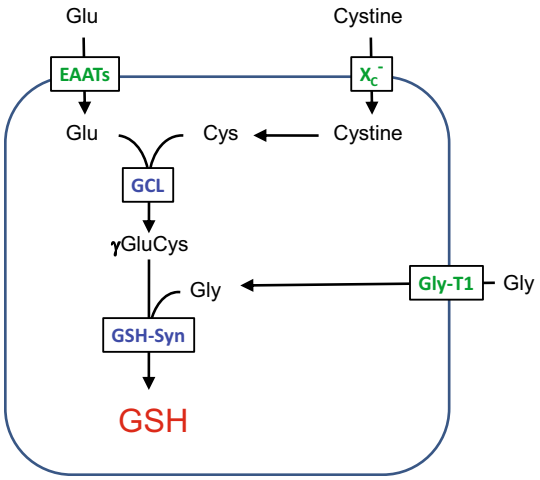

Fig. 1 Synthesis of GSH in astrocytes. GSH is synthesized by the consecutive reactions of glutamate cysteine ligase (GCL) and GSH synthetase (GSH-Syn) from the amino acid substrates glutamate (Glu), cysteine (Cys) and glycine (Gly). Preferred extracellular amino acid precursors for astrocytic GSH synthesis are the GSH substrates glutamate and glycine which are taken up into astrocytes by the excitatory amino acid transporters (EAATs) 1 or 2 and by the glycine transporter GlyT1, respectively. The cysteine required as substrate for GSH synthesis is predominately derived from extracellular cystine which is taken up into astrocytes by the cystine-glutamate antiporter X_C^-

The cellular content of GSH is determined by the rates of its synthesis and consumption. Concerning the synthesis of GSH, the activity of the enzymes involved in GSH synthesis, the intracellular availability of the substrates glutamate, cysteine and glycine as well as a feedback inhibition of GCL by high cellular GSH concentrations determine the rate of GSH synthesis. Accordingly, various compounds and treatments which foster GSH synthesis increase cellular GSH contents in cultured astrocytes (Table 1). In addition, also the inhibition of GSH consumption, for example the inhibition of GSH export, can contribute to an elevated cellular GSH content. At least cultured astrocytes from mice that are deficient of the multidrug resistance protein (Mrp) 1, the transporter predominantly responsible for astrocytic GSH export, contain higher specific GSH contents compared to cultures from wildtype mice [43].

The transcription of the GCL and GSH-Syn genes is regulated by the cis-acting antioxidant response element (ARE) which itself is controlled by the nuclear transcription factor erythroid-2-related factor 2 (Nrf2) [44, 45]. Under unstressed conditions Nrf2 is present in the cytosol in its inactive form, bound and targeted for degradation by the Kelch-like ECH-associated protein 1 (KEAP1). During oxidative stress or in presence of Nrf2 activators, Nrf2 is released from KEAP1 and translocates into the nucleus where it induces the transcription of genes of various

Table 1 Substances that increase GSH contents in astrocytes

Substance	Reference
Acrylonitrile	[157]
Ammonium	[90, 158]
Anethole dithiolethione	[159]
Arsenite	[91]
Arsenate	[89]
Cadmium chloride	[89, 91]
Catalpol	[160]
Copper chloride	[89, 92]
Copper oxide nanoparticles	[93]
Curcumin	[41, 87]
1[2-Cyano-3,12-dioxool-eana-1,9(11)-dien-28-oyl] trifluoroethylamide	[46]
1,25-Dihydroxyvitamine D3	[161]
Dimethylfumarate	[162]
Epigallocatechin-3-gallate	[137]
Ethylpyruvate	[48]
Fibroblast growth factor 1	[95]
Glutamate	[163]
GSH ethyl ester	[164]
3H-1,2-dithiole-3-thione	[165]
High glucose	[70]
Methylmercury	[166]
Monomethylfumarate	[47]
Quercetin	[41]
Resveratrol	[167]
Tumor necrosis factor α	[168]
Tertiary butyl hydroquinone	[41, 49, 95]
Tetrathiomolybdate	[93]
Thyroid hormone	[169]

This table lists compounds and treatments that have been reported to increase cellular GSH levels in cultured astrocytes within an incubation period of 24 h

detoxifying and antioxidant enzymes [44, 45]. In astrocytes, synthesis of both GCL and GSH-Syn, as well as of other proteins involved in GSH-dependent detoxification processes, are stimulated via the Nrf2 pathway during oxidative stress and by the presence of Nrf2-activators [44, 46]. Accordingly, a number of different compounds which activate the Nrf2-ARE pathway increase the GSH content of cultured astrocytes [41, 44, 46–51].

In addition to the activity of GSH synthesis enzymes, the availability of the amino acid precursors determines and limits astrocytic GSH synthesis [1, 35]. The substrate amino acids for GSH synthesis can either be directly taken up from the extracellular space or they can be generated from cellular or extracellular precursor molecules. Astrocytes express the sodium-dependent excitatory amino acid transporter (EAAT) 1 and 2 [52] and the glycine transporter GlyT1 [53] which allow efficient accumulation of the GSH substrates glutamate and glycine (Fig. 1). The best extracellular source of the cysteine required for astrocytic GSH synthesis is cystine which is reduced after uptake in astrocytes to cysteine [54, 55]. Astrocytes take up cystine predominately by the cystine-glutamate antiporter X_C^-, but also other transporters have been discussed to contribute to this import [56, 57]. In addition to these amino acids, astrocytes can use a large number of other amino acids, metabolites or peptides as precursors of the GSH synthesis substrates glutamate, cysteine or glycine [1, 58].

Astrocytes need GSH as substrate for various pathways (Fig. 2). GSH is essential for the detoxification of radicals and peroxides (Fig. 3) as well as the reactive carbonyls formaldehyde and methylglyoxal (Fig. 4). In these pathways GSH is not consumed as it is regenerated by the final enzymatic steps of the respective pathways (Figs. 3, 4). In contrast, the conjugation of GSH by GSH-S-transferases (GSTs) to electrophilic compounds and the export of GSH from the cells (Fig. 2) lower cellular GSH contents and require GSH synthesis to replenish a high cytosolic GSH concentration.

GSH Redox Cycling

Radicals and peroxides are continuously generated in oxygen-consuming cells and a network of cellular antioxidative mechanisms prevent an accumulation of such reactive oxygen species (ROS) and ROS-induced oxidative damage of cellular macromolecules. GSH is involved in two important types of antioxidative reactions (Fig. 3a–c). GSH can react directly with radicals such as superoxide or hydroxyl radicals in non-enzymatic reactions, thereby reducing these radicals (Fig. 3a). In addition, GSH serves as electron donor for the reduction of organic hydroperoxides (Fig. 3b) or hydrogen peroxide (Fig. 3c) in reactions catalyzed by glutathione peroxidases (GPx). In both types of reactions, GSH is oxidized to glutathione disulfide (GSSG) (Fig. 3a–c).

For vertebrates, 8 isoforms of GPx have been described which differ in their catalytic center, their substrate preference and in their subcellular localization [59]. Of those isoforms, GPx1 is expressed in cultured astrocytes and contributes substantially to the detoxification of hydrogen peroxide and organic hydroperoxides [60, 61]. In addition, cultured astrocytes express GPx3 and enhance the secretion of this isoform after exposure to angiogenin [62]. In vivo, astrocytes strongly upregulate the expression of GPx4 after brain injury [63].

The GSSG generated by GSH-dependent reduction of ROS is reduced by the flavoenzyme glutathione reductase (GR) (Fig. 3d). This enzyme is expressed and active in

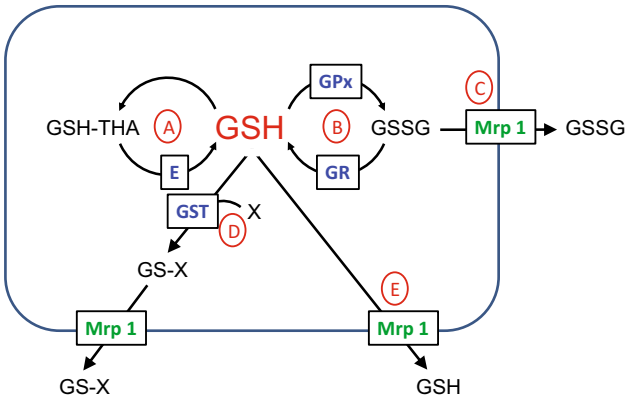

Fig. 2 GSH-dependent metabolism in astrocytes. *A* GSH reacts with carbonyls (formaldehyde or methylglyoxal) to GSH-thiohemiacetals (GSH-THA) which serve as substrates for detoxifying reactions catalyzed by the enzymes (E) alcohol dehydrogenase (ADH) 3 or glyoxalase 1. *B* The GSH-dependent reduction of peroxide by glutathione peroxidases (GPx) produces GSSG which is rapidly reduced by glutathione reductase (GR) to regenerate GSH. *C* If substantial amounts of GSSG accumulate in astrocytes, these cells export GSSG via multidrug resistance protein (Mrp) 1. *D* Conjugation of GSH to electrophilic compounds (X) in reactions that are catalyzed by GSH-S-transferases (GSTs). GSH-conjugates (GS-X) are exported from astrocytes via Mrp1 or other transporters. *E* Mrp1-mediated export of GSH from astrocytes

cultured astrocytes [64, 65]. GR needs NADPH as electron donor [66], which is generated in the cytosol of astrocytes mainly by the pentose phosphate pathway [67–70]. Due to the low micromolar K_M-values of GR for NADPH and GSSG [66], the small amounts of GSSG generated during normal metabolism are rapidly reduced to GSH. Thus, for unstressed conditions GSSG is hardly detectable in astrocytes and the ratio of GSH to GSSG is very high. However, if GR in astrocytes is inhibited by exposure to carmustin or zinc ions, GSSG accumulates in the cells and the normally high ratio of GSH to GSSG decreases [71]. During oxidative stress GR can become rate limiting for GSH-GSSG redox cycling which causes a transient increase in cellular GSSG levels and a corresponding decline in the GSH to GSSG ratio [68, 72, 73]. Under such conditions, GSSG can be exported from astrocytes (Fig. 2) by Mrp1 [43, 74, 75].

GSH Export and Extracellular GSH Metabolism

Cultured astrocytes have been shown to export up to 10 % of their GSH per hour [76]. The astrocytic GSH export is predominately mediated by Mrp1 [43, 77] which belongs to the Mrp family of ATP-driven export pumps [78–80]. In addition to Mrp1, astrocytes express a number of other Mrps and other potential GSH exporters in culture and in vivo [43, 81–86], but the contribution of these transporters in astrocytic export processes remains to be elucidated. GSH has also been shown to be released from astrocytes via gap junction hemichannels under certain conditions [87, 88].

The K_M-value for GSH export from astrocytes is around 25 mM [89] and the cytosolic GSH concentration of untreated astrocytes is around 8 mM [1]. Consequently, treatments which increase the GSH concentration in astrocytes should lead to an increase in the rate of cellular GSH export. This has been confirmed for astrocytes that contained higher GSH contents due to a pre-incubation with ammonium [90], arsenate [89], arsenite [91], cadmium chloride [89, 91], copper chloride [89, 92], copper oxide nanoparticles [93], nitric oxide [94] or with fibroblast growth factor 1 and tertiary butyl hydroquinone [95].

Recently a number of compounds have been reported to strongly stimulate rapid GSH export from viable astrocytes, including formaldehyde [96], arsenate and arsenite [97, 98] and antiretroviral protease inhibitors [99, 100]. Although all of these stimulated GSH export processes were almost completely prevented by inhibition of Mrp1, the molecular mechanism how such structurally very diverse compounds accelerate Mrp1-mediated GSH export

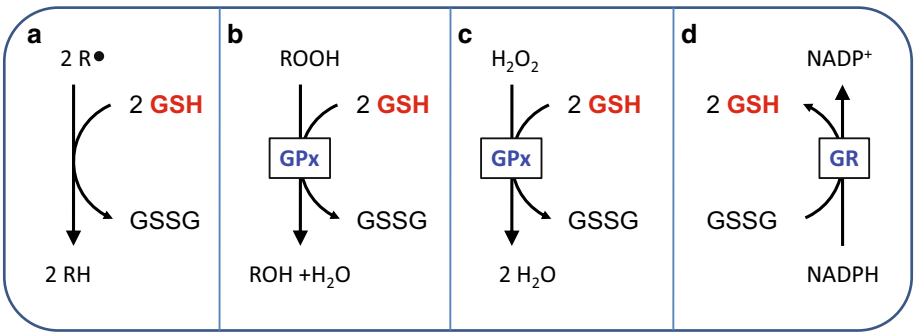

Fig. 3 GSH-GSSG redox cycling in astrocytes. GSH reacts with radicals (R·) in an enzyme-independent reaction (**a**) or with organic hydroperoxides (**b**) or hydrogen peroxide (**c**) in GPx-catalyzed reactions to GSSG and the reduced derivative of the respective reactive oxygen species (ROS). The GSSG generated during the GSH-dependent reduction of ROS (**a**–**c**) is reduced by GR in an NADPH-dependent reaction (**d**)

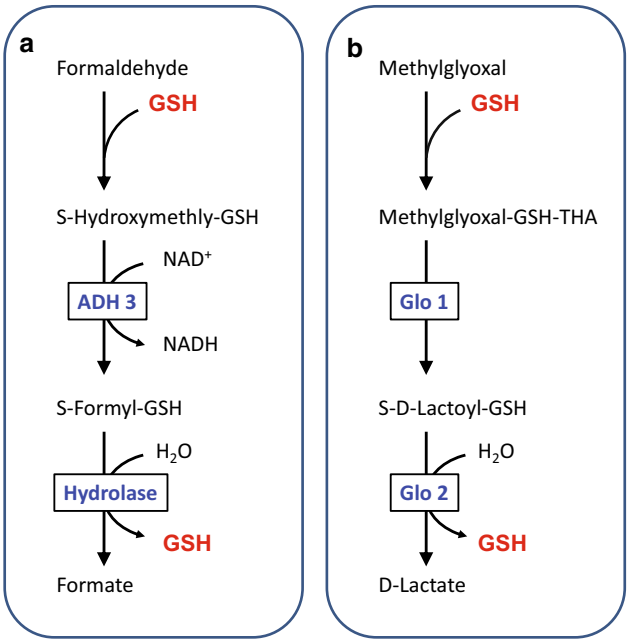

Fig. 4 GSH-dependent detoxification of formaldehyde and methylglyoxal in astrocytes. GSH reacts with formaldehyde (**a**) or methylglyoxal (**b**) in enzyme-independent reactions to the respective thiohemiacetals (THA). S-Hydroxymethyl-GSH is substrate of alcoholdehydrogenase (ADH) 3 and becomes oxidized to S-formyl-GSH which is subsequently hydrolyzed to formate and GSH (**a**). The methylglyoxal-GSH-THA is converted by glyoxalase (Glo) 1 to S-D-lactoyl-GSH which is the substrate of Glo2 to become hydrolyzed to D-lactate and GSH (**b**)

remains unclear. At least for the formaldehyde-stimulated GSH export from astrocytes it was shown that the V_{max}-value for the export was strongly increased while the K_M-values remained unchanged [89], suggesting that formaldehyde treatment of astrocytes may lead to a recruitment of Mrp1 from intracellular vesicles into the plasma membrane. Such a translocation of Mrp1 between intracellular and membrane location has previously been shown for bilirubin-treated astrocytes [101]. Also the upregulation of Mrp1 in astrocytes appears to increase GSH export. At least the upregulation of Mrp1 expression by a treatment of cultured astrocytes with the HIV1 envelope glycoprotein gp120 was accompanied by an elevated export of GSH and GSSG [102].

GSH which has been exported from astrocytes is a substrate of the ectoenzyme γ-glutamyl transpeptidase (γ-GT) [76]. γ-GT transfers the γ-glutamyl moiety of GSH to an acceptor which can be an amino acid, a peptide but also water [103]. Extracellular processing by γ-GT of the GSH released from astrocytes is a crucial step in the supply of GSH precursors from astrocytes to neurons [33]. A product of the reaction catalyzed by astrocytic γ-GT is the dipeptide CysGly which is either taken up into astrocytes by the dipeptide transporter PepT2 [104] or can be cleaved by the neuronal aminopeptidase N [105] to provide cysteine and glycine to neurons. These amino acids are efficiently taken up by neurons [106, 107] and can be used as substrates for neuronal GSH synthesis [33, 55].

GSH in Astrocytic Detoxification Processes

GSH and Peroxide Detoxification

Among the peroxides that are continuously produced by the cellular metabolism, hydrogen peroxide is quantitatively the most important one. This peroxide is formed predominantly by disproportionation of superoxide which can occur enzyme-independently or is catalyzed by superoxide dismutases [22]. Superoxide itself is generated in mitochondria as byproduct of respiratory chain complexes and enzymes [108] and as product of the NADPH oxidase reaction [109]. Hydrogen peroxide is also formed in the reactions of some oxidases, for example monoamine oxidases [110]. A second class of cellular peroxides are organic hydroperoxides which include stereospecifically-defined prostaglandin and leukotrine hydroperoxides that are products of cyclooxygenases and lipooxygenases, respectively, as well as various hydroperoxides that are generated by unspecific oxidation of polyunsaturated fatty acids in membranes by radical-mediated lipid peroxidation [111].

Cellular hydrogen peroxide is detoxified in astrocytes predominantly by the enzymes GPx and catalase [22]. Cultured astrocytes efficiently dispose of exogenous hydrogen peroxide with half-times in the minute range [72, 112–114]. The specific clearance rates of astrocytes for hydrogen peroxide are similar to values reported for cultured neurons and microglial cells but higher than those found for cultured oligodendrocytes [22, 113, 115, 116]. In hydrogen peroxide clearance both the GSH system and catalase are involved and the capacity of each of the two contributing enzyme systems is sufficiently high to compensate at least in part for an impairment of the other system [72, 112]. Cultured astrocytes also efficiently dispose of exogenous organic hydroperoxides such as tertiary butyl hydroperoxide (tBHP) and cumene hydroperoxide (CHP) with half-times similar to that for hydrogen peroxide [61, 68, 73, 114, 117]. As inhibition of catalase does not affect the clearance of organic hydroperoxides by astrocytes, only the GSH system appears to be involved in the disposal of these peroxides [61, 68, 73, 117]. This view is supported by the strong increase in the half-time of the peroxides in astrocytes that had been pre-incubated with BSO to lower cellular GSH contents, with mercaptosuccinate to inhibit GPx or in the absence of glucose which

slows NADPH regeneration by pentose phosphate pathway [68, 73].

Another class of enzymes which can contribute to the cellular peroxide disposal are peroxiredoxins [2, 118]. Concerning GSH dependent processes, especially peroxiredoxin 6 is of interest as this peroxiredoxin requires for peroxide reduction GSH and involves GSTs [2]. Peroxiredoxin 6 is expressed in cultured astrocytes and its expression is upregulated by the Nrf2 activator tertiary butyl hydroquinone which accelerates the clearance of tBHP and increases the resistance of astrocytes towards hydrogen peroxide and tBHP [114].

The ability of cultured astrocytes to detoxify hydrogen peroxide is affected by ageing. At least cultured astrocytes derived from old mice have a slower hydrogen peroxide clearance rate than astrocytes cultured from young mice [119]. This may be caused by a lowered GR activity and an increased GSH export rate observed for astrocytes cultured from old mice [119].

GSH-GSSG redox cycling is the base of the GSH-dependent peroxide detoxification in astrocytes. During the GPx-catalyzed reduction of peroxides, GSH becomes oxidized to GSSG (Fig. 3b, c) which is subsequently reduced to GSH by GR (Fig. 3d). For cultured astrocytes this is demonstrated by the transient occurrence of GSSG in cultures that had been exposed to hydrogen peroxide [60, 72, 117, 120], tBHP [73] or CHP [61, 68]. Among the different isoforms of GPx, especially the cytosolic isoform GPx1 appears to be important for astrocytic peroxide detoxification as astrocytes cultured from GPx1-deficient mice dispose hydrogen peroxide and CHP much slower than wild type cells, did not show a transient increase in cellular GSSG contents and were more vulnerable towards peroxide mediated toxicity [60, 61].

Severe oxidative stress or conditions that make GR rate limiting for GSH-GSSG redox cycling lead to a strong accumulation of cellular GSSG. Under such conditions, astrocytes export efficiently GSSG via Mrp1. This has been demonstrated for astrocytes which have been exposed to chronic hydrogen peroxide stress [43, 74], to high concentrations of dopamine [75] or to zinc chlorid [71].

GSH as Cofactor in the Detoxification of Reactive Carbonyls

GSH is the essential substrate for the cellular pathways that detoxify the reactive carbonyls formaldehyde and methylglyoxal. These carbonyls are endogenously generated during normal metabolism and react non-enzymatically with GSH to thiohemiacetals which serve as substrates for an enzymatic conversion of the respective carbonyl to a less reactive compound which can be released from cells (Fig. 4). Efficient detoxification of formaldehyde and methylglyoxal appears to be especially important for the brain as disturbances of the respective metabolic pathways have been connected with neurological disorders and ageing [121–123]. Recent studies suggest that astrocytes have a prominent role in the detoxification of formaldehyde and methylglyoxal in brain.

Formaldehyde

Formaldehyde is generated in substantial amounts in the human body during normal metabolism. The formaldehyde concentration in blood is around 0.1 mM [124], while formaldehyde concentrations in brain have been reported to be even higher [125]. The steady state formaldehyde concentrations in brain are maintained low due to the effective action of enzymes which oxidize formaldehyde to formate [122]. Cellular formaldehyde oxidation involves either the mitochondrial aldehyde dehydrogenase (ADH) 2 and/or the cytosolic GSH-dependent formaldehyde dehydrogenase (ADH3) [122]. The ADH3 reaction requires the presence of S-hydroxymethyl-GSH which is generated by the enzyme-independent reaction of formaldehyde with GSH (Fig. 4a). Oxidation of S-hydroxymethyl-GSH by ADH3 generates S-formyl-GSH which is hydrolyzed by a thiolase to regenerate GSH and to generate formate [122].

Cultured astrocytes contain the mRNAs of enzymes which generate and oxidize formaldehyde including ADH3 [126], suggesting that astrocytes are able to produce and to detoxify formaldehyde. Indeed, cultured astrocytes remove exogenously applied formaldehyde very efficiently with a rate of around 0.2 μmol/(h × mg) [126]. The K_M-value for formaldehyde clearance by cultured astrocytes is around 0.19 mM, suggesting that the cytosolic ADH3 which has a low micromolar K_M-value for its substrate S-hydroxymethyl-GSH contributes substantially to astrocytic formaldehyde oxidation [126].

Astrocytes convert the majority (>90 %) of exogenously applied formaldehyde to formate that is subsequently exported from the cells [126], indicating that the S-formyl-GSH generated by the ADH3 reaction is rapidly hydrolyzed in these cells to GSH and formate. The observed rate of formaldehyde oxidation to formate by astrocytes is with 0.23 μmol/(h × mg) in a range similar to that of glucose consumption (0.73 μmol/(h × mg) [126], underlining the high capacity of astrocytes to detoxify formaldehyde. A similar formaldehyde clearance rate was recently reported for cultured neurons, although these cells release less formate than astrocytes [127]. The formate generated via formaldehyde oxidation may also be harmful for brain cells, as formate-induced inhibition of mitochondrial respiration is the likely reason for the observed accelerated glycolytic flux in formaldehyde-treated astrocytes and neurons [126, 127].

Methylglyoxal

Methylglyoxal is a ubiquitous product of cellular glucose metabolism and is predominately formed by non-enzymatic decomposition of the glycolysis intermediates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate [128]. In order to avoid unspecific glycation reactions of proteins, the reactive methylglyoxal is efficiently detoxified in cells by the GSH-dependent glyoxalase system which consists of the two enzymes glyoxalase 1 and glyoxalase 2. The enzyme-independent reaction of methylglyoxal with GSH generates a thiohemiacetal that is the substrate of glyoxalase 1 (Fig. 4b). This enzyme converts the thiohemiacetal to the thioester S-D-lactoyl-GSH which is subsequently hydrolyzed by glyoxalase 2 to GSH and D-lactate [128].

In brain, especially astrocytes appear to have a highly efficient glyoxalase system to detoxify methylglyoxal. Both glyoxalase 1 and glyoxalase 2 are strongly expressed in cultured astrocytes and the specific activity determined for this enzyme is higher in astrocytes than in cultured neurons [129]. Strong immunoreactivity for glyoxalase 1 was also found for astrocytes in mouse and rat brain [129, 130]. Cultured astrocytes convert exogenously applied methylglyoxal more efficiently to D-lactate than neurons [129]. Also the higher toxic potential of methylglyoxal to cultured neurons compared with cultured astrocytes as well as the protection by astrocytes of co-cultured neurons against methylglyoxal induced toxicity demonstrates the high capacity of astrocytes to detoxify this carbonyl [129].

Conjugation of GSH with Endogenous Compounds and Xenobiotics

GSH is an essential substrate for the cellular detoxification of endogenous and exogenous compounds by GSTs which covalently conjugate GSH via its thiol group to the respective electrophilic substrate (Fig. 2). Although this type of detoxification lowers the cellular GSH concentration, it is of advantage as GSH-conjugates are in most cases less toxic than their precursors and will be actively exported from cells by Mrps [79, 85]. Endogenous substrates of GSTs are for example intermediates of the eicosanoid metabolisms but also lipid peroxides and reactive aldehydes such as 4-hydroxynonenal [131]. In addition, various electrophilic compounds of exogenous origin (xenobiotics) are substrates of GSTs [132]. For cultured astrocytes a large number of reactive compounds have been reported to rapidly lower the cellular GSH content (Table 2). However, it is currently not known to which extent astrocytic GSTs contribute to the rapid reactions of such compounds with GSH.

Table 2 Substances that acutely lower astrocytic GSH contents

Compound	References
Acrylonitrile	[170]
3-Bromopyruvate	[171]
Chloroacetate	[172]
Diethylmaleate	[173–176]
Dimethylfumarate	[51, 162]
Deoxyribose	[177]
Ethacrinic acid	[139]
Formaldehyde	[96]
4-Hydroxynonenal	[50, 137]
Jodoacetamide	[178]
Jodoacetate	[175, 178]
Methylmercury	[166]
Monochlorobimane	[179]
Monomethyfumarate	[162]
Tetrachlorocarbon	[173]
L-trans-pyrrolidine-2,4-dicarboxylate	[180]

This table lists compounds that have been reported to decrease cellular GSH levels in cultured astrocytes within minutes to hours

GSTs represent a large superfamily of enzymes which are encoded by a polymorphic gene superfamily [133, 134]. GSTs differ in their cellular localization (cytosolic, microsomal or mitochondrial). However, already for the cytosolic GSTs seven subfamilies (alpha, mu, pi, sigma, theta, omega and zeta) have been classified [133, 134]. Little is known on the expression and functions of the various isoforms of GSTs in brain astrocytes. Rodent astrocytes were shown to express mu- and alpha-type GSTs as well as microsomal GSTs [135–138]. GST activity was also found in mitochondrial fractions of cultured astrocytes [139]. Chromatographic methods allowed to separate the cytosol of cultured astrocytes into several fractions which differed regarding their GST activity towards various GST substrates [140]. The expression of members of different GSTs in astrocytes is likely to be regulated separately. At least a treatment of astrocytes with conditioned media from activated microglial cells increased the expression of GST-pi1 and GST-mu3, while treatment with conditioned media from non-activated microglial cells only increased GST-pi1 but decreased GST-mu3 expression [141]. As another trigger ethyl pyruvate was identified to elevate GST levels in primary astrocytes via activation of Nrf2 [48].

Alterations in the expression of GST isoenzymes in brain have been connected to neurological disorders including Alzheimer's disease [142], epilepsy [143] and Parkinson's disease [144, 145]. However, it remains to be elucidated whether the expression or activity of astrocytic GSTs are altered in such diseases.

Conclusions and Perspectives

Astrocytes have a key function in the GSH-dependent detoxification processes in brain. These cells have a high capacity for GPx-catalyzed peroxide reduction, for the detoxification of methylglyoxal and formaldehyde, as well as for the GST-catalyzed conjugation reactions of xenobiotics and endogenous compounds. As all these processes require sufficiently high concentrations of intracellular GSH in astrocytes, the different detoxification processes may under certain conditions interfere with each other and even compete for the substrate GSH. For example, an acute decline of cellular GSH by an alkylating substance will lower GSH-dependent peroxide reduction. Furthermore, impairments of astrocytic GSH synthesis, recycling and export are likely to affect the GSH-dependent detoxification processes in astrocytes which may harm these cells directly but may also increase the toxic potential of oxidants and toxins for other brain cells. At least the protection by astrocytes of co-cultured neurons against various toxins is compromized, if astrocytes contain low levels of GSH [146–148].

Most of the data described in this review article were obtained in experiments performed on cultured primary astrocytes which are considered as a suitable model system to investigate properties and metabolic functions of astrocytes [149, 150]. Confirmation of a given cell culture result on GSH-dependent detoxification processes for the in vivo situation is in many cases difficult to obtain and remains a challenge. Nevertheless, results from future studies on the GSH-dependent pathways in astrocytes in vivo are highly warranted.

Although GSH synthesis takes place exclusively in the cytosol, the presence of GSH has been reported for many other cellular compartment, including nucleus [151], mitochondria [152] and lysosomes [153]. For astrocytes, at least the presence of GSH and GSH-dependent enzymes in mitochondria as well as the transport of GSH in astrocytic mitochondria have been investigated [139, 154, 155]. Future studies are now required to elucidate in more detail the subcellular distribution of GSH in astrocytes, the particular functions of GSH in the different compartments and the transport mechanisms which are responsible for intracellular trafficking of GSH in astrocytes.

Impairments in GSH-dependent detoxification processes in astrocytes as well as the supply of GSH precursors by astrocytes to neurons are likely to contribute to disturbances in brain GSH homeostasis as well as to neural damage and cognitive impairments reported for neurological disorders which have been connected with alteration in GSH metabolism in the brain. Accordingly, strategies which would help astrocytes to maintain a high cellular GSH concentration, including delivery of GSH as pro-drugs or with carriers [156], could prove beneficial for the defence of the brain against oxidative stress and toxins.

Conflict of interest The authors declare that they have no conflict of interest.

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