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Review

# Multiple Roles of Glutathione in the Central Nervous System

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**Glutathione is a storage form of cysteine and protects against reactive oxygen species and potentially toxic xenobiotics in the central nervous system. Marked reductions in intracellular or intramitochondrial glutathione are associated with cell death. Enzymes involved in glutathione metabolism are very active in the choroid plexus, and astrocytes maintain a high concentration of glutathione. Astrocytes probably play an important role in regulating cerebral sulfur/glutathione metabolism and in protecting the brain against noxious chemicals. Oxidative stress contributes to age-related neurodegenerative diseases. Patients with inborn errors of glutathione metabolism often exhibit progressive neurological problems. Therefore, increasing brain glutathione levels may have therapeutic benefits.**

*Key words:* Anti-oxidants / γ-Glutamyl cycle / Glutathione / Glutathione disulfide / Reactive oxygen species.

## Introduction

Glutathione (GSH) was first discovered by J. de Rey-Pailhade over 100 years ago and its structure (i.e. L-γ-glutamyl-L-cysteinylglycine) was deduced in the 1930s. [See Meister (1989) for a discussion of historical perspectives.] GSH is present in most mammalian cells at concentrations ranging from 0.5 to 12 mM (~ 2–3 mM in whole brain) (Cooper, 1997).

GSH may exert its anti-oxidant activity synergistically with both ascorbate (Jain *et al.*, 1994; Meister, 1995) and vitamin E. Vitamin E is essential for normal neurological function and is both a free radical scavenger and structural stabilizer (see discussion in Heslop *et al.*, 1996). The link between GSH and vitamin E is apparent from the fact that dietary selenium (an essential component of glutathione peroxidase) is known to prevent many symptoms of vitamin E deficiency (Heslop *et al.*, 1996). Dehydroascorbate resulting from oxidation of ascorbate is reduced by GSH (and NADPH) in brain (Rose, 1993). Patients with disorders of GSH metabolism apparently exhibit an increased reliance on ascorbate as an antioxidant, and many display progressive neurological disease. These observations underscore the importance of GSH for normal brain function. [See Cooper (1997) for a recent review].

Generally, GSH serves as:

a) A non-toxic storage form of cysteine,
b) an enzyme cofactor,
c) a component in metabolic (and transport) pathways, and
d) a protectant against reactive oxygen species (ROS) and potentially harmful xenobiotics.

(For discussions on the physiological roles of GSH see, for example, Meister and Anderson, 1983; Meister, 1989). A scheme that shows interrelationships among these functions is presented in Figure 1. The general properties of GSH are briefly discussed below, followed by a more detailed description of its metabolism in brain and possible therapeutic interventions designed to increase GSH.

## Glutathione Is a Non-Toxic Storage Form of Cysteine

An important physiological role of GSH is as a carrier and storage form of cysteine. Elevated cysteine/cystine is excitotoxic, possibly by interfering with the *N*-methyl-D-aspartate (NMDA) receptor. Cysteine also has more generalized toxic effects through possession of a reactive sulfhydryl group that forms hemithioketals with α-keto acids and hemithioacetals with aldehydes. An example of reactivity with aldehydes is the formation of a hemithioacetal with pyridoxal 5′-phosphate. This adduct cyclizes to a thiazolidinone thereby inhibiting some key pyridoxal 5′-phosphate-containing enzymes, such as glutamate decarboxylase. In contrast, the sulfhydryl of GSH is less reactive, permitting concentrations in mammalian tissues to be ~10–100 times higher than those of cysteine. (Reviewed by Cooper, 1997).

## Cofactor Functions

GSH is an essential cofactor for a number of enzymes including formaldehyde dehydrogenase, glyoxylase, maleylacetoacetate isomerase, dehydrochlorinase, and prostaglandin endoperoxidase isomerase. In these reactions, GSH is not consumed but presumably plays a critical role in the catalytic mechanism (Meister, 1989).

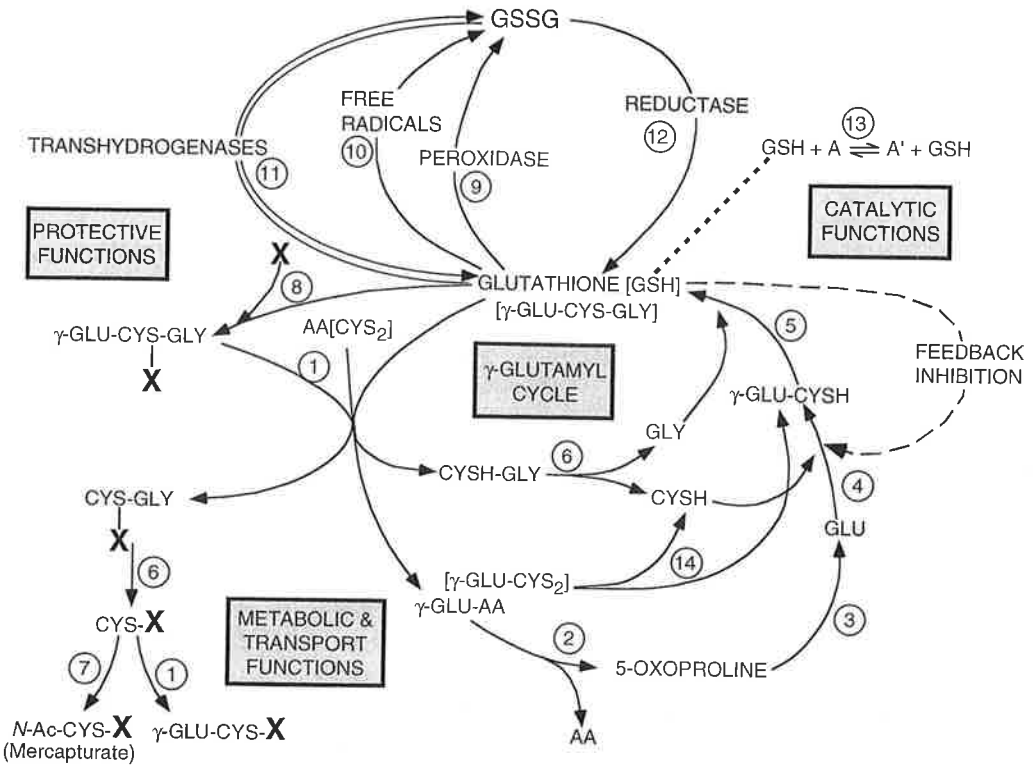


**Fig. 1** Metabolism of Glutathione (GSH).
(1) γ-Glutamyltranspeptidase; (2) γ-glutamylcylotransferase; (3) 5-oxoprolinase; (4) γ-glutamylcysteine synthetase; (5) glutathione synthetase; (6) cysteinylglycine dipeptidase; (7) L-cysteine *S*-conjugate *N*-acetyltransferase; (8) glutathione *S*-transferase; (9) glutathione peroxidase; (10) free radical quenching (probably non-enzymatic); (11) glutathione transhydrogenase; (12) glutathione disulfide (GSSG) reductase; (13) enzymatic reactions in which GSH is required as a cofactor but is not consumed; (14) transport of γ-glutamylcystine and reduction to γ-glutamylcysteine. AA, amino acids; CYSH, cysteine; CYS2, cystine; **X** = compounds that form conjugates with glutathione. Modified from Meister (1989).

## Metabolism of GSH and Possible Transport Functions

In 1970, Orlowski and Meister noted that enzymes involved in GSH metabolism may be linked in such a way as to form a process for the recycling of the constituent amino acids of the tripeptide. This process was named the γ-glutamyl cycle (Figure 1). GSH is synthesized by the consecutive actions of the ATP-dependent enzymes γ-glutamylcysteine synthetase (reaction 4) and glutathione synthetase (reaction 5). Levels of GSH are regulated in part by feedback inhibition of γ-glutamylcysteine synthetase by GSH. In the presence of a suitable amino acid [AA] acceptor, GSH is catabolized by the action of γ-glutamyltranspeptidase (reaction 1) to yield a γ-glutamyl amino acid [γ-GLU-AA] and cysteinylglycine [CYSH-GLY]. The γ-GLU-AA is converted to free amino acid and 5-oxoproline by the action of γ-glutamylcyclotransferase (reaction 2). 5-oxoproline is converted back to glutamate by the ATP-dependent 5-oxoprolinase reaction (reaction 3). The glutamate released in this process can then be reused in the synthesis of GSH, thus completing the cycle with the glutamate component of GSH. The cysteinylglycine released in the γ-glutamyltranspeptidase reaction is hydrolyzed by the action of a dipeptidase (reaction 6) to glycine and cysteine, completing the cycle with the glycine and cysteine components of GSH. Cystine is an especially active amino acid substrate of γ-glutamyltranspeptidase yielding γ-glutamylcystine [γ-GLU-$CYS_2$] (reaction 1, square brackets) which in turn is reduced to γ-glutamylcysteine [γ-GLU-CYSH] and cysteine (reaction 14). Note that when cystine is used as the amino donor, the cycle is short-circuited by bypassing reactions 3 and 4 (Thompson and Meister, 1975).

Orlowski and Meister (1970) suggested that the positioning of γ-glutamyltranspeptidase on the cell surface and of the other enzymes of GSH metabolism within the cell permits the γ-glutamyl cycle to play a role in the translocation of amino acids across the cell membrane. Certainly, strong evidence suggests that the cycle operates *in vivo* (Griffith and Meister, 1979). Moreover, the constituent enzymes of the γ-glutamyl cycle are most active in tissues, such as the kidney, where transport of amino acids is especially prominent (Meister and Anderson, 1983). However, many amino acid transporters have now been characterized and found to be unrelated to the γ-glutamyl cycle (e.g. Tate *et al.*, 1992). The cycle is now recognized to play a minor role in transport of amino acids across cell membranes. Perhaps the γ-glutamyl cycle is too energetically expensive (requiring the hydrolysis of 3 ATPs at steps

3, 4 and 5) and spacially too diffuse for efficient translocation of most amino acids. A possible exception is cystine, because as noted above this amino acid is an excellent substrate of γ-glutamyltranspeptidase.

Current opinion holds that the γ-glutamyl cycle has a major role in metabolism of leukotrienes, estrogens and prostaglandins (depicted as **X** in Figure 1) through *S*-conjugate formation. Here, we discuss the role of the γ-glutamyl cycle in leukotriene metabolism. Leukotriene $A_4$ ($LTA_4$) is formed from arachidonic acid that is released from membrane phospholipids in response to a variety of immunological and inflammatory stimuli. $LTA_4$ is either hydrolyzed to the leukocyte stimulator leukotriene $B_4$ ($LTB_4$) or is converted consecutively to glutathione *S*-conjugate ($LTC_4$), γ-glutamylcysteine *S*-conjugate ($LTD_4$) and cysteine *S*-conjugate ($LTE_4$) by the actions of a specialized form of glutathione *S*-transferase, cysteinylglycine dipeptidase and L-cysteine *S*-conjugate *N*-acetyltransferase, respectively (Figure 1, reactions 8→1→6). $LTC_4$. $LTD_4$ and $LTE_4$ are now known to be components of the 'slow acting substance of anaphylaxis' and are involved in pulmonary smooth muscle contraction, mucous secretion, vasoconstriction and vascular permeability (Nicholson, 1993). The physiological effects of the leukotriene *S*-conjugates are terminated by conversion of the cysteine *S*-conjugate to the mercapturate [*N*-acetyl cysteine *S*-conjugate (*N*-Ac-CYS-**X**); Figure 1, reaction 7] which is excreted. Alternatively, the cysteine *S*-conjugate may enter directly into the γ-glutamyl cycle as a substrate of γ-glutamyltranspeptidase. This reaction generates the γ-glutamylcysteine *S*-conjugate which in turn is a substrate of the cyclotransferase (reactions 1 and 2) effectively translocating the cysteinyl *S*-conjugate.

In addition, the γ-glutamyl cycle and associated reactions are involved in the detoxification of potentially poisonous xenobiotics (again depicted as **X** in Figure 1) (reactions 8→1→6→7). This pathway is discussed in more detail later in the review.

In brain, the enzymes of the γ-glutamyl cycle (Figure 1, reactions 1 through 6) are especially abundant in the choroid plexus (Tate *et al.*, 1973). The specific activities are comparable to those in the kidney, suggesting that the cycle is involved in some sort of transport process in the brain. GSH is present in the cerebrospinal fluid (CSF) at μM concentrations, and the choroid plexus plays a role in the formation and recycling of GSH in the CSF (Anderson *et al.*, 1989). γ-Glutamyltranspeptidase is present in brain capillaries and is regarded as a marker for capillaries. However, γ-glutamyltranspeptidase is also present in astrocytes and neurons (Makar *et al.*, 1994). Special aspects of GSH turnover and metabolism in brain are described later in the review.

## Protective Functions

The mammalian, adult brain has a very high energy demand, normally relying almost entirely on the oxidative metabolism of glucose to meet its energy demands. Most (> 90%) of the glucose taken up by the brain is fully oxidized to $CO_2$. This very high capacity to oxidize glucose suggests that the brain may generate ROS at an appreciable rate. The brain contains relatively low levels of some anti-oxidant defenses (e.g., catalase) and a high lipid content. The combination of high levels of ROS production, highly susceptible targets, and relatively low levels of defense mechanisms suggests that the brain may be especially vulnerable to oxidative stress. GSH is a major protectant in the brain against oxidative stress by interacting directly with ROS or by participating in enzyme-catalyzed redox cycling reactions.

An important enzyme in glutathione redox reactions is glutathione peroxidase (eq. 1) which catalyzes the reduction of potentially toxic $H_2O_2$ (or lipid peroxides; ROOH) to $H_2O$ (or ROH) with the concomitant conversion of GSH to glutathione disulfide (GSSG). Catalase also converts $H_2O_2$ to $H_2O$, but this enzyme is not present in mitochondria of most tissues (with the notable exception of heart) and cannot detoxify lipid peroxides. Therefore, glutathione peroxidase is especially significant in protecting these organelles against $H_2O_2$ generated from incomplete reduction of $O_2$ (Benzi and Moretti, 1995). [The importance of glutathione peroxidase is underscored by recent work of Zeevalk *et al.* (1997) who showed that mice overexpressing this enzyme are resistant to oxidative damage resulting from treatment with malonate (an inhibitor of succinate dehydrogenase). Addition of malonate to GSH-depleted mesencephalic cultures elicited a toxic response (Zeevalk *et al.*, 1997).] Mitochondria also contain glutathione disulfide reductase (eq. 2) completing a glutathione redox cycle. A relatively large pool of GSH is maintained in mitochondria through the action of a high-affinity GSH uptake system (Mårtensson *et al.*, 1990). Depletion of this pool in preweanling rats leads to damaged brain mitochondria and death (Jain *et al.*, 1991; Meister, 1995).

$$2\,GSH + H_2O_2\ (or\ ROOH) \rightarrow GSSG + 2\,H_2O\ (or\ ROH + H_2O) \quad (1)$$

$$GSSG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+ \quad (2)$$

GSH has been known for many years to play a crucial role in mitochondria by helping maintain normal function and structure (Hunter *et al.*, 1964). If mitochondria are not suitably protected against metabolic insults (including those induced by ROS) the organelles may become irreversibly damaged through a process culminating with induction of a mitochondrial permeability transition (mPT). As summarized by Gunter *et al.* (1994), the mPT is characterized by a sudden increase in the permeability of the mitochondrial inner membrane to small ions and molecules (with $M_r$ values < 1500). This increase is associated with the complete collapse of the mitochondrial membrane potential ($\Delta\Psi$) and colloid-osmotic swelling of the mitochondrial matrix.

Agents that can precipitate the mPT include compounds that oxidize pyridine nucleotides, deplete matrix GSH (Gunter *et al.*, 1994; Reed and Savage, 1995) or increase the GSSG/GSH ratio (Beatrice *et al.*, 1984) thereby creating stress. GSH detoxifies 4-hydroxyhexenal (a lipid peroxidation byproduct) – the most potent inducer of the mPT yet identified (Kristal *et al.*, 1996). Until recently, almost all work on the mPT was carried out on liver and heart mitochondria. However, work from our laboratory has shown that a PT can be induced in isolated rat brain mitochondria and in mitochondria *in situ* in primary cultures of astrocytes (Kristal and Dubinsky, 1997). Some evidence suggests that mitochondrial dysfunction is a primary event in the cascade leading to glutamate excitotoxicity (White and Reynolds, 1996; Schinder *et al.*, 1996). Taken together these findings suggest that GSH and the glutathione redox cycle play a crucial role in maintaining mitochondrial integrity in brain and other organs.

The glutathione redox cycle is present in the cytosol of brain cells (Cooper, 1997). In addition, the brain contains a phospholipid hydroperoxide glutathione peroxidase. Moreover, certain cytosolic glutathione *S*-transferases also catalyze GSH-dependent reduction of lipid peroxides. Lipids are protected against free radical damage by $\alpha$-tocopherol (vitamin E), which quenches free radical propagation by formation of $\alpha$-tocopheroxyl radical (Figure 1, reaction 10), and by other lipophilic scavengers such as the ubiquinols. The $\alpha$-tocopheroxyl radical does not accumulate, in part because it can be re-reduced nonenzymatically to $\alpha$-tocopherol by GSH (Meister, 1989). This reaction and those catalyzed by glutathione peroxidase, glutathione *S*-transferases possessing peroxidase activity, phospholipid hydroperoxide glutathione peroxidase, and transhydrogenases (eq. 3) all result in the formation of GSSG.

$$R'SSR'' + 2\,GSH \longleftrightarrow R'SH + R''SH + GSSG \qquad (3)$$

Under normal conditions, oxidative stress puts little 'strain' on the glutathione redox cycle, and the concentration of GSSG in most tissues is usually low. Indeed, in rat forebrain the concentration of GSSG (in GSH equivalents) is normally < 1.0% that of GSH (Cooper, 1997; Cooper *et al.*, 1980). The low GSSG/GSH ratio is presumably due to strong GSSG reductase activity in brain and rapid synthesis of NADPH from $NADP^+$. About 3–5% of cerebral glucose is converted to $CO_2$ via the oxidative branch of the pentose phosphate pathway – providing a ready source of NADPH for reduction of GSSG (Baquer *et al.*, 1988). The pentose phosphate pathway is up-regulated in CNS cells exposed to $H_2O_2$ (Ben-Yosef *et al.*, 1996). In addition to the pentose phosphate pathway, other possible sources of NADPH for reduction of GSSG include the reactions catalyzed by the two malic enzymes and by isocitrate dehydrogenase.

In addition to its role in protecting tissues against ROS, GSH is important in the detoxification of potentially harmful xenobiotics. GSH forms *S*-conjugates with electrophilic compounds in reactions that are catalyzed by, or accelerated by, a large family of glutathione *S*-transferases. These enzymes are mostly cytosolic with the exceptions of a single microsomal enzyme and possibly a single mitochondrial enzyme. [Leukotriene $C_4$ synthase is a specialized membrane-bound glutathione *S*-transferase (Nicholson, 1993).] The glutathione *S*-conjugate formed from the action of these enzymes is converted to the corresponding L-cysteinylglycine-*S*-conjugate and then to the L-cysteine-*S*-conjugate by the consecutive actions of $\gamma$-glutamyltranspeptidase and dipeptidase. The L-cysteine-*S*-conjugate is then acetylated to the corresponding mercapturate (*N*-acetyl-L-cysteine-*S*-conjugate) which is excreted, completing the detoxification process. [The mercapturate pathway is depicted in Figure 1 to the side of the $\gamma$-glutamyl cycle as reactions 8→1→6→7).] However, the mercapturate pathway can also sometimes lead to bioactivation (generation of a toxin). Halogenated cysteine *S*-conjugates that generate highly reactive sulfhydryl-containing fragments through the action of cysteine *S*-conjugate $\beta$-lyases (eq. 4) are nephrotoxic. Dichloroacetylene is both nephrotoxic and neurotoxic. The compound is an excellent substrate of microsomal glutathione *S*-transferase yielding *S*-(1,2-dichlorovinyl)glutathione (DCVG) which is transformed to *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC). The latter conjugate is converted to pyruvate, ammonia and a reactive (toxic) sulfhydryl-containing fragment by the action of cysteine *S*-conjugate $\beta$-lyases. The neurotoxicity of dichloroacetylene may be due to formation of the glutathione *S*-conjugate and subsequent metabolism to a toxic fragment exclusively within the brain. However, it is also conceivable that dichloroacetylene is converted to glutathione- and cysteine *S*-conjugates elsewhere in the body and transported to the brain. The blood-brain barrier (BBB) possesses uptake systems for both DCVC (probably the L-type transporter) and DCVG. For a discussion see Cooper (1994).

$$RSCH_2CH(NH_2)CO_2H + H_2O \downarrow \qquad (4)$$
$$CH_3C(O)CO_2H + NH_3 + HSR$$

The mercapturate pathway is present in the brain (see below), where it is presumably important for the metabolism of endogenous substances (such as leukotrienes) and in detoxifying reactions. However, the role of the pathway in detoxifying (and toxifying) reactions in the brain has not been extensively studied.

## Origin of GSH in the Brain

The metabolism of GSH is intimately linked with overall sulfur homeostasis in the brain. The concentration of GSH in the brain (~2–3 mM) is much greater than that in the blood (~15 μM) or cerebrospinal fluid (~5 μM). Therefore, the brain must have an avid system for accumulating GSH, or more likely, of synthesizing it *in situ*. Some evidence suggests that the brain is able to take up GSH (Kaplowitz *et al.*, 1996), but other evidence suggests that uptake from

the blood is not the major source of brain GSH (Jain *et al.*, 1991). Cysteine is taken up into the brain on the L-type carrier (Oldendorf and Szabo, 1976; Wade and Brady, 1981). Cystine is apparently not transported by this mechanism, but cystine moieties may enter the brain as cystinyl bisglycine and γ-glutamylcyst(e)ine both of which are ready sources of free cysteine (Jain *et al.*, 1991). Cysteine taken up by the brain is used for protein synthesis and for the synthesis of GSH. Cysteine can also be released from brain. Isolated brain capillaries contain an ASC-type transporter on the abluminal surface that is presumably involved in the egress of cysteine (Tayarani *et al.*, 1987). Methionine is readily taken up into brain on the L-type transporter (Oldendorf and Szabo, 1976). This methionine is used for protein synthesis and as a source of methyl groups. The extent to which this methionine is a source of cysteine sulfur and indirectly of GSH sulfur in the brain is not clear.

## Metabolic Compartmentation of GSH Metabolism within the Brain

In the brain, glutamine is synthesized in a small, rapidly turning over pool of glutamate (primarily in astrocytes) that is kinetically distinct from a larger, more slowly turning over pool of glutamate (primarily in neurons). Tracer studies, histochemical studies, and studies of cells in culture all suggest that GSH formation is similarly compartmented. In brain, GSH is synthesized from glutamate primarily, but not exclusively, in the small pool (astrocytes) and astrocytes maintain a large pool of this tripeptide. Nerve endings also probably contain appreciable levels of GSH. Several authors have measured GSH levels in cultured astrocytes and neurons. Without exception all these studies have found high levels of GSH in cultured astrocytes (2–20 mM). The reported levels in neurons tend to be more variable but generally appreciably less than in astrocytes. [Reviewed by Cooper (1997).] Enzymes of GSH synthesis and turnover are present in neurons in culture (Makar *et al.*, 1994). As described below, compartmentation of GSH metabolism in the brain has important physiological consequences. GSH metabolism in brain is shown schematically in Figure 2.

## A Role for Astrocytic GSH in Neuroprotection

### Astrocytes and the Detoxification of Glutamate and Cysteine

Excess glutamate in the extracellular space is neurotoxic (Olney *et al.*, 1971). Astrocytes contain relatively strong uptake systems for glutamate, glycine and cysteine. Dringen and Hamprecht (1996) have suggested that under physiological conditions the synthesis of GSH from glutamate in the presence of glycine and cysteine in these cells may be a way of disposing potentially toxic glutamate. The same system would also dispose of potentially neurotoxic cysteine.

### Astrocytes and Metallothioneins

Metallothioneins are low $M_r$ proteins that contain high levels of cysteine, serine and lysine residues but no aromatic amino acid residues. These proteins have been suggested to play a role as free radical scavengers and in protection against UV and X-ray damage (Dunn *et al.*, 1988), presumably as a result of high cysteine content. Metallothioneins are especially rich in mouse, rat, monkey and human astrocytes and are inducible by cytokines (Ebadi

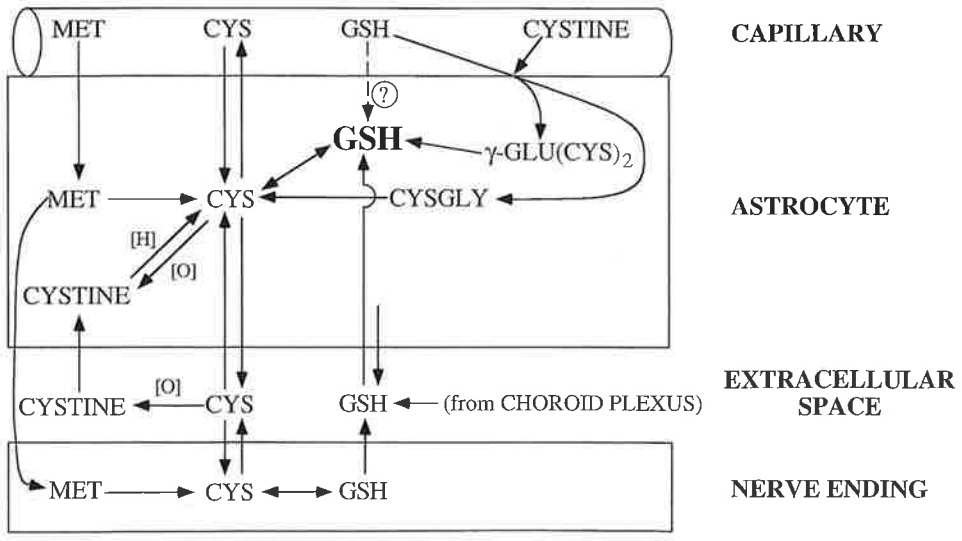


**Fig. 2** Compartmentation of Sulfur Metabolism in the Brain.
Note the proposed central importance of GSH within the astrocytes. The transfer of sulfur from methionine to cysteine occurs via the transsulfuration pathway and is denoted as MET → CYS. Neurons contain GSH and the enzymes necessary for its synthesis (e.g. Makar *et al.*, 1994). However, this pool of GSH is probably small compared with that of astrocytes in the majority of neurons and is not depicted in the Figure.

*et al.*, 1995). Four isoforms (MT-I, MT-II, MT-III, MT-IV) are known to be present in mammalian tissues. MT-I and MT-II are ubiquitously expressed, and these isoforms are known to protect against metal toxicity. MT-IV is expressed in tissues containing stratified squamous epithelia (Ebadi *et al.*, 1995, 1996).

MT-III is expressed almost exclusively in the brain and is thought to regulate brain zinc homeostasis, which itself appears to be regulated in part through redox reactions. One scenario is that an oxidative challenge to the brain will result in increased GSSG formation. GSSG causes the release of free zinc from MT-III, which in turn stimulates zinc-dependent processes and zinc-dependent enzymes. Up to a point this release of zinc may be protective, but beyond a threshold value the release of zinc may be deleterious (e.g. by inhibiting -SH-dependent enzymes) (Ebadi *et al.*, 1995, 1996; Maret, 1995). Inasmuch as astrocytes contain large pools of GSH and metallothioneins, these cells may be especially important in controlling the redox-driven zinc homeostasis in the brain.

### Astrocytes and the Mercapturate Pathway

Earlier immunohistochemical studies suggested that glutathione *S*-transferases in the brain were present exclusively in astrocytes. However, some of these enzymes are now known to occur in the choroid plexus, oligodendrocytes and in selected neuronal cell populations. Nevertheless, it is apparent that glutathione *S*-transferases are particularly abundant in astrocytes (especially in the end feet) and ependymal cells (Lowndes *et al.*, 1994; Makar *et al.*, 1994; Cooper, 1997). This location of glutathione *S*-transferases suggests the possibility that astrocytes and ependymal cells in the brain form a first line of defense against potentially toxic xenobiotics that can diffuse across the BBB and CSF-brain barrier, respectively (Cammer *et al.*, 1989). Possibly, glutathione *S*-transferases also participate in transport of substances into and out of the brain. Such bidirectional transport could affect hormonal control over processes such as myelination and neuronal growth and could facilitate removal of endogenous as well as exogenous toxins from the central nervous system (CNS) (Cammer *et al.*, 1989). However, as noted above, the extent to which the glutathione S-transferases and the mercapturate pathway are involved in detoxification reactions in the brain is unknown and the pathway may actually lead, on occasion, to bioactivation of xenobiotics.

Possible additional roles of glutathione *S*-transferases in the brain include protection of the myelin sheath against toxic substances, removal of epoxyeicosatrienoic acid derivatives from cells, and participation in the local metabolism of prostaglandins and leukotrienes (Cammer *et al.*, 1989). $LTC_4$ and $LTB_4$ are present in brain and these compounds may have a role in the secretion of luteinizing hormone from the anterior pituitary (Sammuelsson *et al.*, 1987).

Quantitatively, in the whole body, the most important site for reaction of electrophiles with GSH is the liver. The glutathione *S*-conjugates formed by this process in the liver are released to the bile. In the intestinal lumen they are converted in part to the cysteine *S*-conjugate. The cysteine *S*-conjugates enter first the portal vein and then eventually the general blood circulation. In the kidney the cysteine *S*-conjugates are *N*-acetylated to the mercapturate and excreted (Dekant *et al.*, 1994). In future work it will be interesting to determine how the enzymes of the mercapturate pathway in the brain conform with this general scheme. As noted already, enzymes involved in the formation of glutathione *S*-conjugates and enzymes of the γ-glutamyl cycle are present in the choroid plexus. If cysteine *S*-conjugate *N*-acetylases are similarly located then the data would suggest excretion of mercapturates to the CSF is analogous to excretion of mercapturates from kidney into urine. Evidently, the role and dynamics of the mercapturate pathway in brain are fertile areas for further research.

### Astrocytes and ROS

In addition to maintaining high levels of GSH, astrocytes in culture have relatively high levels of α-tocopherol and enzymes of GSH metabolism (Makar *et al.*, 1994). This finding suggests that astrocytes may have a special role in the brain in protecting neurons against ROS (cf. Han *et al.*, 1996). The ability of astrocytes to detoxify ROS may be particularly important for diffusible species such $NO^{\bullet}$. Astrocytes in culture are much more resistant to ROS than are neurons (Bolaños *et al.*, 1995; Han *et al.*, 1996; Ben-Yosef *et al.*, 1996) and recent evidence suggests that both catalase and glutathione peroxidase are important in these cells for detoxifying external $H_2O_2$ (Dringen and Hamprecht, 1997). GSH biosynthesis may be up-regulated in astrocytes exposed to ROS (Ben-Yosef *et al.*, 1996; Han *et al.*, 1996). β-Amyloid has been shown to induce apoptosis and oxidative stress in neurons *in vitro*. For example, depletion of GSH by Aβ25–35 leads to oxidative stress in rat cortical neurons (Müller *et al.*, 1997). Whether stimulation of the antioxidative defense systems of astrocytes in Alzheimer's disease is of therapeutic value remains to be determined.

Despite the generally protective role of astrocytes, under some pathophysiological conditions (e.g. reflow after stroke) these cells may exacerbate injury to neurons. The activity of inducible NO synthase may increase in stressed astrocytes. This induction would in turn be predicted to cause increased $NO^{\bullet}$ production and increased $ONOO^{-}$ formation, resulting in damage to nearby neurons (Barker *et al.*, 1996) and interference with mitochondrial energy metabolism (Bolaños *et al.*, 1997). Such mitochondrial dysfunction may occur in certain neurodegenerative diseases (Bolaños *et al.*, 1997).

## Sulfur Trafficking between Astrocytes and Neurons

An understanding of the processes involved in sulfur homeostasis and sulfur trafficking in the CNS must take into account the following three observations:

1) Astrocytic end feet surround capillaries – controlling in part the flow of many substances, including sulfur-containing amino acids, across the BBB;
2) GSH is a major store of cysteine in the brain; and
3) astrocytes contain a large pool of GSH.

These observations underscore the central importance of GSH in sulfur homeostasis in the CNS (Figure 2). As noted above, some GSH may enter the astrocytes by transport intact across the BBB. It is likely, however, that the astrocytic GSH pool is maintained largely by ingress of cysteine which is converted to GSH directly. Inasmuch as cysteine movement across the BBB is bidirectional, the GSH pool in the astrocytes may be regarded as a cysteine/sulfur buffer that can be enlarged or depleted as dictated by the requirements of the whole brain. This buffering is disrupted by metabolic insults. For example, ischemia to the rat forebrain results in depleted GSH levels (Cooper *et al.*, 1980). This depletion is presumably due to lack of ATP which will seriously restrict GSH synthesis but not catabolism. In brain, the ischemia-induced loss of GSH is matched almost stoichiometrically by an increase in cysteine (Slivka and Cohen, 1993). The accumulation of cysteine may be substantial and contribute to ischemia-induced neurotoxicity (Slivka and Cohen, 1993).

Astrocytes also presumably act normally to buffer neuronal metabolism of sulfur-containing amino acids. Neuronal requirements for sulfur-containing amino acids are probably met in part by uptake of cysteine and methionine released from astrocytes (Figure 2). Cysteine in the extracellular space is readily oxidized non-enzymatically to cystine, which can be taken up by astrocytes, but not by neurons (Sagara *et al.*, 1996). Inasmuch as elevated levels of both cysteine and cystine are neurotoxic, the concentrations of these two compounds in the extracellular space must be tightly regulated.

Cysteine and GSH are readily interconvertible via reactions of the γ-glutamyl cycle, but cysteine is not formed anew by this process. Net synthesis of cysteine can, however, occur via the transsulfuration pathway (Figure 2, MET→CYS). Methionine is a source of methyl groups (via its conversion to *S*-adenosylmethionine), and as noted above, methionine is readily taken up across the BBB by the L-transporter (Oldendorf and Szabo, 1976). Methylation reactions are extensive in the brain, generating *S*-adenosylhomocysteine which in turn is converted to homocysteine. The complete transsulfuration pathway provides the machinery for the removal of potentially toxic homocysteine, generation of *de novo* cysteine, and recycling of methionine carbon and sulfur. Inborn errors of methionine adenosyltransferase, and thus abnormalities in the transsulfuration pathway, can lead to demyelinating disease (Ubagai *et al.*, 1995). As with cysteine, the astrocytes presumably control the flow of methionine from blood to neurons. Evidently, the importance of the transsulfuration pathway in the brain is yet another fertile area for further research.

Extracellular GSH in the CNS is provided by the choroid plexus (Anderson *et al.*, 1989), astrocytes (Yudkoff *et al.*, 1992; Sagara *et al.*, 1996; Dringen *et al.*, 1997), and possibly nerve endings (Zängerle *et al.*, 1992). Sagara *et al.* (1996) could find no evidence for uptake of GSH by neurons, suggesting that astrocytes regulate the levels of this tripeptide in the extracellular space.

The GSH in the extracellular space may be involved in neuronal signaling. Astrocytes have high-affinity binding sites for GSH that may regulate physiological responses such as G-protein coupling, activation of second messengers, regulation of protein kinases, and $Ca^{2+}$ release (Guo and Shaw, 1992). Some evidence has also been presented that GSH may be a modulator of glutamatergic transmission by acting as a selective agonist for the *N*-methyl-D-aspartate (NMDA) recognition domain of the NMDA receptor ionophore (Ogita *et al.*, 1995). These observations suggest that the concentration of GSH may regulate physiological signals.

Finally, the central nervous system contains large amounts of taurine. This amino acid may be involved, for example, in osmoregulation and neurotransmitter modulation (Huxtable, 1989). Taurine is synthesized from cysteine via sulfur oxidation/decarboxylation. Inasmuch as GSH is a storage form of cysteine the tripeptide may have a regulatory role in the synthesis and disposition of taurine in the brain.

## Cerebral GSH in Aging and Disease

Several studies have shown that the levels of GSH and of 'total' glutathione (i.e., GSH plus GSSG) in the rodent brain decline with age and that the brains of older rodents are more susceptible to peroxidative stress (reviewed by Benzi and Moretti, 1995). Several reports also suggest that the ratio of GSSG to GSH increases with age, but because GSH is easily oxidized during tissue extraction the results of these studies must be viewed cautiously (Cooper, 1997).

A large body of evidence suggests the involvement of ROS in several neurodegenerative diseases [e.g., Parkinson's disease (PD)]. GSH levels are decreased in substantia nigra of PD patients (Sian *et al.*, 1994) and of individuals with incidental Lewy bodies considered presymptomatic for PD (Dexter *et al.*, 1994). Although some evidence suggests that Alzheimer's disease (AD) is associated with ROS, no evidence has been found that GSH levels are altered in the brains of these patients compared with the levels in the brains of age-matched controls (Makar *et al.*, 1995). However, as noted above, Aβ25–35 causes a decrease in GSH in neuronal cells *in vitro*. Perhaps the contrast between AD and PD is due to differences in the chemical nature of the ROS or to the site(s) of their generation

in brain. Decreased levels of GSH are associated with decreased survival rate in patients with HIV (Herzenberg *et al.*, 1997). The concentrations of *S*-adenosylmethionine and GSH are decreased in the CSF of HIV patients exhibiting neurological symptoms, suggesting impaired cerebral GSH metabolism/transsulfuration (Castagna *et al.*, 1995).

## Animal Models of GSH Deficiency

Many methods have been devised to interfere with the turnover of GSH. For example, GSH synthesis may be inhibited by buthionine sulfoxide, a specific inhibitor of γ-glutamylcysteine synthetase (reviewed by Meister, 1995; Cooper, 1997). Other methods designed to decrease GSH include administration of diamide (oxidizes GSH), cyclohexene-1-one or cycloheptene-1-one (deplete free GSH by forming glutathione *S*-conjugates) (for original references see Benzi and Moretti, 1995; Cooper, 1997). Interestingly, challenges with diethyldithiocarbamate (a copper chelator and inhibitor of superoxide dismutase), paraquat (induces superoxide formation), or aminotriazole (inhibits catalase) lead to a small, but significant increase in GSH in the forebrains of rats (Benzi and Moretti, 1995). Perhaps, brain tissue *in vivo*, as was noted earlier for astrocytes in culture, up-regulates GSH synthesis in the face of an oxidative challenge.

In addition to causing an oxidative stress, depletion of brain GSH can lead to enhanced toxicity toward xenobiotics. For example, the agrochemical intermediate L-2 chloropropionic acid is acutely neurotoxic to rats, causing cerebellar edema and locomotor dysfunction (Wyatt *et al.*, 1996). The compound destroys cerebellar granule cells. Toxicity toward these cells is exacerbated by depletion of GSH with buthionine sulfoximine. On the other hand, glutathione isopropyl ester (a precursor of GSH; see the following section) is protective (Wyatt *et al.*, 1996).

## Possible Therapeutic Benefits Associated with Increasing Glutathione Levels

The role of GSH in neuroprotection suggests that raising tissue GSH levels may be therapeutically beneficial. Administration of relatively large amounts of GSH to experimental animals, however, does not result in appreciable increases in tissue GSH because of poor penetration into cells (Meister, 1995; Jain *et al.*, 1991). In animal models of GSH deficiency, GSH levels can be restored by administration of *N*-acetylcysteine (*N*-Ac) or 2-oxothiazolidine-4-carboxylate (OTC), two compounds that are converted to cysteine *in vivo*. GSH esters are also GSH precursors *in vivo* (reviewed by Cooper, 1997). In addition, as noted above, ascorbate has a sparing effect on GSH (Meister, 1995; Mårtensson and Meister, 1992; Jain *et al.*, 1994). As a result of these findings ascorbate, OTC, *N*-Ac and GSH esters have all been considered as possible therapeutic agents in diseases associate with oxidative stress. For example, ascorbate and *N*-Ac have been used to treat patients with hereditary glutathione synthetase deficiency (Mårtensson *et al.*, 1989; Jain *et al.*, 1994). Several studies have shown that GSH monoester, *N*-Ac and OTC suppress HIV virus expression in infected human cells (Harekeh *et al.*, 1990; Kabelic *et al.*, 1991). *N*-Ac is especially effective, presumably because it is an excellent precursor of GSH and is also an antioxidant (Raju *et al.*, 1994). Castagna *et al.* (1995) showed that the decrease in *S*-adenosylmethionine and GSH in the CSF of HIV patients could be redressed by parenteral administration of *S*-adenosylmethionine. The above mentioned studies suggest that regimens designed to correct the imbalance in sulfur-containing amino acids and increase tissue GSH status may be useful in treating HIV patients and patients with inborn errors of GSH metabolism. Whether such regimens will prove robust in treating other diseases associated with oxidative stress or exposure to toxic xenobiotics remains to be determined.

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