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Metabolism and functions of glutathione in brain

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Dedicated to Professor Bernd Hamprecht on occation of his 60th birthday.

Abstract

The tripeptide glutathione is the thiol compound present in the highest concentration in cells of all organs. Glutathione has many physiological functions including its involvement in the defense against reactive oxygen species. The cells of the human brain consume about 20% of the oxygen utilized by the body but constitute only 2% of the body weight. Consequently, reactive oxygen species which are continuously generated during oxidative metabolism will be generated in high rates within the brain. Therefore, the detoxification of reactive oxygen species is an essential task within the brain and the involvement of the antioxidant glutathione in such processes is very important. The main focus of this review article will be recent results on glutathione metabolism of different brain cell types in culture. The glutathione content of brain cells depends strongly on the availability of precursors for glutathione. Different types of brain cells prefer different extracellular glutathione precursors. Glutathione is involved in the disposal of peroxides by brain cells and in the protection against reactive oxygen species. In coculture astroglial cells protect other neural cell types against the toxicity of various compounds. One mechanism for this interaction is the supply by astroglial cells of glutathione precursors to neighboring cells. Recent results confirm the prominent role of astrocytes in glutathione metabolism and the defense against reactive oxygen species in brain. These results also suggest an involvement of a compromised astroglial glutathione system in the oxidative stress reported for neurological disorders.

Contents

Abbreviations: ADT, anethole dithiolethione; ALS, amyotrophic lateral sclerosis; ATP, adenosine triphosphate; bFGF, basic fibroblast growth factor; BDNF, brain-derived neurotrophic factor; BSO, buthionine sulfoximine; CHP, cumene hydroperoxide; CysGly, cysteinylglycine; $(CysGly)_{ox}$, oxidised cysteinylglycine; GDNF, glial cell line-derived neurotrophic factor; GPx, glutathione peroxidases; GR, glutathione reductase; GSH, glutathione; GST, glutathione-S-transferase(s); GSSG, glutathione disulfide; γGluCys, γ-glutamylcysteine; γGT, γ-glutamyl transpeptidase; LT, leukotriene; L-dopa, L-2,4-dihydroxyphenylalanine; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAC, N-acetylcysteine; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); NGF, nerve growth factor; OTC, 2-oxothiazolidine-4-carboxylate; PD, Parkinson's disease; ROS, reactive oxygen species; SOD, superoxide dismutase(s); tBHP, tertiary butylhydroperoxide.

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1. Introduction

Oxidative stress occurs as a consequence of an alteration in the equilibrium of the production of reactive oxygen species (ROS) and antioxidative processes in favor of the production of ROS. ROS include non-organic molecules, such as the superoxide radical anion ($O_2^-\bullet$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO•), as well as organic molecules such as alkoxyl and peroxyl radicals. ROS are continuously generated during oxidative metabolism. In order to avoid damage caused by ROS, such as lipid peroxidation, protein modification, and DNA strand breaks, mechanisms exist which remove ROS or prevent the generation of ROS (Sies, 1991; Halliwell and Gutteridge, 1999). For example, the removal of superoxide and H_2O_2 prevents the generation of hydroxyl radicals, which are formed by the iron-catalyzed Fenton Reaction or by the Haber–Weiss-Reaction (Winterbourn, 1995; Wardman and Candeias, 1996) and are the most reactive species within the ROS family.

Compared to other organs the brain has some disadvantages regarding the generation and the detoxification of ROS. (i) The cells of the human brain utilize 20% of the oxygen consumed by the body but constitute only 2% of the body weight (Clarke and Sokoloff, 1999), indicating the potential generation of a high quantity of ROS during oxidative phosphorylation in brain. (ii) A high content of iron has been reported for some brain areas (Gerlach et al., 1994), which can catalyze the generation of ROS. (iii) The brain is rich in lipids with unsaturated fatty acids, targets for lipid peroxidation (Porter, 1984; Halliwell, 1992). (iv) The brain contains only low to moderate activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) compared to liver or kidney (Cooper, 1997; Ho et al., 1997). In addition, the loss of neurons in adult brain cannot generally be compensated by generation of new neurons.

Oxidative stress generated by ROS appears to be connected with the loss of neurons during the progression of neurodegenerative diseases, i.e., Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis (ALS) (Bains and Shaw, 1997; Cadet and Brannock, 1998; Reiter, 1998; Sun and Chen, 1998). These facts underline the importance of an effective antioxidant system for brain function during a long human life.

Evidence is growing that glutathione plays an important role in the detoxification of ROS in brain. Glutathione deficiency induced in newborn rats by application of an inhibitor of γ-glutamylcysteine (γGluCys) synthetase, buthionine sulfoximine (BSO), leads to mitochondrial damage in brain (Jain et al., 1991). Furthermore, reduction of the brain glutathione content by BSO enhances the toxic effects of insults that are associated with elevated production of ROS, i.e., ischemia (Mizui et al., 1992) or treatment with 1-

methyl-4-phenylpyridinium (MPP^+) (Wüllner et al., 1996) or 6-hydroxydopamine (Pileblad et al., 1989). Such results have to be considered in the context of the pathogenesis of Parkinson's disease (PD) where a lowered glutathione content has been found in the *substantia nigra pars compacta* (Sofic et al., 1992; Sian et al., 1994a).

2. Functions and basic metabolism of glutathione

2.1. Functions of glutathione

The tripeptide glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine) is the cellular thiol present in concentrations up to 12 mM in mammalian cells (Cooper, 1997). It has important functions as antioxidant, is a reaction partner for the detoxification of xenobiotica, is a cofactor in isomerization reactions, and is a storage and transport form of cysteine (Meister and Anderson, 1983; Cooper, 1997). In addition, glutathione is essential for cell proliferation (Poot et al., 1995) and maintains the thiol redox potential in cells keeping sulfhydryl groups of proteins in the reduced form (Cotgreave and Gerdes, 1998). In addition, recent results suggest that glutathione plays a role in the regulation of apoptosis (van den Dobbelsteen et al., 1996; Ghibelli et al., 1998; Hall, 1999).

The glutathione system is very important for the cellular defense against ROS. A high intracellular concentration of glutathione protects against a variety of different ROS. GSH reacts directly with radicals in nonenzymatic reactions (Saez et al., 1990; Winterbourn and Metodiewa, 1994) and is also an electron donor in the reduction of peroxides catalyzed by GPx (Chance et al., 1979). It should be noted that the glutathione system is only part of the cellular defense system against ROS. Other enzymes, such as SOD and catalase, as well as antioxidants, such as ascorbate and α-tocopherol, are also involved in ROS detoxification. These compounds, their mechanism of action as well as their interaction with the glutathione system have been reviewed (Di Mascio et al., 1991; Meister, 1994; Yu, 1994; Winkler et al., 1994; Jacob, 1995; Wilson, 1997; Wolf et al., 1998; Gate et al., 1999).

2.2. Synthesis of glutathione

Glutathione is synthesized in vivo by the consecutive action of two enzymes (Meister, 1974; Fig. 1). γGluCys synthetase uses glutamate and cysteine as substrates forming the dipeptide γGluCys which is combined with glycine in a reaction catalyzed by glutathione synthetase to generate GSH. Adenosine triphosphate (ATP) is a cosubstrate for both enzymes. The intracellular level of glutathione is regulated by a feedback inhibition of γGluCys synthetase by the endproduct GSH (Richman and Meister, 1975; Misra and Griffith, 1998). Therefore, cellular synthesis and consumption of glutathione are balanced.

2.3. Glutathione metabolism

During detoxification of ROS glutathione is involved in two types of reactions: (i) GSH reacts nonenzymatically with radicals such as the superoxide rad-

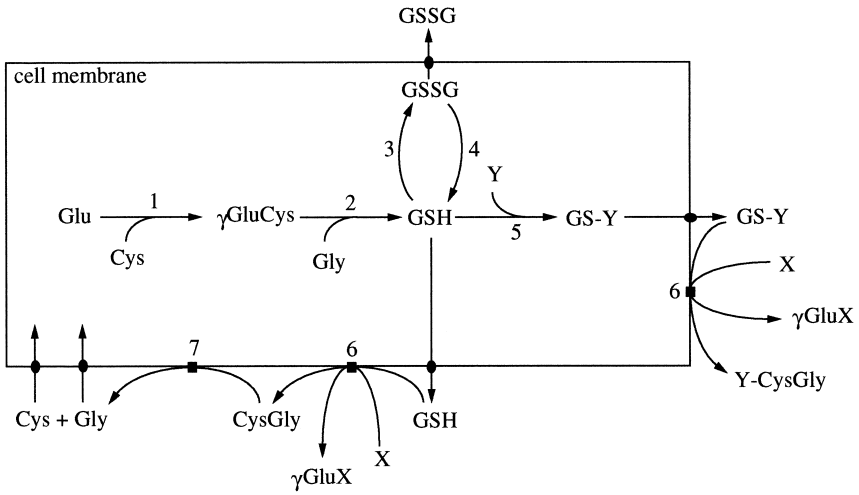

Fig. 1. Metabolism of glutathione. This figure describes the part of glutathione metabolism which is the focus of the present review. For a detailed review on other metabolic pathways related to glutathione see (Meister and Anderson, 1983; Cooper, 1997; Cooper and Kristal, 1997). X represents an acceptor of the γ-glutamyl moiety transferred by γGT from glutathione, Y a substrate of glutathione-*S*-transferase(s). 1, γ-glutamylcysteine synthetase; 2, glutathione synthetase; 3, glutathione peroxidase(s); 4, glutathione reductase; 5, glutathione-*S*-transferase(s); 6,γ-glutamyl transpeptidase; 7, ectopeptidase(s).

ical anion, nitric oxide or the hydroxyl radical (Saez et al., 1990; Clancy et al., 1994; Winterbourn and Metodiewa, 1994; Singh et al., 1996) and (ii) GSH is the electron donor for the reduction of peroxides in the GPx reaction (Chance et al., 1979). The final product of the oxidation of GSH is glutathione disulfide (GSSG). Within cells GSH is regenerated from GSSG by the reaction catalyzed by glutathione reductase (GR) (see Fig. 2). The biochemical properties of the enzymes GR and GPx have been extensively studied. To date, four selenocysteine-containing isozymes of GPx are known (Ursini et al., 1995). In addition, a selenium-independent GPx has recently been identified (Ghyselinck et al., 1993; Vernet et al., 1996). The product of the GPx reaction is GSSG which is a substrate of the flavoenzyme GR. This enzyme transfers electrons from nicotinamide adenine dinucleotide phosphate (reduced from) (NADPH) to GSSG, thereby regenerating GSH (see Fig. 2). The structure, functions and mechanism of GR have been reviewed in detail (Schirmer et al., 1989; Lopez-Barea et al., 1990). More recently, the cDNA of mammalian GR (Tutic et al., 1990) as well as the genomic structure of the mouse GR gene have been reported (Tamura et al., 1997).

During the reactions catalyzed by GPx and GR glutathione is not consumed but recycled (see Fig. 1). In contrast, during the generation of glutathione-*S*-conjugates by glutathione-*S*-transferases (GST) (Commandeur et al., 1995; Salinas and Wong, 1999) or by release of glutathione from cells (Akerboom and Sies, 1990; Kaplowitz et al., 1996) the level of total intracellular glutathione is lowered (see Fig. 1). Therefore, the glutathione used for these processes has to be replaced by resynthesis from the constituent amino acids. Extracellular GSH and glutathione conjugates are substrates for the ectoenzyme γ-glutamyl transpeptidase (γGT). This enzyme catalyzes the transfer of the γ-glutamyl moiety from GSH or a glutathione conjugate onto an acceptor molecule, thereby generating the dipeptide CysGly or the CysGly conjugate, respectively (Meister et al., 1981; Commandeur et al., 1995; Taniguchi and Ikeda, 1998). CysGly can be hydrolyzed by ectopeptidases (Tate, 1985) to cysteine and glycine, amino acids which are subsequently taken up by cells and can serve again as substrates for cellular glutathione synthesis (see Fig. 1).

3. Glutathione in the brain

3.1. Transport of glutathione into the brain

During food deprivation the brain appears to be protected against a loss of metabolites including glutathione (Benuck et al., 1995). Presumably, glutathione homeostasis in brain is maintained predominantly by recycling of glutathione constituents within the brain. Nevertheless, precursor for brain glutathione synthesis might be transported across the blood–brain barrier. Such precursors could be amino acids such as glutamine and cysteine (Wade and Brady, 1981; Ennis et al., 1998). In addition, glutathione import from blood into brain through the blood–brain barrier has been reported (Kannan et al., 1990, 1992; Zlokovic et al., 1994; Favilli et al., 1997). However, it is not yet clear whether GSH is transported intact or whether GSH uptake depends on breakdown of GSH initiated by γGT on the luminal side of brain capillaries (Jain et al., 1991; Meister, 1991). Recently, evidence has been presented for the existence of a sodium-dependent GSH transporter in brain capillaries (Kannan et al., 1996) and brain endothelial cells (Kannan et al., 1999). If and to what extent this GSH transporter contributes to the delivery of GSH from blood into the brain and to the glutathione homeostasis of brain remains to be elucidated.

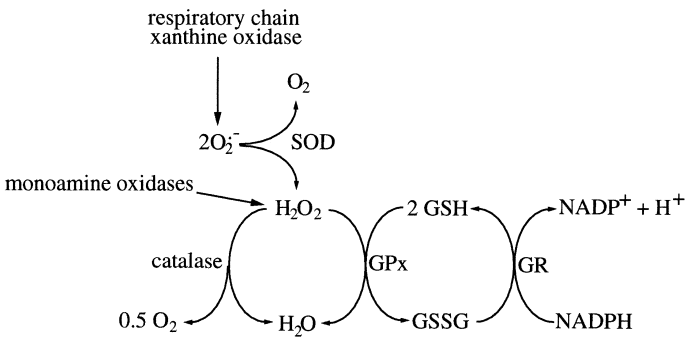

Fig. 2. Generation and disposal of superoxide and hydrogen peroxide. The superoxide generated by the respiratory chain (Radi et al., 1997) or by xanthine oxidase (Hille and Nishino, 1995) is converted by SOD (Fridovich, 1995) to H_2O_2. Monoamine oxidases (Berry et al., 1994) generate additional H_2O_2. This peroxide is disposed of by catalase and/or GPx.

3.2. Glutathione synthesis

Glutathione synthesis in brain cells follows the same pathway as in other tissues (see Fig. 1). Both enzymes involved in glutathione synthesis, γGluCys synthetase and glutathione synthetase, have been found in brain with the highest specific activity in the choroid plexus (Tate et al., 1973; Okonkwo et al., 1974). However, the specific activities of these enzymes in whole brain are lower than those reported for kidney and liver (Sekura and Meister, 1977; Oppenheimer et al., 1979).

Recently, the available data on glutathione concentrations in different brain areas of various species have been summarized (Cooper, 1997). The concentration of glutathione in the brain is in a range of 1 to 3 mM. Direct application into rat brain of the glutathione precursors γGluCys (Pileblad and Magnusson, 1992) or 2-oxothiazolidine-4-carboxylate (OTC) (Mesina et al., 1989) increased the brain glutathione level. Low-dose γ-irradiation enhanced the expression of γGluCys synthetase and, subsequently, led to an elevated glutathione content in brain (Kojima et al., 1998). In contrast, treatment of rats or mice with BSO, an inhibitor of γGluCys synthetase, decreased the glutathione content of the brain (Jain et al., 1991; Andersen et al., 1996; Wüllner et al., 1996). At the cellular level glutathione has been found by histochemical (Slivka et al., 1987; Philbert et al., 1991) and immunohistochemical techniques (Amara et al., 1994; Hjelle et al., 1994; Maybodi et al., 1999) to be present in neurons and glial cells. The glutathione concentration in brain astrocytes appears to be higher than that in neurons, whereas ascorbate concentrations are higher in neurons (Rice and Russo-Menna, 1998).

3.3. Glutathione metabolism

Activities of GPx and GR have been reported for brain homogenates (for overview see: Cooper, 1997). The specific activities of these enzymes are lower in brain than in some other tissues. For example, in mouse brain the specific activity of cytosolic GPx is less than 5% that of kidney and liver and the specific activity of GR is 32 and 65% that of kidney and liver, respectively (Ho et al., 1997). In sections from rat brain GPx immunoreactivity has been found predominantly in neurons in cortex, hippocampus and cerebellum (Ushijima et al., 1986). In contrast, more recently GPx in rat brain was localized predominantly in microglial cells, and only a faint homogeneous staining for GPx was reported for neurons, astrocytes and the neuropil (Lindenau et al., 1998). In mouse brain sections GPx is localized in both neurons and in glial cells (Trepanier et al., 1996). Highly immunoreactive neurons have been observed in layer II of the cerebral cortex, in the CA1 region, the dentate gyrus, and the pontine nucleus (Trepanier et al., 1996). In contrast, in human brain only weak GPx-immunoreactivity has been reported in astrocytes and in neurons (Takizawa et al., 1994). However, in another study GPx staining has exclusively been found in glial cells (Damier et al., 1993). An increase in GPx immunoreactivity in glial cells has been reported for human brain in the marginal area around infarction (Takizawa et al., 1994) and around surviving dopaminergic neurons in parkinsonian brains (Damier et al., 1993). In conclusion, the localization of GPx in the different cell types of the brain has not been unequivocally resolved so far.

GR has been purified from brain and is a dimer of identical subunits (Acan and Tezcan, 1989; Gutterer et al., 1999). The monomer has an apparent molecular mass of about 50 kDa. The purified brain enzyme has K_M values for its substrates NADPH and GSSG in the micromolar range (Acan and Tezcan, 1991; Gutterer et al., 1999). GR from sheep brain is inhibited by Cd^{2+} (Acan and Tezcan, 1995). In brain sections GR-immunoreactivity has been localized in neurons, whereas the detectability of GR immunoreactivity in glial cells depended on the species investigated (Knollema et al., 1996). In brain cell cultures astroglial cells showed, at best, weak staining for GR. In contrast, strong immunoreactivity for GR has been detected in cultured neurons, microglial cells, and oligodendroglial cells (Gutterer et al., 1999).

Glutathione-consuming processes have also been described for brain. A variety of isoenzymes of GST are expressed in brain (Awasthi et al., 1994). Of the three classes of GST (α, μ, π), the α-class is expressed in astrocytes, neurons, and ependymal cells (Abramovitz et al., 1988; Johnson et al., 1993), the μ-class in neurons and astrocytes (Tansey and Cammer, 1991; Johnson et al., 1993; Philbert et al., 1995), and the π-class exclusively in oligodendrocytes (Cammer et al., 1989; Tansey and Cammer, 1991; Philbert et al., 1995).

Extracellular glutathione has been monitored in brain by microdialysis (Orwar et al., 1994; Yang et al., 1994; Lada and Kennedy, 1997). These studies indicate that brain cells are able to release glutathione. The concentration of extracellular glutathione is elevated during ischemia (Orwar et al., 1994; Yang et al., 1994). Little information is available on the cellular origin and the mechanism of GSH release in brain. One of the cloned hepatic GSH-transporters has been reported to be expressed in brain (Kaplowitz et al., 1996). However, since the molecular identity of this transporter has been disputed (Lee et al., 1997; Li et al., 1997b), the mechanism of GSH release by brain cells requires further investigation. In brain slices release of glutathione was induced by depolarization and it was concluded that neurons are the glutathione-releasing cell type in brain (Zängerle et al., 1992). However, in cultures of brain cells glutathione release has so far only

been reported for astrocytes (Yudkoff et al., 1990; Sagara et al., 1996; Dringen et al., 1997a).

A variety of γ-glutamyl peptides has been found in brain (Kakimoto et al., 1964; Kanazawa et al., 1965; Reichelt, 1970; Sandberg et al., 1994) indicating the presence of γGT as the generating enzyme. γGT has been purified from rat brain and occurs in multiple forms (Reyes and Barela, 1980). The specific activity of γGT in brain shows regional variability being highest in the choroid plexus (Tate et al., 1973; Okonkwo et al., 1974). The enzyme is strongly expressed in brain capillaries (Orlowski et al., 1974) where both endothelial cells and pericytes display immunoreactivity on staining with γGT antibodies (Ghandour et al., 1980; Frey et al., 1991). In addition to capillaries, in brain sections immunoreactivity for γGT has also been detected on glial and ependymal cells, whereas neurons showed only weak staining (Shine and Haber, 1981; Philbert et al., 1995).

Glutathione and γGT in brain capillaries have been hypothesized to play a role in amino acid transport across the blood–brain barrier (Orlowski et al., 1974). However, this concept is probably no longer tenable. Very recently it was demonstrated that even the transport into porcine brain microvessels of cystine, one of the best substrates of γGT (Thompson and Meister, 1975), was not affected by inhibition of γGT (Wolff et al., 1998). Therefore, a role of γGT in brain capillaries might rather be connected with glutathione metabolism of the capillaries themselves (Wolff et al., 1998). The concept of the involvement of γGT in amino acid uptake from blood into the brain has been replaced by the concept that γGT functions in detoxification (mercapturic acid pathway) and leukotriene (LT) C_4 catabolism (Frey, 1993). Nevertheless, a regulatory function of components of the γ-glutamyl cycle on amino acid transport has been suggested (Vina et al., 1989). Indeed, 5-oxo-L-proline which is generated from a γ-glutamyl compound activates the amino acid transport systems $B^{0,+}$ and A at the abluminal membrane of brain endothelial cells (Lee et al., 1996). Besides by synthesis, the intracellular concentration of 5-oxo-L-proline is regulated by the activity of 5-oxo-L-prolinase. This enzyme, which converts 5-oxo-L-proline to glutamate, has recently been localized in brain microcapillaries, endothelial cells and pericytes (Jäger et al., 1999).

3.4. Special functions of extracellular glutathione in the brain

Besides the general functions of glutathione (Cooper, 1997; Cooper and Kristal, 1997) this tripeptide appears to have some special functions in the brain. GSH has been considered a neurohormone based on the grounds of the following findings: (i) the extracellular presence of glutathione in brain, (ii) the release of glutathione from brain slices upon stimulation (Zängerle et al., 1992), (iii) the specific binding of glutathione to extracellular receptors (Guo et al., 1992; Lanius et al., 1994), (iv) the stimulation of a signal cascade in astrocytes (Guo et al., 1992), and (v) the induction of sodium currents in neocortex (Shaw et al., 1996). The effects of GSH in the synaptic transmission of the mammalian brain have been reviewed recently (Janaky et al., 1999).

Extracellular GSH serves as substrate for γGT, possibly detoxifying compounds such as glutamate, since an increase in extracellular γ-glutamyl glutamate and other γ-glutamyl dipeptides was detected by striatal microdialysis after experimental ischemia (Orwar et al., 1994) and after depolarisation or incubation under anoxia/aglycemia of brain slices (Li et al., 1996, 1999). γ-Glutamyl peptides as well as glutathione itself have been discussed as agonists and modulators of glutamate receptors in brain (Varga et al., 1994, 1997; Ogita et al., 1995; Janaky et al., 1999). It should be borne in mind, however, that an elevated extracellular concentration of GSH has been reported to increase neuronal vulnerability to hypoxia and glucose deprivation and to enhance excitotoxicity (Regan and Guo, 1999a, 1999b).

An important function of GSH, GSTs and γGT in brain may be their involvement in leukotriene metabolism. LTC_4, a product of the GST reaction, and LTD_4, which is generated from LTC_4 via the γGT reaction, display neuroendocrine and excitatory functions in brain, respectively. At picomolar concentrations LTC_4 stimulates the release of luteinizing hormone from isolated anterior pituitary cells (Hulting et al., 1985). LTD_4 induces a prolonged excitation of rat cerebellar Purkinje neurons (Palmer et al., 1981). Therefore, glutathione-metabolizing enzymes might be involved in local hormonal signaling in brain.

In addition to these functions, glutathione released by brain cells may, at least in part, contribute to the maintainance of the glutathione level in the cerebrospinal fluid (Anderson et al., 1989) and may be a precursor for the synthesis of glutathione in neurons (Dringen et al., 1999a).

4. Cell cultures as models for the investigation of neural glutathione metabolism

During recent years glutathione metabolism of brain cells has been predominantly studied in primary cultures enriched for one brain cell type. From experiments performed on such cultures ample information is available regarding glutathione metabolism of astroglial cells, less is known on the glutathione metabolism of neurons. On the other hand little is known about

the glutathione metabolism of oligodendroglial and microglial cells.

4.1. Astroglial cells

4.1.1. Glutathione content and synthesis

The glutathione content of astroglial cultures prepared by various methods from the brains of several species has been reported to be in the range between 16 nmol/mg protein (Raps et al., 1989) to 50 nmol/mg protein (Devesa et al., 1993). In our hands, the cytosolic glutathione concentration of astroglial cultures is about 8 mM (Dringen and Hamprecht, 1998). Glutathione levels in cultured astroglial cells can be modulated by a variety of treatments (see Table 1). Glutathione levels decline, if the synthesis of GSH is inhibited by BSO, after application of SH-reagents such as dimethyl maleate or ethacrynic acid, and under stress conditions. In contrast, glutathione levels increase (see Table 1) after application of glutathione precursors, after treatments leading to an increased uptake of the precursor cystine (Sagara et al., 1996) or after incubation with the glutathione synthesis-inductor anethole dithiolethione (ADT) (Drukarch et al., 1997a; Dringen et al., 1998e).

Both enzymes involved in glutathione synthesis, namely γGluCys synthetase and glutathione synthetase, are present in cultured astrocytes (Makar et al., 1994). As noted above, glutathione synthesis depends on the concentration of intracellular glutathione, since the activity of γGluCys synthetase is feedback inhibited by GSH (Richman and Meister, 1975; Misra and Griffith, 1998). Consequently, the requirements for glutathione synthesis in astroglial cells can be conveniently investigated after depletion of the cellular glutathione. Depletion can be achieved by starvation (Dringen and Hamprecht, 1996) providing a model system that can be used to test for the capability of astroglial cells to restore glutathione levels from various exogenous precursors (Dringen and Hamprecht, 1996, 1998).

The glutathione content of cultured astroglial cells is limited by the availability of glutamate (Dringen and Hamprecht, 1996). However, glutamine, aspartate, asparagine, ornithine and proline can serve in addition to glutamate as precursors for the glutamate-moiety in astroglial glutathione (Dringen and Hamprecht, 1996). *N*-acetylcysteine (NAC), cystathionine, and OTC serve as donors for the intracellular cysteine essential for astroglial glutathione synthesis, whereas methionine cannot (Kranich et al., 1998). The best cysteine donor for astroglial cells appears to be cystine (Kranich et al., 1996, 1998). The glycine moiety of astroglial glutathione can be derived from exogenous glycine or serine (Dringen and Hamprecht, 1996; Dringen et al., 1998c).

In addition to amino acids, astroglial cells utilize a variety of dipeptides as precursors for glutathione (Dringen and Hamprecht, 1998). In millimolar concentration the dipeptide γGluCys is taken up intact into astroglial cells and serves directly as a substrate for glutathione synthetase, bypassing the reaction of γGluCys synthetase (Dringen et al., 1997b). The dipeptide CysGly, the product of the γGT reaction, is reused by cultured astroglial cells even when present in micromolar concentrations (Dringen et al., 1997b).

Table 1
Modulation of the glutathione content in astroglial cultures

Substance/treatment	Effect[a]	References
BSO	−	Devesa et al. (1993), Peuchen et al. (1996), Barker et al. (1996), Dringen and Hamprecht (1997)
Diethyl maleate	−	Yudkoff et al. (1990), O'Connor et al. (1995), Desagher et al. (1996), Juurlink et al. (1998)
Ethacrynic acid	−	Huang and Philbert (1996)
Ethanol	−	Montoliu et al. (1995)
Ischemia	−	Juurlink et al. (1996)
Nitric oxide	−	Garcia-Nogales et al. (1999)
Starvation	−	Dringen and Hamprecht (1996), Papadopoulos et al. (1997), Dringen et al. (1998e)
tBHP	−	O'Connor et al. (1995), Peuchen et al. (1996), Dringen et al. (1998a)
ADT	+	Drukarch et al. (1997b), Dringen et al. (1998e)
bFGF	+	Hou et al. (1997)
$CdCl_2$	+	Sagara et al. (1996)
L-dopa	+	Han et al. (1996)
γGluCys	+	Pileblad et al. (1991)
Glucose oxidase	+	Sagara et al. (1996)
Glutamate	+	Dringen and Hamprecht (1996)
$NaAsO_2$	+	Sagara et al. (1996)
OTC	+	Aschner et al. (1994)
1,25-dihydroxyvitamin D_3	+	Garcion et al. (1999)

[a] −, Decrease; +, increase.

The peptide transporter PepT2 is expressed in these cultures (Dringen et al., 1998d; tom Dieck et al., 1999) and is responsible for the uptake of CysGly. After intracellular hydrolysis of CysGly the cysteine and glycine generated serve as substrates for astroglial glutathione synthesis (Dringen et al., 1998d).

4.1.2. Release of glutathione

Release of glutathione has been reported so far only for murine astroglial cultures (Yudkoff et al., 1990; Sagara et al., 1996; Dringen et al., 1997a). In contrast, no release of glutathione was found from astroglial cells derived from chicken brain (Makar et al., 1994). The low concentrations of glutathione (up to 3 μM) in the media conditioned by astroglial cultures (Yudkoff et al., 1990; Juurlink et al., 1996; Yonezawa et al., 1996) has been explained by cell death induced by the medium change (Juurlink et al., 1996). However, recently it has been shown that the release of glutathione from astroglial cells has been underestimated due to the consumption of extracellular glutathione by the ectoenzyme γGT. When this enzyme was inhibited, the extracellular concentration of glutathione increased at a constant rate of 2.1 nmol/(h × mg protein) matching well the rate of 3.2 nmol/(h × mg protein) calculated (Dringen et al., 1997a) from the kinetic data reported previously for the glutathione release from astroglial cells (Sagara et al., 1996). Astroglial cultures release within 1 h about 10% of their intracellular glutathione (Dringen et al., 1997a), which has continuously to be resynthesized from its precursors in order to maintain a constant cellular concentration. These data and the reported half-life of about 5 h for astroglial glutathione (Devesa et al., 1993) indicate that the release of glutathione from astroglial cells is quantitatively the most important process that consumes astroglial glutathione.

The rate of release of glutathione from astroglial cells depends on the intracellular glutathione content and follows apparent Michaelis–Menton kinetics (Sagara et al., 1996). The release is partially inhibited by mercurials (Sagara et al., 1996), but, in contrast to the glutathione release from hepatocytes (Aw et al., 1984), is not inhibited by methionine (Dringen, unpublished results). The calculated K_M value for the efflux of GSH from cultured astroglial cells (36 mM; Sagara et al., 1996) is one order of magnitude higher than that found for the glutathione release from hepatocytes (Aw et al., 1986). These data indicate that different mechanisms for glutathione release exist in hepatocytes and brain astroglial cells.

The glutathione released by astroglial cells is predominately GSH (Sagara et al., 1996). However, it cannot be excluded that astroglial cells are also able to release GSSG, as has been described for several cell types and tissues (Akerboom and Sies, 1990). Such a release of GSSG might contribute to the loss of total intracellular glutathione observed after application of peroxides (O'Connor et al., 1995; Peuchen et al., 1996; Dringen and Hamprecht, 1997; Dringen et al., 1998a; Kussmaul et al., 1999).

4.1.3. Disposal of peroxides

Cultured astroglial cells very efficiently dispose of exogenous H_2O_2 (Desagher et al., 1996; Dringen and Hamprecht, 1997) and organic hydroperoxides (Dringen et al., 1998a, 1998b; Kussmaul et al., 1999). The ability of astroglial cultures to clear H_2O_2 increases with the age of the culture (Papadopoulos et al., 1998). H_2O_2 as well as organic hydroperoxides, like tertiary butyl hydroperoxide (tBHP) or cumene hydroperoxide (CHP), are substrates of GPx. Indeed, a rapid oxidation of glutathione was found after application of peroxides to astroglial cultures (Dringen and Hamprecht, 1997; Dringen et al., 1998a, Kussmaul et al., 1999). In addition to GPx, catalase is involved in the detoxification of H_2O_2 (Desagher et al., 1996; Dringen and Hamprecht, 1997). However, inhibition of catalase had only a small influence on the clearance of this peroxide, whereas the inhibition of GPx and catalase strongly reduced the capability of astroglial cells to dispose of H_2O_2 (Dringen and Hamprecht, 1997). These findings indicate that the glutathione system of astroglial cultures can substitute for the function of catalase in H_2O_2 clearance. Catalase does not accept organic hydroperoxides as substrates under the conditions used. Therefore, the glutathione system is responsible and sufficient for the rapid disposal of tBHP and CHP by astroglial cultures (Dringen et al., 1998a; Kussmaul et al., 1999).

The GSSG produced in astroglial cells during the GPx reaction is reduced by GR. Since GR requires NADPH as electron donor, the detoxification of peroxides is linked to the availability and the regeneration of NADPH. As in other cells and tissues, in astroglial cells the pentose phosphate pathway appears to be the predominant source for regeneration of NADPH. Glucose deprivation of astroglial cells caused a small but significant reduction in their capability to detoxify H_2O_2 (Dringen and Hamprecht, 1997) and a large increase in the half-time for the clearance of tBHP (Dringen et al., 1998a) or CHP (Kussmaul et al., 1999). These findings are in accordance with reports showing that the pentose phosphate pathway in cultured astroglial cells is strongly activated during the detoxification of H_2O_2 (Ben-Yoseph et al., 1994, 1996). However, other pathways for the generation of NADPH have to be considered as well, since the NADPH-producing cytosolic malic enzyme (Kurz et al., 1993) and isocitrate dehydrogenases (Juurlink, 1993) are expressed in astroglial cells.

4.2. Neurons

4.2.1. Glutathione content and synthesis

In the first report on glutathione levels in cultured neurons it has been claimed that neurons have at best marginal amounts of glutathione (Raps et al., 1989). However, more recent reports demonstrate that cultured neurons contain glutathione in amounts of up to 40 nmol/mg protein (Pileblad et al., 1991). Nevertheless, cultured neurons appear to contain less glutathione than astroglial cells (Raps et al., 1989; Makar et al., 1994; Huang and Philbert, 1995; Dringen et al., 1999b). The differences in the reported glutathione levels of neurons might be attributed to differences in preparation techniques, to species differences, or to different culture conditions. In addition, the use of different brain areas for the preparation of the cultures influences the glutathione levels of astroglial cells and neurons. Neurons prepared from the cortex contain less glutathione than astroglial cultures from cortex. In contrast, neuronal and astroglial cultures prepared from striatum or mesencephalon contain almost identical levels of glutathione (Langeveld et al., 1996). With regard to the culture conditions especially the content of cysteine or cysteine precursors in the culture medium determines the glutathione level in neurons, since neurons are not able to use the cystine present in most culture media, but rather rely on the availability of cysteine for their glutathione synthesis (Sagara et al., 1993; Kranich et al., 1996). In contrast to cysteine, the availability of glutamine or glycine does not limit neuronal glutathione synthesis (Dringen et al., 1999a). In addition to cysteine, brain neurons are able to use the cysteine donors CysGly, γGluCys, and NAC as precursors for glutathione (see Table 2). The presence of OTC or methionine did not increase neuronal glutathione levels (Dringen and Hamprecht, 1999). Cultured dendrotomized spinal cord neurons utilize γGluCys, NAC, and OTC as precursor for their GSH synthesis (Lucas et al., 1998). The limited availability of endogenous cysteine as precursor for glutathione synthesis in neurons is also evident by the increase in GSH content found after inhibition of protein synthesis, a process competing for amino acids (Ratan et al., 1994). The glutathione content in cultured neurons declines (see Table 2) in the presence of BSO, β-amyloid peptide, agonists of glutamate receptors, the SH-reagent ethacrynic acid, and haloperidol, a compound prescribed for schizophrenia.

Among exogenous precursors of glutathione, the dipeptide CysGly may be the most important, since it is generated from extracellular GSH in the γGT reaction. CysGly is efficiently utilized by neurons in micromolar concentrations (Dringen et al., 1999a). The concentrations of cysteine, CysGly and γGluCys leading to half-maximal glutathione level are lower in neurons (Dringen et al., 1999a) than in astroglial cells (Dringen et al., 1997b), indicating that neurons are more efficient in utilizing these compounds than astroglial cells. BSO inhibits the utilization of both dipeptides in neurons (Dringen et al., 1999a), demonstrating that both dipeptides are hydrolyzed before their constituent amino acids serve as substrates for glutathione synthesis. This finding for neuronal glutathione synthesis from γGluCys contrasts with the situation described for kidney (Anderson and Meister, 1983), brain (Pileblad and Magnusson, 1992), and for cultured astroglial cells (Dringen et al., 1997b), where γGluCys can bypass the γGluCys synthetase reaction. The mechanism by which CysGly and γGluCys are utilized by neurons has not yet been elucidated. These peptides could be taken up into neurons by a peptide transporter as has been described for astroglial cells (Dringen et al., 1998d). Alternatively, the dipeptides could be hydrolyzed by a neuronal ectopeptidase generating amino acids, which subsequently are taken up as precursors for glutathione synthesis. However, an extracellular hydrolysis of the dipeptides would create extracellular cysteine and the neurotransmitters glutamate and glycine, toxic effects of which (Choi, 1988;

Table 2
Modulation of the glutathione content in cultured neurons

Substance	Effect[a]	References
β-amyloid peptide	−	Müller et al. (1997), White et al. (1999)
BSO	−	Grasbon-Frodl et al. (1996), Li et al. (1997a), White et al. (1999), Wüllner et al. (1999)
Ethacrynic acid	−	Wüllner et al. (1999)
Glutamate	−	Almeida et al. (1998)
Haloperidol	−	Sagara (1998)
Kainate	−	Oyama et al. (1997)
CysGly	+	Dringen et al. (1999a)
Cysteine	+	Sagara et al. (1993), Kranich et al. (1996)
γGluCys	+	Pileblad et al. (1991), Dringen et al. (1999a)
NAC	+	Dringen and Hamprecht (1999)

[a] −, Decrease; +, increase.

Olney et al., 1990; Puka-Sundvall et al., 1995) have to be considered.

4.2.2. Detoxification of peroxides

Hydrogen peroxide has been reported to be especially toxic to brain neurons (Mischel et al., 1997; Abe and Saito, 1998). This peroxide causes apoptotic cell death of cultured neurons (Whittemore et al., 1995; Hoyt et al., 1997). Nevertheless, neurons in culture are able to dispose of H_2O_2, but apparently astroglial cultures have a higher capacity than neurons to detoxify this peroxide (Desagher et al., 1996; Dringen et al., 1999b). Evidence has been presented that the neuronal defense against H_2O_2 is mediated primarily by the glutathione system (Desagher et al., 1996). Indeed, application of H_2O_2 to neurons causes a rapid oxidation of GSH. Removal of the peroxide is followed by an almost complete regeneration of the original ratio of GSH to GSSG within minutes (Dringen et al., 1999b). The apparent difference in the velocity of the disposal of H_2O_2 by the cells in primary neuronal and astroglial cultures becomes insignificant, if the differences in protein content of the cultures are taken into consideration (Dringen et al., 1999b). For the rapid clearance of H_2O_2 by neurons both glutathione peroxidase and catalase are essential and, in contrast to the situation in astroglial cultures (Dringen and Hamprecht, 1997), the glutathione system in neurons cannot functionally compensate for the loss of the catalase reaction (Dringen et al., 1999b). The lower efficiency of the neuronal glutathione system of peroxide detoxification compared to that of astroglial cells is also demonstrated by the reduced ability of cultured neurons to dispose of CHP (Dringen et al., 1999b) and by the increased susceptibility towards H_2O_2 by cultured neurons obtained from mice deficient of cytosolic GPx (de Haan et al., 1998).

4.3. Oligodendroglial cells

Oligodendroglial cells and their precursors appear to be very vulnerable to ROS (Kim and Kim, 1991; Husain and Juurlink, 1995; Back et al., 1998), presumably since they contain low amounts of glutathione and high concentrations of iron (Thornburne and Juurlink, 1996; Juurlink, 1997; Juurlink et al., 1998). Oligodendroglial cells survive in culture only in the presence of either cysteine or cystine. Without either of these amino acids the glutathione content declines and the cells die. Cell death can be prevented in the presence of free radical scavengers (Yonezawa et al., 1996; Back et al., 1998) or in the presence of NO donors with half-times in the hour range (Rosenberg et al., 1999). In addition, H_2O_2 has been reported to be highly toxic for oligodendrocytes in culture, an effect which can be partially abolished by presence of NAC (Richter-Landsberg and Vollgraf, 1998). These data indicate that the capacity to detoxify ROS via an intact glutathione system is essential for the survival of oligodendrocytes and their precursors in culture.

4.4. Microglial cells

Little is known about glutathione metabolism of microglial cells in culture. Microglial cells are a minor constituent in astroglia-rich cultures. In these cultures microglial cells express the H_2O_2-reducing enzymes GPx and catalase (Noack et al., 1999). By fluorescence labeling techniques it was shown that among the different glial cells types in astroglia-rich cultures microglial cells contain the highest amount of glutathione (Chatterjee et al., 1999) and stain most strongly for GR (Gutterer et al., 1999). From astroglia-rich primary cultures microglia-rich secondary cultures can be generated which contain approximately 90% microglial cells. Such microglial cultures contain, when compared to neuron-rich and astroglia-rich cultures, a higher glutathione content, higher specific activities of GPx and GR, and a lower specific activity of catalase (Hirrlinger et al., 1999, 2000). Since microglial cells are known to release on activation the radicals superoxide (Colton and Gilbert, 1987; Sankarapandi et al., 1998) and nitric oxide (Minghetti and Levi, 1998), these cells are in immediate contact to the reactive compounds they generate. A prominent glutathione system in microglial cells may be essential for defense against these radicals, since GSH reacts directly with NO and superoxide in nonenzymatic reactions (Saez et al., 1990; Clancy et al., 1994; Winterbourn and Metodiewa, 1994; Singh et al., 1996) and is also an electron donor in the GPx-catalyzed reduction of H_2O_2 (Chance et al., 1979) and peroxynitrite (Arteel et al., 1999), ROS which are generated spontaneously from NO and superoxide.

4.5. Interactions between different brain cell types

In vivo the different types of brain cells are in close contact to each other. Therefore, information obtained on primary cultures enriched for one of the brain cell types does not necessarily reflect the in vivo situation. Evidence is growing that especially between astrocytes and neurons an intensive metabolic exchange occurs (Tsacopoulos and Magistretti, 1996; Wiesinger et al., 1997; Robinson et al., 1998; Hertz et al., 1999). Such interactions appear also to be important regarding cerebral glutathione homeostasis and in the protection of the brain against xenobiotics and oxidative stress (Cooper, 1998).

4.5.1. Detoxification of reactive oxygen species

Astrocytes are considered to play an important role in the defense of the brain against ROS, since they

contain higher levels of various antioxidants than other brain cell types (Peuchen et al., 1997; Juurlink, 1997; Wilson, 1997). This view is supported by the findings that cultured neurons are more vulnerable to damaging compounds such as H_2O_2, peroxynitrite or 6-hydroxydopamine than cultured astroglial cells (Bolanos et al., 1995; Ben-Yoseph et al., 1996; Abe and Saito, 1998; Iwata-Ichikawa et al., 1999), although a contribution of different cell densities in such effects has to be considered. One reason for the reported higher vulnerability of neurons appears to be a lower glutathione content in these cells compared to astroglial cells (Bolanos et al., 1995; Dringen et al., 1999b), a view supported by the compromised resistance of glutathione-deprived astroglial cells against peroxynitrite (Barker et al., 1996).

In coculture astroglial cells support other brain cell types in the defense against ROS. Cocultured astroglial cells protect neurons, retinal ganglion cells, and oligodendrocytes against the ROS-induced toxicity of various compounds and treatments (see Table 3). In addition, astroglial cells contribute to the defense systems against oxidative stress of endothelial cells. In an in vitro model of the blood–brain barrier astroglial cells have been reported to increase the activities of SOD, catalase and GPx, and subsequently lower the amount of radical-mediated lipid peroxidation after hypoxia (Schroeter et al., 1999).

Even at a cellular ratio of 1 astroglial cell to 20 neurons a significant protection against H_2O_2 toxicity towards neurons has been observed (Desagher et al., 1996). Neurons in culture become damaged by extracellular ROS (Drukarch et al., 1998) which can be detoxified by astroglial cells. Glutathione is important for this function, since the protective function of astroglial cells is diminished, when these cells contain low glutathione levels (Drukarch et al., 1997b).

The ability of astroglial cells to protect neurons against H_2O_2 appears to be predominantly related to the capacity of these cells to remove the peroxide (Desagher et al., 1996). However, it cannot be excluded that part of the protection provided by astroglial cells is also due to the release of pyruvate from astroglial cells (Selak et al., 1985) which protects neurons against H_2O_2-toxicity (Desagher et al., 1997). In addition, other antioxidants (Wilson, 1997), metal ion-chelating metallothioneins (Aschner, 1996) as well as interastrocytic gap junction communication (Blanc et al., 1998) may contribute in the prominent role of astrocytes in the defense of brain against ROS.

4.5.2. Glutathione metabolism

For the synthesis of glutathione a metabolic interaction between neurons and astroglial cells takes place. These two cell types do not compete for the substrates used best as precursor for glutathione synthesis (Kranich et al., 1996). The availability of cysteine determines strongly the level of neuronal glutathione. The presence of astroglial cells maintains (Sagara et al., 1993) or even increases glutathione levels in cultured neurons (Bolanos et al., 1996; Dringen et al., 1999b). These results indicate that in the absence of astroglial cells at least one precursor which is provided from the astroglial cells to the neurons limits neuronal glutathione synthesis. Cysteine has been reported to be released from astroglial cells in cystine-containing culture medium (Sagara et al., 1993). However, such a release has to take place against the sodium gradient which enables astroglial cells to efficiently take up cysteine (Sagara et al., 1993) and use it in micromolar concentration as a precursor for glutathione (Dringen and Hamprecht, 1996). The appearance of cysteine in the culture medium of astroglial cells can also be explained as a consequence of the release of GSH (see Section 4.1.2) and the liberation of cysteine from cystine by forming mixed disulfides with GSH (Deneke et al., 1995).

In the rat retina a rapid redistribution of glutathione during ischemia from Müller glial cells to neurons has been reported and a transfer of glutathione from glia

Table 3
Protection by cocultured astroglial cells of neural cells against toxic effects of various compounds

Cell type protected	Toxic compound/treatment	References
Oligodendrocytes	Norepinephrin	Noble et al. (1994)
	Epinephrin	Noble et al. (1994)
	H_2O_2	Noble et al. (1994)
Retinal ganglion cells	NO plus superoxide	Lucius and Sievers (1996)
	Iron ions	Lucius and Sievers (1996)
Cerebellar neurons	Dopamine	Hochman et al. (1998)
Cortical neurons	γ-radiation	Noel and Tofilon (1998)
Mesencephalic neurons	H_2O_2	Langeveld et al. (1995)
	L-dopa	Han et al. (1996), Mena et al. (1997)
	6-hydroxydopamine	Bronstein et al. (1995), Hou et al. (1997)
Striatal neurons	H_2O_2	Desagher et al. (1996)

to neurons was hypothesized (Schütte and Werner, 1998). A direct transfer of glutathione from astrocytes to neurons would explain the increase in glutathione level of cocultured neurons, but uptake of intact glutathione into neurons has not been observed (Sagara et al., 1996). However, the dipeptide CysGly, which is generated from extracellular GSH by the γGT reaction (Dringen et al., 1997a), is efficiently utilized in micromolar concentrations as a precursor for neuronal glutathione (Dringen et al., 1999b). Inhibition of γGT totally prevented the astroglia-induced effect on the glutathione content in neurons (Dringen et al., 1999b) demonstrating that most likely CysGly is the glutathione precursor provided by astroglial cells to neurons (see Fig. 3).

Excess extracellular CysGly will not be wasted, since other brain cells should be able to utilize this dipeptide as glutathione precursor. At least glutathione-deprived astroglial cells are able to utilize CysGly (Dringen et al., 1997b). In the extracellular space CysGly is likely to be oxidized quickly by oxygen to its oxidation product $(CysGly)_{ox}$. The constitutent amino acids of this compound are not lost for neural glutathione synthesis, since $(CysGly)_{ox}$ can be recycled, at least by cultured astroglial cells, to precursors for glutathione (Dringen et al., 1997b).

The hypothesis presented here for the metabolic interactions involved in glutathione metabolism between astrocytes and neurons (see Fig. 3) is supported by recent results obtained with brain slices. After onset of hypoxia the concentration of cysteine in the superfusion solution of brain slices increased strongly, an effect which was almost prevented in the presence of the γGT-inhibitor acivicin (Li et al., 1999). These data suggest that the cysteine found has been generated from the GSH released by the consecutive reactions of γGT and a dipeptidase.

Fig. 3 shows our hypothesis for the metabolic interactions between astrocytes and neurons regarding glutathione metabolism. With the release of glutamine by astroglial cells (Hertz et al., 1999) and the extracellular generation of CysGly from glutathione astroglial cells provide for neurons all three constituent amino acids of glutathione. These interactions suggest several options for modulation of the neuronal glutathione content. (i) The glutathione content of astroglial cells determines the velocity of GSH release (Sagara et al., 1996). Therefore, treatments leading to an elevation of astrocytic glutathione level will increase GSH efflux and could subsequently increase the availability of precursors for neuronal glutathione. (ii) The activity of γGT regulates the extracellular concentration of GSH and the generation of CysGly. The expression of this enzyme is controlled by the use of multiple promotors (Taniguchi and Ikeda, 1998) which might allow strong modulation of expression in various cell types. For astrocytes in vitro and in vivo it has been shown that 1,25-dihydroxyvitamin D_3 increases activity of γGT (Garcion et al., 1996, 1999). Such an upregulation could increase the extracellular concentration of the γGT-product CysGly. (iii) Modulation of the yet unknown pathway(s) of utilization of CysGly by neur-

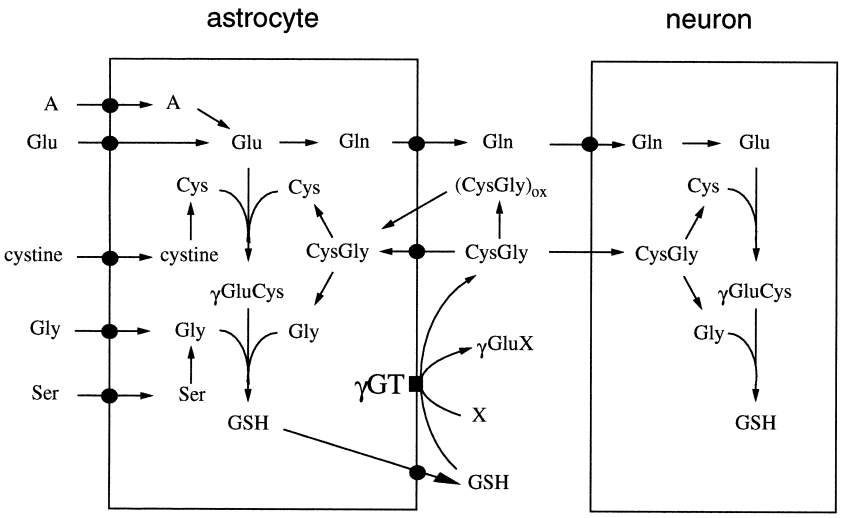

Fig. 3. Scheme of the proposed metabolic interaction between astrocytes and neurons in glutathione metabolism. Astroglial cells use various exogenous substrates as precursors for glutathione. The GSH released from astroglial cells is substrate for the astroglial ectoenzyme γGT. CysGly, generated by the γGT reaction, serves as a precursor of neuronal glutathione. In addition, glutamine is released from astrocytes and used by neurons as precursor for the glutamate necessary for glutathione synthesis. A represents amino acids which are metabolized by astroglial cells to generate glutamate. X represents an acceptor of the γ-glutamyl moiety transferred by γGT from glutathione. It is not known whether the hydrolysis of CysGly for neuronal utilization occurs in the extracellular space or after uptake of the dipeptide in the neurons. Also the mechanism of utilization of $(CysGly)_{ox}$ by astroglial cells is not yet known.

ons might also influence the concentration of glutathione in neurons. Preliminary experiments point to the involvement of an ectopeptidase in this process (Dringen, unpublished results). This result is supported by the decrease in cysteine concentration in the perfusion fluid of hippocampal slices afer inhibition of γGT (Li et al., 1999) which suggests the involvement of a peptidase in the extracellular procession of the GSH released.

4.5.3. Effect of neurotrophic factors on neuronal glutathione metabolism

In addition to low molecular-weight antioxidants such as GSH, glia-derived neurotrophic factors might contribute to the protection of neurons by glial cells. The toxic effects of a variety of ROS-generating compounds on cultured primary neurons or a neuronal cell line was completely prevented by neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) (Mena et al., 1997; Gong et al., 1999). Plateled-derived growth factor, basic fibroblast growth factor (bFGF), nerve growth factor (NGF) and BDNF protect cultured neurons, neuroblastoma and PC12 cells against ROS by upregulation of the concentration of glutathione and/or the activities of enzymes involved in the defense against ROS (Spina et al., 1992; Pan and Perez-Polo, 1993, 1996; Jackson et al., 1994; Cheng and Mattson, 1995; Mattson et al., 1995; Gong et al., 1999). In addition, glia-conditioned medium contains an unidentified glia-derived diffusible factor which enhances neuronal resistance to oxidative stress by increasing transcription of γGluCys synthetase (Iwata-Ichikawa et al., 1999). However, it has to be stressed that an increased activity of γGluCys synthetase does not necessarily increase cellular glutathione levels. Even in cells severalfold overexpressing this enzyme an elevation in the concentration of glutathione was only observed after increasing the supply of the glutathione precursor cysteine (de Saint Vincent et al., 1999). Neurotrophic factors can also influence the detoxification of ROS in vivo. At least for GDNF it has been demonstrated that infusion of this factor into the brain increased the activities of GPx, SOD and catalase (Chao and Lee, 1999).

4.5.4. Harmful interactions at situations with a compromised glutathione system

Notwithstanding the protective effects of neighboring astroglia, harmful events mediated by glia-derived compounds can also take place. Release from activated glial cells of toxic compounds has been described which contributes to neuronal injury (Chao et al., 1996; Aschner et al., 1999; Heales et al., 1999). For example, NO released from glutathione-deprived astrocytes, might compromise oxidative phosphorylation in neighboring neurons (Barker et al., 1996). Indeed, the presence of lipopolysaccharide-treated astroglial cells increased the toxicity of tyrosine hydroxylase-positive neurons against 6-hydroxydopamine (Bronstein et al., 1995) and caused an NO-dependent loss of ATP in cocultured neurons (Bolanos et al., 1996). The possibility that glia can release neuronotoxic compound(s) under certain conditions is supported by recent results showing that glutathione-depleted glial cells generate ROS most likely via the lipoxygenase pathway which leads to degeneration of cocultured neurons (Mytilineou et al., 1999).

5. Glutathione deficiency and neurodegeneration

The balance between generation of ROS and antioxidative processes can become disturbed as reported for aging (Benzi and Moretti, 1995; Mo et al., 1995; Beckman and Ames, 1998) and several neurological disorders (see Table 4). Available literature on oxidative stress and neurological disorders, as well as the involvement of the glutathione system in such processes, has recently been reviewed (for references see Table 4). Here, only alterations in the glutathione system in Parkinson's disease (PD) will be discussed, since best evidence has been presented for PD regarding a disturbed glutathione metabolism as an important factor contributing to the pathogenesis of a neurodegenerative disease.

PD is characterized by a progressive degeneration of dopaminergic neurons in the *substantia nigra pars compacta*. The etiology of the disease is unknown, but biochemical analysis of *post mortem* tissues provides evidence for oxidative stress in the *substantia nigra* during the disease. Glutathione content in this brain region is decreased by 40–50% compared to controls (Sofic et al., 1992; Sian et al., 1994a). Moreover, this region exhibits increased lipid peroxidation (Jenner et al., 1992) and an increased content in iron (Riederer et al., 1989; Sofic et al., 1991; Gerlach et al., 1994; Hirsch and Faucheux, 1998). On the cellular level a significant loss of GSH in the surviving nigral neurons has been reported (Pearce et al., 1997). The importance of the decline in glutathione level during the progression of PD is underscored by the lowered glutathione level in the *substantia nigra* found for incidental Lewy body disease, a presymptomatic form of PD (Dexter et al., 1994). The elevated ratio of GSSG to GSH in PD (Sian et al., 1994a) is consistent with the concept of oxidative stress as an important component in the pathogenesis of PD. The lowered glutathione content appears to be the first indicator for oxidative stress during the progression of PD (Nakamura et al., 1997) preceding even the inhibition of complex I of the respiratory chain (Dexter et al., 1994). In addition to the

lower glutathione level, alterations in the specific activities of enzymes involved in glutathione metabolism and the defense against ROS have been reported. In the parkinsonian brain the specific activity of γGT is increased selectively in the *substantia nigra* (Sian et al., 1994b). This increase may reflect an attempt to locally conserve the availability of glutathione precursors in order to prevent a further decrease in the level of the antioxidant glutathione. Of other enzymes involved in glutathione metabolism the activity of GPx may be decreased (Kish et al., 1985) or unaltered (Sian et al., 1994b). An increase in GPx-immunoreactivity in glial cells around surviving dopaminergic neurons in PD has been reported (Damier et al., 1993). In addition, catalase activity has been found to be decreased in the *substantia nigra* in PD (Ambani et al., 1975).

A loss of GSH alone appears not to be responsible for the nigrostriatal damage in PD, since reduction of brain GSH by chronical infusion of BSO did not reduce the number of dopaminergic neurons (Toffa et al., 1997). The GSH depletion may rather enhance the susceptibility of brain cells against other harmful events, such as the reduction of mitochondrial energy production. A synergistic effect of a lowered intracellular concentration of glutathione and a reduced ATP production in increasing the susceptibility of dopaminergic neurons has been described in vitro and in vivo (Zeevalk et al., 1997, 1998).

A deficiency of complex I of the mitochondrial respiratory chain has been reported for PD (Schapira et al., 1990; Mizuno et al., 1998). Reduction of complex I activity can be acquired during life by mutations in the mitochondrial genome which codes for subunits of the respiratory complexes (Mizuno et al., 1998; Cassarino and Bennet, 1999; Kösel et al., 1999). In addition, inhibition of complex I takes place in the presence of MPP^+ (Nicklas et al., 1985). A treatment of brain with this compound or its precursor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes loss of dopaminergic neurons and PD-like syndroms (Langston et al., 1983; Bezard et al., 1999). Physiological compounds able to influence complex I activity are nitric oxide and its toxic metabolite peroxynitrite. Complex I is particularly susceptible to peroxynitrite when the cellular glutathione level is compromised (Barker et al., 1996; Bolanos et al., 1996). In addition, other complexes of the respiratory chain are inhibited by NO and peroxynitrite (Heales et al., 1999).

Mitochondria are considered to play an important role in the pathogenesis of neurological diseases (Cassarino and Bennet, 1999). Mitochondrial GSH is especially important for neuronal survival. Reduction of the cytosolic glutathione level by BSO caused no immediate reduction in viability of neuronal cells, whereas reduction in both the cytosolic and the mitochondrial GSH levels by application of ethancrynic acid led to inhibition of respiratory complexes (Seyfried et al., 1999) and neuronal degeneration (Wüllner et al., 1999). These results suggest that a lowered glutathione concentration and a reduced ATP production could synergistically contribute to the oxidative stress and the neuronal loss in PD.

Evidence has been presented that oxidative stress might originate in glial cells rather than in neurons and that alterations in glial functions may be important contributors to the pathogenesis of neurodegenerative diseases like PD (Jenner and Olanow, 1998). Glial cells surrounding dopaminergic neurons in brain may be involved in the selective vulnerability of these neurons by scavenging ROS and/or releasing compounds such as NO or cytokines (Hirsch et al., 1998). In addition, glial cells themselves have been implicated in the reduced level of glutathione in PD, since the extent of glutathione loss cannot simply be explained by the loss of nigral neurons (Jenner and Olanow, 1998). A compromised astroglial glutathione system could contribute to a lower defense capacity in brain against ROS and subsequently to increased susceptibility of astrocytes themselves. Such a scenario might take place at least in experimental focal ischemia, where death of astrocytes procedes delayed neuronal death (Liu et al., 1999). In addition, due to insufficient glial detoxification by a compromised glutathione system, reactive glia-derived compounds could be released which might be toxic for neighboring cells.

Many different therapeutical treatments for PD have been applied during the past or are currently under investigation (Dunnet and Björklund, 1999). Since a

Table 4
Oxidative stress in neurological disorders

Disorder	Selected review articles
ALS/Motor neuron disease	Facchinetti et al. (1998), Robberecht and van den Bosch (1998), Cookson and Shaw (1999)
Alzheimer's disease	Retz et al. (1998), Behl (1999), Markesbery and Carney (1999)
Huntington's disease	Browne et al. (1999)
Brain ischemia	Love (1999)
Schizophrenia	Smythies (1997)
Parkinson's disease	Jenner (1998), Jenner and Olanow (1998), Jellinger (1999)

compromised glutathione system appears to be an early event during the pathogenesis of PD, improvement of either glutathione levels in neural cells or of activities of enzymes involved in glutathione metabolism have been considered as treatment strategies. In mice overexpressing GPx, the toxic effect on dopaminergic neurons of intracerebroventricularly injected 6-hydroxydopamine is drastically reduced (Bensadoun et al., 1998). In addition, GPx-overexpressing PC12 cells are more resistant to L-2,4-dihydroxyphenylalanine (L-dopa) and tBHP than are control cells (Kim-Han and Sun, 1998). To up-regulate the glutathione system in brain an application of neurotrophic factors (Williams, 1995; Skaper and Walsh, 1998; Gash et al., 1998) or application of glutathione precursors such as NAC (Martinez et al., 1999) have been considered.

6. Conclusions

The importance of glutathione for function and survival of neural cells has been demonstrated in vivo and in vitro. Cultures of primary neural cells are good models for individual types of brain cells. However, for studies of these cultured cells to be meaningful the metabolic properties of the cell type investigated in culture must be confirmed for the normal physiological environment in vivo.

All cells possess a network of antioxidants and enzymes which are involved in defense against ROS. However, the contribution in ROS detoxification of the various components of this network may differ in different brain cell types. Consequently, alterations in enzyme activities or in the concentrations of small molecular weight antioxidants as well as the availability of precursors for glutathione synthesis and NADPH regeneration may contribute to the susceptibility or to the resistance against ROS of the different brain cell types under physiological and pathophysiological conditions.

Coculture experiments have convincingly demonstrated that different brain cell types strongly influence each other regarding glutathione metabolism and defense against ROS. However, such interactions are difficult to study in vivo. To address such questions the availability in future of conditional, cell type specific transgenic or knock out mice for enzymes involved in glutathione metabolism should prove helpful.

The importance of astroglial cells for the defense of the brain against ROS and especially the function of astroglial glutathione metabolism has become evident at least for cell culture models. Such results suggest that in vivo a compromised astroglial glutathione system may contribute to a lower defense capacity of the brain against ROS and subsequently to increased susceptibility to ROS of astrocytes themselves and of neighboring cells.

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