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Critical Review

Regulation of Neuronal Glutathione Synthesis

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Abstract. The brain is among the major organs generating large amounts of reactive oxygen species and is especially susceptible to oxidative stress. Glutathione (GSH) plays critical roles as an antioxidant, enzyme cofactor, cysteine storage form, the major redox buffer, and a neuromodulator in the central nervous system. GSH deficiency has been implicated in neurodegenerative diseases. GSH is a tripeptide comprised of glutamate, cysteine, and glycine. Cysteine is the rate-limiting substrate for GSH synthesis within neurons. Most neuronal cysteine uptake is mediated by sodium-dependent excitatory amino acid transporter (EAAT) systems, known as excitatory amino acid carrier 1 (EAAC1). Previous studies demonstrated EAAT is vulnerable to oxidative stress, leading to impaired function. A recent study found EAAC1-deficient mice to have decreased brain GSH levels and increased susceptibility to oxidative stress. The function of EAAC1 is also regulated by glutamate transporter associated protein 3-18. This review focuses on the mechanisms underlying GSH synthesis, especially those related to neuronal cysteine transport via EAAC1, as well as on the importance of GSH functions against oxidative stress.

Keywords: glutathione, cysteine, excitatory amino acid carrier 1 (EAAC1), glutamate transporter associated protein (GTRAP) 3-18, oxidative stress

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Invited article

Introduction

Glutathione (GSH) was discovered in 1888 by de Rey Pailhade as an extract from baker's yeast, called "philothione", that had the ability to reduce sulphur to hydrogen sulphide (1). The GSH level in bread dough is an important determinant of strength and extensibility, both of which depend on the redox state of sulphydryl groups. Its structure was identified in the 1930s (1) and was later recognized as "the most important non-protein thiol" (2). After GSH was "rediscovered" in the 1970s, a number of studies demonstrated the importance of GSH in cell biology. GSH plays a critical role in protecting cells from oxidative stress and xenobiotics, as well as maintaining the thiol redox state, most notably in the central nervous system (CNS) (3). In this review, we will discuss the biosynthesis, functions, and regulation of GSH in the CNS, with particular emphasis on the mechanisms by which neuronal GSH synthesis is regulated. Given this background, we will expand our discussion to the involvement of GSH deficiency in neurodegenerative diseases.

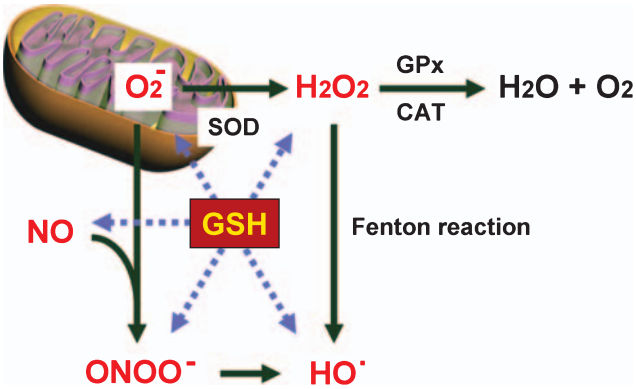

Fig. 1. Glutathione (GSH)-dependent protection against oxidative stress. GSH is a major antioxidant in the brain, which non-enzymatically reacts with superoxide, nitric oxide, hydroxyl radical, and peroxynitrite (dotted arrow). GSH also reacts with H_2O_2 or other peroxides catalyzed by GPx/CAT. Abbreviations are as follows: O_2^-: superoxide, H_2O_2: hydrogen peroxide, GPx: glutathione peroxidase, CAT: catalase, NO: nitric oxide, $ONOO^-$: peroxynitrite, $HO^·$: hydroxyl radical.

Oxidative stress in the central nervous system

The oxygen requirements of the human brain account for 20% of the oxygen consumed by the body, despite the brain being only 2% of body weight. The brain is among the major organs generating large amounts of reactive oxygen species (ROS). Approximately 2% – 4% of the oxygen consumed by mitochondria is diverted to form superoxide (4). Superoxide dismutase (SOD) converts superoxide to hydrogen peroxide (H_2O_2), which is subsequently converted to water and molecular oxygen by GSH peroxidase (GPx) or catalase (3) (Fig. 1). However, H_2O_2 can also react with iron via the Fenton reaction to form hydroxyl radicals, which cause lipid peroxidation (5). The brain also has an abundance of nitric oxide (NO) produced by neuronal NO synthase (nNOS). Neither superoxide nor NO is particularly toxic in vivo, while the reaction of superoxide with NO generates the toxic oxidant peroxynitrite ($ONOO^-$) (6 – 8). $ONOO^-$ can diffuse 10,000 times farther than the hydroxyl radical and is produced by a reaction that is a million times faster than the Fenton reaction (9). The presence of $ONOO^-$ leads to the oxidation of proteins, lipids, and DNA, as well as the nitration of amino acids, mainly tyrosine, and readily inactivates mitochondrial enzymes, resulting in energy production failure (8). ROS-induced lipid peroxidation leads to the conversion of polyunsaturated fatty acids to highly reactive aldehydes such as 4-hydroxynonenal (4-HNE) (10). 4-HNE can react with cysteine, histidine, and lysine residues on protein, by the process of Michael addition (10), to irreversibly form the pyrrol adduct. 4-HNE inhibits the enzymatic activity of GPx and thereby increases H_2O_2 levels (11).

Compared with other organs, the brain is especially vulnerable to oxidative stress because it has lower SOD, catalase, and GPx activities, while it contains an abundance of lipids with unsaturated fatty acids that are targets of lipid peroxidation (3). In addition, the brain GSH concentration is lower than those of the liver, kidney, spleen, or small intestine (12). Brain GSH depletion leads to increased productions of superoxide, hydroxyl radicals, and H_2O_2 (13). Treatment with L-buthionine sulfoximine (BSO) a specific inhibitor of glutamate-cysteine ligase (GCL), the rate-limiting enzyme of GSH biosynthesis, leads to GSH depletion (14). Decreased intracellular GSH due to BSO treatment worsened oxidative damage in vivo (15), while increased intracellular GSH due to *n*-acetylcysteine (NAC) treatment ameliorated this damage (16). The intracellular GSH pool is important for limiting oxidative stress–induced neuronal injury.

GSH functions

GSH is a major antioxidant in the brain (3), with a concentration of approximately 2 – 3 mM, which is much higher than that in blood or cerebrospinal fluid (CSF) (17). GSH exerts its functions via several mechanisms.

First, GSH non-enzymatically reacts with superoxide (18), NO (19), hydroxyl radical (20), and $ONOO^-$ (21) (Fig. 1). In particular, GSH has a higher ability to scavenge superoxide than NAC or cysteine (22). Furthermore, there is no known enzymatic defense against hydroxyl radicals, making GSH the only compound capable of scavenging these radicals (20).

Second, GSH serves as an essential cofactor for a number of enzymes. GSH works as an electron donor for the reduction of H_2O_2 or other peroxides catalyzed by GPx (4). The brain has a relatively high level of GPx as compared with that of catalase, while the liver has high levels of both (23). H_2O_2 is reduced to H_2O by the reaction of GPx with GSH, which is oxidized to GSH disulfide (GSSG) (3). GSSG is then reduced back to GSH, a step catalyzed by GSH reductase with NADPH, and is then reused as a GPx substrate. The relative ratio of the reduced/oxidized forms is over 100 under normal conditions, but is decreased to 49 under stressed conditions (23). It serves as an indicator of the cellular redox environment (24). GSH reacts with various endogenous and xenobiotic compounds mediated by glutathione-*S*-transferase (GST) (12) to form mixed disulfides, which are exported to the outside of the cell. GSH can also react with 4-HNE via the action of GST to form the GSH-HNE adduct (25). This process

plays an important role in cellular detoxification.

A third important role of GSH is serving as a carrier/storage form for cysteine. Cysteine itself has neurotoxic effects mediated by free radical generation, increasing extracellular glutamate, and triggering over-activation of *N*-methyl-D-aspartate (NMDA) receptors (26). GSH is a non-toxic cysteine storage form with 10 – 100 times higher concentrations in mammalian tissues than cysteine (17). Approximately one-third to one-half of the total liver GSH serves as a cysteine reservoir that can be released, when necessary (27).

Fourth, GSH is the major redox buffer and maintains intracellular redox homeostasis. Under conditions of oxidative stress, GSH can lead to the reversible formation of mixed disulfides between protein thiol groups (*S*-glutathionylation), a process critical for preventing irreversible oxidation of proteins (28). Thus, GSH modulates a variety of protein functions via *S*-glutathionylation.

Fifth, GSH can serve as a neuromodulator/neurotransmitter. GSH binds via its gamma-glutamyl moiety to NMDA receptors (29). GSH is thought to exert dual (agonistic/antagonistic) actions on neuronal responses mediated by NMDA receptors in the brain. GSH also serves as an endogenous NO reservoir to form *S*-nitrosoglutathione (GSNO) (30). GSNO can release NO under certain conditions with biological effects, while GSNO has a protective effect in the brain under oxidative stress conditions (31).

In addition, GSH is also required for cell proliferation and neuronal differentiation (32, 33).

GSH synthesis

GSH is a tripeptide comprised of glutamate, cysteine and glycine (Fig. 2). The majority of GSH in a cell remains in the cytoplasm, its site of synthesis (34). Mitochondria also contain 5% – 15% of total cellular GSH (35), although they cannot themselves synthesize GSH because they lack GCL activity (34). GSH synthesis requires two enzymatic steps involving ATP. GCL, also known as γ-glutamylcysteine synthetase, catalyzes the first, that is, rate-limiting enzymatic, step in GSH synthesis (3). GCL mediates the first reaction between glutamate and cysteine to form a dipeptide, γ-glutamylcysteine (γGluCys), which in turn reacts with glycine catalyzed by GSH synthetase (GS) to produce GSH (3). GSH regulates its own synthesis via feedback inhibition of GCL (36). GCL is composed of a catalytic subunit, GCLC, and a modulatory subunit, GCLM. GCLC, not GCLM, has all the enzymatic activity and is also subject to feedback inhibition by GSH (37). GCLC knockout mice showed embryonic lethality, demonstrating that this gene is essential for embryonic development (38). GCLM knockout mice are viable, but in the absence of the GCLM subunit, GCLC is catalytically inefficient, leading to a GSH decrease (39). GS mediates the addition of glycine to γGluCys to form GSH. Although

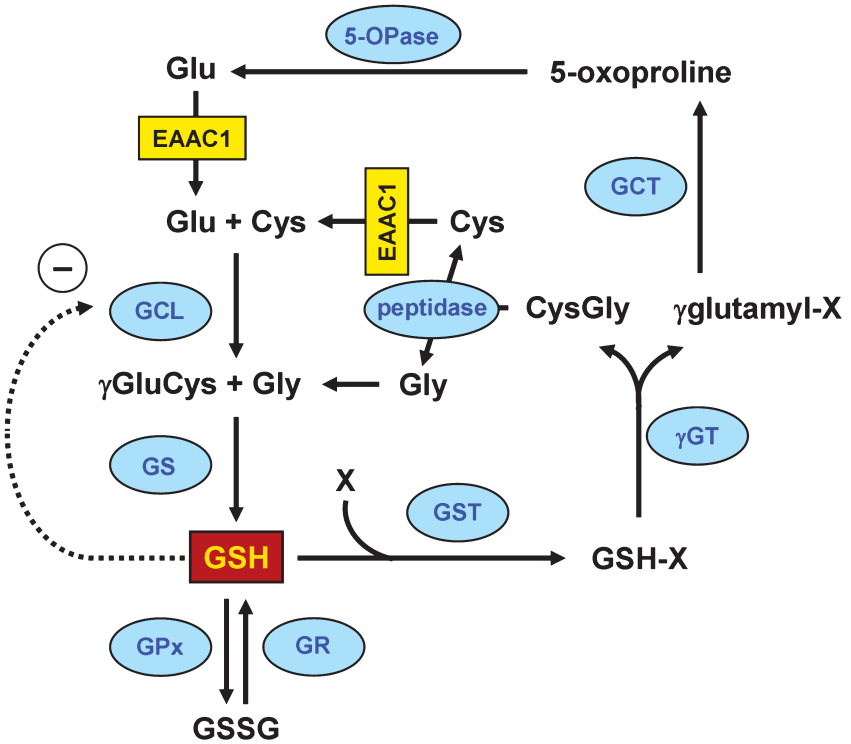

Fig. 2. Metabolism of glutathione (GSH). GSH synthesis requires three amino acids (glutamate, cysteine, and glycine) and two enzymatic steps involving ATP. In neurons, glutamate/cysteine uptake is mediated by EAAC1. GCL catalyzes the first, i.e., the rate-limiting enzymatic, step in GSH synthesis. GSH regulates its own synthesis via feedback inhibition of GCL (dotted arrow). Abbreviations are as follows: Glu: glutamate, Cys: cysteine, Gly: glycine, γGluCys: γ-glutamylcysteine, CysGly: cysteinylglycine, GSSG: glutathione disulfide, X: compounds that can form conjugates with GSH, GCL: γ-glutamylcysteine ligase, GS: glutathione synthase, GPx: glutathione peroxidase, GR: glutathione reductase, GST: glutathione-*S*-transferase, γGT: γ-glutamyltransferase, GCT: γ-glutamylcylotransferase, 5-OPase: 5-oxoprolinase.

GS activity is inhibited by $ONOO^-$ (21), the precise regulatory mechanism remains uncertain. Some reports describe exposure to ROS and nitrogen species as raising the GSH content by increasing the GSH synthesis rate (40, 41). GSH depletion can result in short-term increases in both GCL activity and GSH synthesis.

Cysteine is the rate-limiting substrate for neuronal GSH synthesis (42). Extracellular supplies of the other two precursors, glutamate and glycine, do not increase GSH synthesis (3, 42) because of their higher intracellular concentrations. Another precursor for the glutamate moiety of GSH is glutamine (43). The activity of glutaminase, which generates glutamate from glutamine, is reportedly higher in neurons than in astrocytes (44). In the absence of cysteine, however, no increase in the neuronal GSH concentration was observed when glutamine was supplied (42). Methionine is an important amino acid for producing cysteine via the trans-sulfuration pathway, which supplies approximately 50% of the cysteine needed for GSH synthesis in the liver (45). However, the brain trans-sulfuration pathway is thought to be negligible because the pathway-related enzymatic activity and RNA expression were found to be much lower than those in the liver (46). A primary culture experiment demonstrated incubation with cysteine or NAC, but not methionine, to increase the neuronal GSH level (47). Therefore, the availability of cysteine alone, that is, none of the other amino acids, is important for neuronal GSH synthesis.

Cysteine uptake

In primary neuron culture, approximately 90% of the total cysteine uptake is mediated by sodium-dependent systems, mainly the excitatory amino acid transporter (EAAT), also known as system X_{AG}- (48). The neutral amino acid transporter system ASC takes up three amino acids, alanine, serine, and cysteine, in a sodium-dependent manner. System ASC transporters, ASCT1 and ASCT2, have been identified in humans and mice (49, 50). Neurons express only ASCT1, while astrocytes express both ASCT1 and ASCT2 (51). In neurons, cysteine uptake is not suppressed by system ASC substrates, but is suppressed by EAAT substrates (52, 53). System ASC thus appears to play a minor role in neuronal cysteine uptake (48, 52).

Neurons rely mainly on extracellular cysteine from astrocytes for GSH synthesis (42, 54) because they lack a means of direct GSH uptake. In the CNS, astrocytes store high levels of GSH, with concentrations reaching up to 8 mM (55), and release GSH (56). Astrocytes can export approximately 10% of their intracellular GSH within 1 h (57) and continuously re-synthesize GSH from a variety of dipeptides serving as precursors (58). A recent report demonstrated that gap junction hemichannels mediate this GSH release from astrocytes (59). GSH is released by astrocytes and then cleaved into a γ-glutamyl moiety and a dipeptide CysGly via the reaction with γ-glutamyl transpeptidase (γGT) (57). The dipeptide CysGly is hydrolyzed by neuronal dipeptidase into cysteine and glycine (42, 60). Neurons utilize cysteine but not cystine for GSH synthesis, whereas glial cells utilize both (43, 54). The CSF cysteine concentration was much higher than that of cystine (56). The availability of cysteine determines neuronal GSH synthesis (42). In addition to cysteine, neurons can utilize the cysteine donor dipeptides γGluCys and CysGly for GSH synthesis (42), although it is still unclear how these dipeptides are taken up into neurons.

Cystine uptake

Cystine, an oxidized form of two cysteines with a disulfide linkage, is utilized as a substrate for GSH synthesis in some types of brain cells (61). Cystine is taken up into the cell in exchange for glutamate via system xc-, which can be inhibited by a high extracellular glutamate concentration (62). System xc- is present on astrocytes, microglia, retinal Muller cells, and Bergmann glial cells in the cerebellum (63, 64). Neurons reportedly could not utilize cystine adequately for their own GSH synthesis (43, 54, 56), and cystine uptake activity was especially important for maintaining the GSH level in astrocytes (43). System xc- is a sodium-independent antiporter composed of two subunits, xCT and 4F2hc (65). The former subunit correlates more directly with the system xc- activity expressed in regions facing the CSF, suggesting a role in redox buffering of the cysteine/cystine balance in the CSF (65, 66). Mice lacking the xCT subunit were recently reported to show no change in brain GSH contents (67). Mature neurons mainly take up cysteine via system X_{AG}- (43, 48, 54), whereas immature neurons take up cystine (68) via system xc-, for GSH synthesis. There are in fact reports on system xc- activity in neurons in vitro (62, 69). In a cell culture system, system xc- activity was enhanced under atmospheric oxygen conditions because it can be induced by oxygen (62) or by oxidative stress. Therefore, cystine transport might also show greater enhancement in vitro than in vivo (62).

EAATs

EAATs play an important role in removing extracellular glutamate in the CNS (70, 71). There are five sodium-dependent EAATs termed glutamate aspartate

transporter (GLAST, also known as EAAT1), glutamate transporter-1 (GLT-1, also known as EAAT2), excitatory amino acid carrier 1 (EAAC1, also known as EAAT3), EAAT4, and EAAT5 (71). GLAST and GLT-1 are localized primarily to astrocytes; and, EAAC1, EAAT4, and EAAT5 are localized to neurons. EAAT4 and EAAT5 are restricted to cerebellar Purkinje cells and the retina, respectively, whereas EAAC1 is expressed throughout the CNS. EAATs form homomultimers, mainly trimers, and each of the subunits works independently (72). Each subunit has eight transmembrane domains with two membrane inserted loops, which are the key functional regions of the transporter (73). EAATs can co-transport three Na^+ and one H^+ with each glutamate and counter-transport one K^+ (70). Knockdown of the expression of GLAST or GLT-1 in rats using antisense oligonucleotides increased the extracellular glutamate concentration, whereas EAAC1 knockdown mice showed no increase in extracellular glutamate (74). EAAC1 is localized diffusely over cell bodies and processes, whereas the astrocyte glutamate transporters are clustered in glutaminergic synapses (75). These findings suggest that clearing extracellular glutamate is not a major role of EAAC1. EAAT can transport not only excitatory amino acids, for example, glutamate and aspartate, but also cysteine (76). In particular, EAAC1 can transport cysteine at a rate comparable to that of glutamate with an affinity 10 – 20-fold higher than that of GLAST or GLT-1 (76). Partial knock-down of EAAC1 resulted in approximately 20% decreases in cysteine uptake and the GSH contents of cultured neurons (53). Recently, another study demonstrated EAAC1 deficient mice to show 30% – 40% decreases in brain GSH contents, increased oxidant levels, and increased vulnerability to oxidative stress (16). EAAC1-deficient mice showed brain atrophy and pronounced behavioral abnormalities at advanced ages but not when younger. It is possible that these abnormalities are attributable to impaired neuronal GSH metabolism and that dysfunction of EAAC1 might be responsible for age-related neurodegenerative diseases.

Regulation of EAAC1

EAAC1 expression on the plasma membrane accounts for approximately 20% of the total intracellular EAAC1 under normal conditions (77). When activated, EAAC1 translocates to the cell surface, with no change in *de novo* synthesis (77, 78). EAAC1 is up-regulated by serum- and glucocorticoid-inducible kinase (SGK1) (79) and by phosphoinositide-dependent kinase (PDK1) (80), while being negatively modulated by the δ-opioid receptor (81), glutamate transporter associated protein 3-18 (GTRAP3-18, see discussion below) (82), and the phosphoinositide 3-kinase (PI3K) inhibitor wortmannin (78). Platelet-derived growth factor (PDGF) activates EAAC1 through Akt/PI3K activation in C6 glioma cells (77, 83, 84) (Fig. 3). Protein kinase C (PKC),

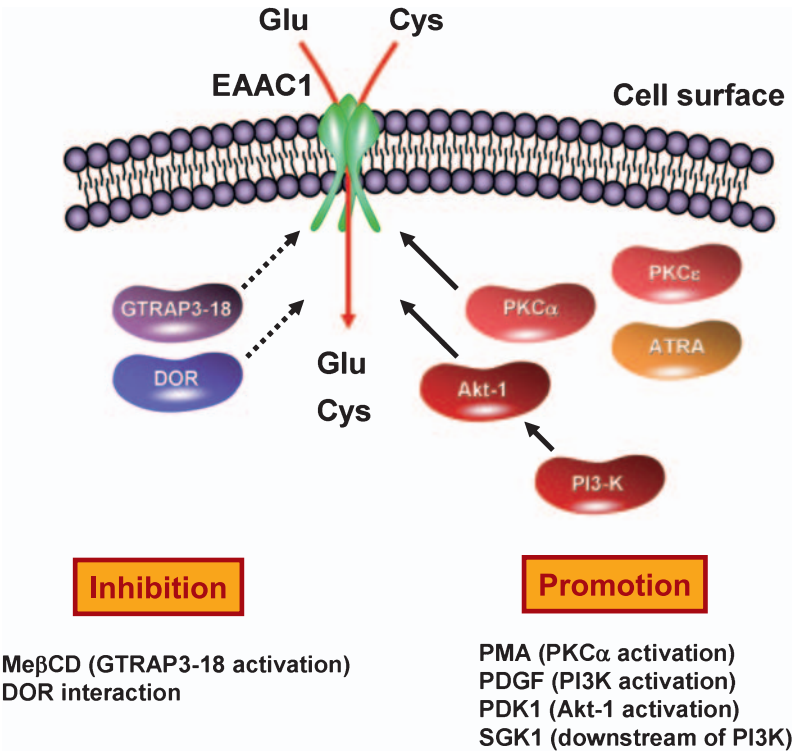

Fig. 3. EAAC1 regulatory mechanisms. EAAC1 is up-regulated (solid arrow) by SGK1, PDK1, and PDGF through Akt/PI3K activation. PKCα or ε activation also induces EAAC1 up-regulation with or without membrane translocation, respectively. ATRA increases the expression of EAAC1 mRNA, thereby raising the level of EAAC1 protein. EAAC1 is down-regulated (dotted arrow) by GTRAP3-18 or DOR interaction. Abbreviations are as follows: SGK1: serum- and glucocorticoid-inducible kinase 1, PDK1: phosphoinositide-dependent kinase 1, PDGF: platelet-derived growth factor, PI3K: phosphoinositide 3-kinase? ATRA: *all-trans*-retinoic acid, DOR; δ-opioid receptor.

particularly PKC subtype α, activation is also known to positively regulate the cell surface expression of EAAC1 and glutamate uptake activation, while PKCε mediates the increase in EAAC1 activity without translocation to the membrane (85). Phorbol 12-myristate 13-acetate (PMA) is a PKC activator and increases the cell surface expression and activity of EAAC1 (78), while decreasing those of GLT-1 (86). The carboxyl-terminal domain of EAAC1 is an intracellular tail and plays a critical role in trafficking to the membrane surface. A mutant EAAC1 lacking 20 carboxyl-terminal amino acids did not show trafficking to the cell surface when cells were stimulated with PMA or PDGF (84). Phosphorylation of serine 465 in EAAC1 by PKCα activation increased activity and both redistribution to the plasma membrane and glutamate uptake (87). Another study demonstrated that a short EAAC1 motif, ^{502}YVN^{504}, was necessary for PDGF-induced redistribution to the plasma membrane (84), despite the Tyr residue in this motif not being phosphorylated. Arginine 445 is also important for the control of coupling between glutamate and cations in EAAC1 (88). Mutation of the arginine residue to non-positive residues resulted in decreased glutamate uptake. These studies suggest that the carboxyl-terminal of EAAC1 is required for both membrane trafficking and transport activity.

RTN2B, a member of the reticulon family of proteins, interacts with EAAC1 to enhance the endoplasmic reticulum (ER) exit process and cell surface expression of EAAC1 with increased glutamate transport activity (89). Rab1 is a GTPase, which is activated at the ER exit site, to support ER-Golgi trafficking of EAAC1 (90). *All-trans*-retinoic acid (ATRA) raises the expressions of EAAC1 mRNA and protein, thereby increasing excitatory amino acid transport activity (91). However, further investigations are needed to determine whether these regulatory systems are the same as those controlling GSH synthesis via cysteine uptake in neurons.

GTRAP3-18

GTRAP3-18 is a 188 amino acid protein with a molecular weight of 22,500 (82). GTRAP3-18 was identified by a yeast two-hybrid screening system using the carboxyl-terminal intracellular domain (arginine 438 – phenylalanine 524) of EAAC1 (73, 82). A recent study using GTRAP3-18 transfected cell lines demonstrated GTRAP3-18 to be an integral ER membrane protein necessary for retaining EAAC1 at the ER as a trafficking regulator (89, 92). Another EAAC1-regulating mechanism is phosphorylation of the carboxyl-terminal. Serine 465 is regarded as an important phosphorylation site in EAAC1 for controlling the distribution of EAAC1 between the plasma membrane and the intracellular space (87). Therefore, GTRAP3-18 might inhibit EAAC1 activity by masking the serine 465 residue.

The GTRAP3-18 distribution in brain tissues showed widespread expression co-localized to neurons. GTRAP3-18 is a negative modulator of EAAC1 via direct binding to the C-terminal domain of EAAC1 (82). GTRAP3-18 is located in the ER and prevents EAAC1 maturation by restricting EAAC1 exit from the ER (89, 92). An earlier investigation showed GTRAP3-18 to reduce EAAC1-mediated glutamate transport with no effect on translocation to the cell surface (82), while recent studies demonstrated an inhibitory effect on EAAC1 trafficking from the ER (89, 90). Chronic morphine administration leads to a 3 – 4 fold increase in GTRAP3-18 mRNA (93). Differentiation, heat shock, and oxidative stress also increase the human isoform, termed JWA, of GTRAP3-18 protein (94, 95). However, no studies have focused on whether GTRAP3-18 regulates EAAC1-mediated cysteine transport leading to GSH synthesis. We recently reported that inhibition of GTRAP3-18 expression using antisense oligonucleotides increased the intracellular GSH content in vitro, while the increase in GTRAP3-18 expression using methyl-β-cyclodextrin (MeβCD) led to a decrease in the GSH content without blocking EAAC1 trafficking to the membrane (96). Immunohistochemical analysis revealed GTRAP3-18 to be present in both the plasma membrane and the intracellular compartment. In MeβCD-treated cells, GTRAP3-18 immunoreactivity was augmented in both the cell membrane and the intracellular compartment, whereas MeβCD had no effect on EAAC1 protein expression. Increased expression of GTRAP3-18 rendered cells more vulnerable to the oxidative stress induced by H_2O_2. We have also confirmed this GTRAP3-18 mediated GSH regulation in vivo (97). MeβCD administered by intracerebroventricular (i.c.v.) injection increased hippocampal GTRAP3-18 expression while decreasing the GSH content. I.c.v. injections of siRNA for GTRAP3-18 or EAAC1 decreased hippocampal expressions, leading to increased or decreased GSH contents, respectively. Our results suggest that GTRAP3-18 is a potential target for increasing neuronal GSH levels endogenously.

Antioxidants increasing neuronal GSH

Orally administered GSH is hydrolyzed by dipeptidase in the gastrointestinal tract. Intravenously administered GSH (reduced form) is also rapidly eliminated, with a half-life of only 7 min (98), by reaction with γGT. Only 0.5% of radiolabeled GSH administered by intra-carotid

injection was detectable in brain extracts (99). It is generally considered to be difficult for GSH to cross the blood-brain-barrier (14, 100), although there are some reports describing a so-called "GSH transporter" (101). One trial found intravenous GSH administration for one month to early stage Parkinson's disease (PD) patients to produce significant efficacy persisting 2 – 4 months after this therapy; these are promising results, even though the number of patients was small and there were no controls (102). The precise mechanism underlying GSH transport from blood to the brain remains unknown.

GSH monoethyl ester (GEE) increased intracellular GSH levels in rat mesencephalic culture and striatal GSH levels with continuous i.c.v. injection (100). However, peripheral administration of GEE failed to increase GSH levels in the brain (100). Furthermore, some toxicity due to the ethyl ester moiety was noted with the use of GEE, suggesting that caution is necessary when this compound is employed in vivo (103).

L-2-oxothiazolidine-4-carboxylic acid (OTC), a cysteine precursor, is metabolized by 5-oxoprolinase to L-cysteine with ATP-dependent hydrolysis. OTC also increases the intracellular GSH content of astrocytes (104), but not that of neurons (47). We can speculate that this enzyme activity is absent from neurons. In addition, peripheral OTC administration did not increase GSH levels in the brain (105).

Cysteine has a neurotoxic effect at concentrations high enough to activate NMDA receptors, leading to enhanced glutamate neurotoxicity (106), whereas NAC acts as a precursor for GSH synthesis by supplying cysteine (47) and activates the GSH cycle (107). NAC enters cells readily and is then deacetylated to form L-cysteine regardless of the presence or absence of EAAC1 (16). NAC also exerts a direct chemical effect as an antioxidant, although with less potency than GSH (22). Systemic administration of NAC can deliver cysteine to the brain, thereby raising the GSH level in the CNS (16). There are, in fact, reports of systemic NAC administration being beneficial in animal models with neurological disorders (108, 109). Our recent study (110) also showed NAC to be an effective precursor for GSH synthesis in dopaminergic neurons. NAC pre-administration ameliorated motor dysfunction in addition to restoring GSH levels in MPTP-treated mice. We also found the nitrotyrosine level on EAAC1 to be lower in the midbrains of NAC/MPTP-treated mice than in those of MPTP-treated mice. Although we do not yet know whether NAC would be clinically beneficial in PD, its low toxicity and ease of administration warrant further investigation of this compound.

Neurodegenerative diseases and oxidative stress

It is well established that oxidative stress plays a major role in age-related neurodegenerative diseases (111). Increased levels of nitrotyrosine, a permanent marker of $ONOO^-$ attack on proteins, were previously demonstrated in Alzheimer's disease (AD), PD, and amyotrophic lateral sclerosis (ALS) (8). Increased levels of 4-HNE, the most cytotoxic product of lipid peroxidation (10), have also been documented in AD, PD, ALS, and other neurodegenerative diseases (112, 113). Aging is a critical factor for GSH homeostasis. Several lines of evidence support a GSH decline with aging in the brain (23). GSH depletion enhanced oxidative stress leading to neuronal degeneration (16). In fact, brain GSH is reduced in some age-related neurodegenerative diseases. AD is a leading age-related neurodegenerative disease, which pathogenically involves oxidative stress (114). Brain tissues from AD patients with the ε4 allele of ApoE showed decreased GSH levels as compared with those of age-matched controls or AD patients homozygous for the ε3 allele (115). The GSH level in red blood cells was also found to be decreased (116), while the activity of GST, an enzyme with a protective action against 4-HNE, was decreased in AD patients (117). PD is the second most common neurodegenerative disease after AD. PD is also characterized by decreased GSH concentrations in the substantia nigra (SN) (118). The severity of GSH depletion parallels pathological and/or clinical PD severity (102). Decreased GSH may be an early event in PD progression (119). Progressive supranuclear palsy (PSP) is also an age-related neurodegenerative disease and its pathogenesis is also related to oxidative stress (120). Recently, PSP patients were also shown to have decreased GSH levels in the SN (121). Although recent studies have provided evidence that oxidative stress is involved in these neurodegenerative diseases, the precise mechanisms of declining brain GSH are not fully understood.

Parkinson's model and EAAC1

PD is a progressive, late-onset disease which is affected by dopaminergic neurodegeneration in the SN. The precise etiology of PD remains uncertain, although genetic and/or environmental factors are clearly important in its pathogenesis (122). Oxidative stress is a major factor implicated in the pathogenesis of PD. Previous reports demonstrated increased iron levels in the SN of PD patients (3), leading to hydroxyl radical formation via the Fenton reaction (5). The SN generally contains lower GSH levels than the cortex, cerebellum, hippocampus, or striatum (123) and is therefore

considered to be a target of oxidative stress. Decreasing GSH levels in dopaminergic neurons by BSO potentiates susceptibility to some dopaminergic neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylprydinium (MPP^+), and 6-hydroxydopamine (124, 125). Loss-of-function of GCLM also enhanced dopaminergic neurotoxicity, while GCLM overexpression rescued the neuronal loss in flies overexpressing α-synuclein, a principle component of Lewy bodies that define PD pathologically (126). However, whether cysteine uptake by EAAC1 is suppressed, thereby leading to the decreased GSH synthesis in PD models, remains to be determined.

Previous studies showed glutamate transporters to be vulnerable to oxidative stress and that glutamate uptake is inhibited by pre-incubation with $ONOO^-$ or H_2O_2 in vitro (127). However, little is known about the influence of oxidative stress on the capacity of EAAC1 to function as a cysteine transporter. Pigmented dopaminergic neurons were found to express high levels of EAAC1 in human subjects (128). EAAC1-deficient mice showed age-dependent decreases in the number of dopaminergic neurons in the SN and in motor function (129). It is possible that oxidative stress induces EAAC1 dysfunction in PD. However, to our knowledge, there have been no studies examining EAAC1 in PD patients, nor in any of the currently available PD models.

Recently, we suggested the involvement of EAAC1 dysfunction in an MPTP-treated murine model (110). MPTP is known as to be an exogenous neurotoxin that induces mitochondrial dysfunction leading to increased oxidative stress and dopamine depletion in the striatum (130). MPTP-treated mice showed reduced motor activity, reduced GSH contents, EAAC1 translocation to the membrane, and increased levels of nitrated EAAC1. These changes were reversed by pre-administration of NAC, a membrane-permeable cysteine precursor. $ONOO^-$ is a potent oxidant generated by the reaction between superoxide and NO (6 – 8). In a study conducted by our group, pretreatment with 7-nitroindazole (7-NI), an nNOS inhibitor, also prevented both GSH depletion and nitrotyrosine formation induced by MPTP. Although we recently found 7-NI to be non-specific to nNOS (131), a previous report demonstrated attenuation of MPTP neurotoxicity in nNOS-deficient mice (132). These results suggest a major role of $ONOO^-$ derived from nNOS in MPTP-induced GSH depletion. $ONOO^-$ can oxidize cysteine residues and/or nitrate tyrosine residues on glutamate transporters and thereby impair their functions (127, 133). Our data from acute slice culture experiments demonstrated pre-incubation with H_2O_2 or MPP^+ reduced subsequent cysteine uptake in the midbrain. Similarly, a marked reduction of cysteine uptake was observed in the presence of an EAAC1 inhibitor, but not a GLT-1 inhibitor, suggesting that EAAC1 is the primary cysteine transporter in the midbrain, as has been demonstrated in the hippocampus (16). We also confirmed the inhibition of cysteine uptake by MPP^+ using SHSY-5Y cells, a dopaminergic cell line, in vitro. These results indicate that MPTP neurotoxicity would be enhanced by inhibiting neuronal cysteine uptake leading to impaired GSH synthesis and may explain the GSH decrease that occurs with EAAC1 dysfunction in the midbrains of MPTP-treated mice.

Concluding remarks

In conclusion, increasing the neuronal GSH level, whether endogenously or exogenously, would prevent the progression of some age-related neurodegenerative diseases by protecting against oxidative stress. It is unclear whether exogenous GSH/cysteine supplements are clinically effective, whereas endogenous mechanisms inducing GSH synthesis might be an alternative strategy against neurodegeneration. Cysteine transport via EAAC1 plays an important role in neuronal GSH synthesis. Although the precise mechanism(s) regulating EAAC1 function remains elusive, an agent inhibiting GTRAP3-18 would be a promising approach to increasing the neuronal GSH level endogenously in patients with neurodegenerative diseases.

References

1 Meister A. On the discovery of glutathione. Trends Biochem Sci. 1988;13:185–188.
2 Kosower NS, Kosower EM. The glutathione status of cells. Int Rev Cytol. 1978;54:109–160.
3 Dringen R. Metabolism and functions of glutathione in brain. Prog Neurobiol. 2000;62:649–671.
4 Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 1979;59:527–605.
5 Youdim MB, Ben-Shachar D, Riederer P. Is Parkinson's disease a progressive siderosis of substantia nigra resulting in iron and melanin induced neurodegeneration? Acta Neurol Scand Suppl. 1989;126:47–54.
6 Nakaki T, Fujii T. Nitration modifying function of proteins, hormones and neurotransmitters. Jpn J Pharmacol. 1999;79:125–129.
7 Bian K, Ke Y, Kamisaki Y, Murad F. Proteomic modification by nitric oxide. J Pharmacol Sci. 2006;101:271–279.
8 Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007;87:315–424.
9 Beckman JS. Peroxynitrite versus hydroxyl radical: the role of nitric oxide in superoxide-dependent cerebral injury. Ann N Y Acad Sci. 1994;738:69–75.
10 Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11:81–128.

11 Bosch-Morell F, Flohe L, Marin N, Romero FJ. 4-Hydroxynonenal inhibits glutathione peroxidase: protection by glutathione. Free Radic Biol Med. 1999;26:1383–1387.

12 Commandeur JN, Stijntjes GJ, Vermeulen NP. Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. Pharmacol Rev. 1995;47:271–330.

13 Gupta A, Gupta A, Datta M, Shukla GS. Cerebral antioxidant status and free radical generation following glutathione depletion and subsequent recovery. Mol Cell Biochem. 2000;209:55–61.

14 Jain A, Martensson J, Stole E, Auld PA, Meister A. Glutathione deficiency leads to mitochondrial damage in brain. Proc Natl Acad Sci U S A. 1991;88:1913–1917.

15 Genovese T, Mazzon E, Esposito E, Muia C, Di Paola R, Di Bella P, et al. Role of endogenous glutathione in the secondary damage in experimental spinal cord injury in mice. Neurosci Lett. 2007;423:41–46.

16 Aoyama K, Suh SW, Hamby AM, Liu J, Chan WY, Chen Y, et al. Neuronal glutathione deficiency and age-dependent neurodegeneration in the EAAC1 deficient mouse. Nat Neurosci. 2006;9:119–126.

17 Cooper AJ, Kristal BS. Multiple roles of glutathione in the central nervous system. Biol Chem. 1997;378:793–802.

18 Winterbourn CC, Metodiewa D. The reaction of superoxide with reduced glutathione. Arch Biochem Biophys. 1994;314:284–290.

19 Clancy RM, Levartovsky D, Leszczynska-Piziak J, Yegudin J, Abramson SB. Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: evidence for S-nitrosoglutathione as a bioactive intermediary. Proc Natl Acad Sci U S A. 1994;91:3680–3684.

20 Bains JS, Shaw CA. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Res Brain Res Rev. 1997;25:335–358.

21 Koppal T, Drake J, Yatin S, Jordan B, Varadarajan S, Bettenhausen L, et al. Peroxynitrite-induced alterations in synaptosomal membrane proteins: insight into oxidative stress in Alzheimer's disease. J Neurochem. 1999;72:310–317.

22 Hussain S, Slikker W Jr, Ali SF. Role of metallothionein and other antioxidants in scavenging superoxide radicals and their possible role in neuroprotection. Neurochem Int. 1996;29:145–152.

23 Maher P. The effects of stress and aging on glutathione metabolism. Ageing Res Rev. 2005;4:288–314.

24 Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide /glutathione couple. Free Radic Biol Med. 2001;30:1191–1212.

25 Xie C, Lovell MA, Markesbery WR. Glutathione transferase protects neuronal cultures against four hydroxynonenal toxicity. Free Radic Biol Med. 1998;25:979–988.

26 Janaky R, Varga V, Hermann A, Saransaari P, Oja SS. Mechanisms of L-cysteine neurotoxicity. Neurochem Res. 2000;25:1397–1405.

27 Tateishi N, Higashi T, Naruse A, Nakashima K, Shiozaki H. Rat liver glutathione: possible role as a reservoir of cysteine. J Nutr. 1977;107:51–60.

28 Giustarini D, Rossi R, Milzani A, Colombo R, Dalle-Donne I. S-glutathionylation: from redox regulation of protein functions to human diseases. J Cell Mol Med. 2004;8:201–212.

29 Janaky R, Ogita K, Pasqualotto BA, Bains JS, Oja SS, Yoneda Y, et al. Glutathione and signal transduction in the mammalian CNS. J Neurochem. 1999;73:889–902.

30 Singh RJ, Hogg N, Joseph J, Kalyanaraman B. Mechanism of nitric oxide release from S-nitrosothiols. J Biol Chem. 1996;271:18596–18603.

31 Rauhala P, Lin AM, Chiueh CC. Neuroprotection by S-nitrosoglutathione of brain dopamine neurons from oxidative stress. FASEB J. 1998;12:165–173.

32 Poot M, Teubert H, Rabinovitch PS, Kavanagh TJ. De novo synthesis of glutathione is required for both entry into and progression through the cell cycle. J Cell Physiol. 1995;163:555–560.

33 Sagara J, Makino N. Glutathione induces neuronal differentiation in rat bone marrow stromal cells. Neurochem Res. 2008;33:16–21.

34 Meister A. Mitochondrial changes associated with glutathione deficiency. Biochim Biophys Acta. 1995;1271:35–42.

35 Meredith MJ, Reed DJ. Status of the mitochondrial pool of glutathione in the isolated hepatocyte. J Biol Chem. 1982;257:3747–3753.

36 Richman PG, Meister A. Regulation of gamma-glutamylcysteine synthetase by nonallosteric feedback inhibition by glutathione. J Biol Chem. 1975;250:1422–1426.

37 Seelig GF, Simondsen RP, Meister A. Reversible dissociation of gamma-glutamylcysteine synthetase into two subunits. J Biol Chem. 1984;259:9345–9347.

38 Dalton TP, Chen Y, Schneider SN, Nebert DW, Shertzer HG. Genetically altered mice to evaluate glutathione homeostasis in health and disease. Free Radic Biol Med. 2004;37:1511–1526.

39 Yang Y, Dieter MZ, Chen Y, Shertzer HG, Nebert DW, Dalton TP. J Biol Chem. 2002;277:49446–49452.

40 Rahman I, Bel A, Mulier B, Lawson MF, Harrison DJ, Macnee W, et al. Transcriptional regulation of gamma-glutamylcysteine synthetase-heavy subunit by oxidants in human alveolar epithelial cells. Biochem Biophys Res Commun. 1996;229:832–837.

41 Moellering D, Mc Andrew J, Patel RP, Forman HJ, Mulcahy RT, Jo H, et al. The induction of GSH synthesis by nanomolar concentrations of NO in endothelial cells: a role for gamma-glutamylcysteine synthetase and gamma-glutamyl transpeptidase. FEBS Lett. 1999;448:292–296.

42 Dringen R, Pfeiffer B, Hamprecht B. Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. J Neurosci. 1999;19:562–569.

43 Kranich O, Hamprecht B, Dringen R. Different preferences in the utilization of amino acids for glutathione synthesis in cultured neurons and astroglial cells derived from rat brain. Neurosci Lett. 1996;219:211–214.

44 Hogstad S, Svenneby G, Torgner IA, Kvamme E, Hertz L, Schousboe A. Glutaminase in neurons and astrocytes cultured from mouse brain: kinetic properties and effects of phosphate, glutamate, and ammonia. Neurochem Res. 1988;13:383–388.

45 Beatty PW, Reed DJ. Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. Arch Biochem Biophys. 1980;204:80–87.

46 Ishii I, Akahoshi N, Yu XN, Kobayashi Y, Namekata K, Komaki G, et al. Murine cystathionine gamma-lyase: complete cDNA and genomic sequences, promoter activity, tissue distribution and developmental expression. Biochem J. 2004;381:113–123.

47 Dringen R, Hamprecht B. N-acetylcysteine, but not methionine or 2-oxothiazolidine-4-carboxylate, serves as cysteine donor for the synthesis of glutathione in cultured neurons derived from embryonal rat brain. Neurosci Lett. 1999;259:79–82.
48 Shanker G, Allen JW, Mutkus LA, Aschner M. The uptake of cysteine in cultured primary astrocytes and neurons. Brain Res. 2001;902:156–163.
49 Shafqat S, Tamarappoo BK, Kilberg MS, Puranam RS, McNamara JO, Guadano-Ferraz A, et al. Cloning and expression of a novel Na(+)-dependent neutral amino acid transporter structurally related to mammalian Na+/glutamate cotransporters. J Biol Chem. 1993;268:15351–15355.
50 Utsunomiya-Tate N, Endou H, Kanai Y. Cloning and functional characterization of a system ASC-like Na+-dependent neutral amino acid transporter. J Biol Chem. 1996;271:14883–14890.
51 Yamamoto T, Nishizaki I, Furuya S, Hirabayashi Y, Takahashi K, Okuyama S, et al. Characterization of rapid and high-affinity uptake of L-serine in neurons and astrocytes in primary culture. FEBS Lett. 2003;548:69–73.
52 Chen Y, Swanson RA. The glutamate transporters EAAT2 and EAAT3 mediate cysteine uptake in cortical neuron cultures. J Neurochem. 2003;84:1332–1339.
53 Himi T, Ikeda M, Yasuhara T, Nishida M, Morita I. Role of neuronal glutamate transporter in the cysteine uptake and intracellular glutathione levels in cultured cortical neurons. J Neural Transm. 2003;110:1337–1348.
54 Sagara JI, Miura K, Bannai S. Maintenance of neuronal glutathione by glial cells. J Neurochem. 1993;61:1672–1676.
55 Dringen R, Hamprecht B. Glutathione restoration as indicator for cellular metabolism of astroglial cells. Dev Neurosci. 1998;20:401–407.
56 Wang XF, Cynader MS. Astrocytes provide cysteine to neurons by releasing glutathione. J Neurochem. 2000;74:1434–1442.
57 Dringen R, Kranich O, Hamprecht B. The gamma-glutamyl transpeptidase inhibitor acivicin preserves glutathione released by astroglial cells in culture. Neurochem Res. 1997;22:727–733.
58 Dringen R, Kranich O, Loschmann PA, Hamprecht B. Use of dipeptides for the synthesis of glutathione by astroglia-rich primary cultures. J Neurochem. 1997;69:868–874.
59 Rana S, Dringen R. Gap junction hemichannel-mediated release of glutathione from cultured rat astrocytes. Neurosci Lett. 2007;415:45–48.
60 Dringen R, Gutterer JM, Gros C, Hirrlinger J. Aminopeptidase N mediates the utilization of the GSH precursor CysGly by cultured neurons. J Neurosci Res. 2001;66:1003–1008.
61 Shih AY, Erb H, Sun X, Toda S, Kalivas PW, Murphy TH. Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. J Neurosci. 2006;26:10514–10523.
62 Sagara J, Miura K, Bannai S. Cystine uptake and glutathione level in fetal brain cells in primary culture and in suspension. J Neurochem. 1993;61:1667–1671.
63 Pow DV. Visualising the activity of the cystine-glutamate antiporter in glial cells using antibodies to aminoadipic acid, a selectively transported substrate. Glia. 2001;34:27–38.
64 Qin S, Colin C, Hinners I, Gervais A, Cheret C, Mallat M. System Xc- and apolipoprotein E expressed by microglia have opposite effects on the neurotoxicity of amyloid-beta peptide 1-40. J Neurosci. 2006;26:3345–3356.
65 Sato H, Tamba M, Ishii T, Bannai S. Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. J Biol Chem. 1999;274: 11455–11458.
66 Sato H, Tamba M, Okuno S, Sato K, Keino-Masu K, Masu M, et al. Distribution of cystine/glutamate exchange transporter, system x(c)-, in the mouse brain. J Neurosci. 2002;22:8028–8033.
67 Sato H, Shiiya A, Kimata M, Maebara K, Tamba M, Sakakura Y, et al. Redox imbalance in cystine/glutamate transporter-deficient mice. J Biol Chem. 2005;280:37423–37429.
68 Murphy TH, Schnaar RL, Coyle JT. Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. FASEB J. 1990;4:1624–1633.
69 La Bella V, Valentino F, Piccoli T, Piccoli F. Expression and developmental regulation of the cystine/glutamate exchanger (xc-) in the rat. Neurochem Res. 2007;32:1081–1090.
70 Kanai Y, Hediger MA. The glutamate and neutral amino acid transporter family: physiological and pharmacological implications. Eur J Pharmacol. 2003;479:237–247.
71 Maragakis NJ, Rothstein JD. Glutamate transporters: animal models to neurologic disease. Neurobiol Dis. 2004;15:461–473.
72 Koch HP, Larsson HP. Small-scale molecular motions accomplish glutamate uptake in human glutamate transporters. J Neurosci. 2005;25:1730–1736.
73 Yernool D, Boudker O, Jin Y, Gouaux E. Structure of a glutamate transporter homologue from Pyrococcus horikoshii. Nature. 2004;431:811–818.
74 Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, et al. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron. 1996;16:675–686.
75 Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, et al. Localization of neuronal and glial glutamate transporters. Neuron. 1994;13:713–725.
76 Zerangue N, Kavanaugh MP. Interaction of L-cysteine with a human excitatory amino acid transporter. J Physiol. 1996;493 (Pt 2):419–423.
77 Fournier KM, Gonzalez MI, Robinson MB. Rapid trafficking of the neuronal glutamate transporter, EAAC1: evidence for distinct trafficking pathways differentially regulated by protein kinase C and platelet-derived growth factor. J Biol Chem. 2004;279:34505–34513.
78 Davis KE, Straff DJ, Weinstein EA, Bannerman PG, Correale DM, Rothstein JD, et al. Multiple signaling pathways regulate cell surface expression and activity of the excitatory amino acid carrier 1 subtype of Glu transporter in C6 glioma. J Neurosci. 1998;18:2475–2485.
79 Schniepp R, Kohler K, Ladewig T, Guenther E, Henke G, Palmada M, et al. Retinal colocalization and in vitro interaction of the glutamate transporter EAAT3 and the serum- and glucocorticoid-inducible kinase SGK1 [correction]. Invest Ophthalmol Vis Sci. 2004;45:1442–1449.
80 Rexhepaj R, Grahammer F, Volkl H, Remy C, Wagner CA, Sandulache D, et al. Reduced intestinal and renal amino acid transport in PDK1 hypomorphic mice. Faseb J. 2006;20:2214–2222.
81 Xia P, Pei G, Schwarz W. Regulation of the glutamate transporter EAAC1 by expression and activation of delta-opioid receptor. Eur J Neurosci. 2006;24:87–93.
82 Lin CI, Orlov I, Ruggiero AM, Dykes-Hoberg M, Lee A,

Jackson M, et al. Modulation of the neuronal glutamate transporter EAAC1 by the interacting protein GTRAP3-18. Nature. 2001;410:84–88.

83 Krizman-Genda E, Gonzalez MI, Zelenaia O, Robinson MB. Evidence that Akt mediates platelet-derived growth factor-dependent increases in activity and surface expression of the neuronal glutamate transporter, EAAC1. Neuropharmacology. 2005;49:872–882.

84 Sheldon AL, Gonzalez MI, Robinson MB. A carboxyl-terminal determinant of the neuronal glutamate transporter, EAAC1, is required for platelet-derived growth factor-dependent trafficking. J Biol Chem. 2006;281:4876–4886.

85 Gonzalez MI, Kazanietz MG, Robinson MB. Regulation of the neuronal glutamate transporter excitatory amino acid carrier-1 (EAAC1) by different protein kinase C subtypes. Mol Pharmacol. 2002;62:901–910.

86 Kalandadze A, Wu Y, Robinson MB. Protein kinase C activation decreases cell surface expression of the GLT-1 subtype of glutamate transporter. Requirement of a carboxyl-terminal domain and partial dependence on serine 486. J Biol Chem. 2002;277:45741–45750.

87 Huang Y, Feng X, Sando JJ, Zuo Z. Critical role of serine 465 in isoflurane-induced increase of cell-surface redistribution and activity of glutamate transporter type 3. J Biol Chem. 2006; 281:38133–38138.

88 Borre L, Kanner BI. Arginine 445 controls the coupling between glutamate and cations in the neuronal transporter EAAC-1. J Biol Chem. 2004;279:2513–2519.

89 Liu Y, Vidensky S, Ruggiero AM, Maier S, Sitte HH, Rothstein JD. Reticulon RTN2B Regulates Trafficking and Function of Neuronal Glutamate Transporter EAAC1. J Biol Chem. 2008; 283:6561–6571.

90 Maier S, Reiterer V, Ruggiero AM, Rothstein JD, Thomas S, Dahm R, et al. GTRAP3-18 serves as a negative regulator of Rab1 in protein transport and neuronal differentiation. J Cell Mol Med. In press 2008.

91 Bianchi MG, Gazzola GC, Tognazzi L, Bussolati O. C6 glioma cells differentiated by retinoic acid overexpress the glutamate transporter excitatory amino acid carrier 1 (EAAC1). Neuroscience. 2008;151:1042–1052.

92 Ruggiero AM, Liu Y, Vidensky S, Maier S, Jung E, Farhan H, et al. The Endoplasmic Reticulum Exit of Glutamate Transporter Is Regulated by the Inducible Mammalian Yip6b/GTRAP3-18 Protein. J Biol Chem. 2008;283:6175–6183.

93 Ikemoto MJ, Inoue K, Akiduki S, Osugi T, Imamura T, Ishida N, et al. Identification of addicsin/GTRAP3-18 as a chronic morphine-augmented gene in amygdala. Neuroreport. 2002;13: 2079–2084.

94 Wang NP, Zhou JW, Li AP, Cao HX, Wang XR. [The mechanism of JWA gene involved in oxidative stress of cells.] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2003;21:212–215. (text in Chinese with English abstract)

95 Mao WG, Li AP, Ye J, Huang S, Li AQ, Zhou JW. [Expressions of JWA protein and heat stress protein 70 induced by cell differentiation inducers combined with heat stress in K562 cells.] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2004;22:60–63. (text in Chinese with English abstract)

96 Watabe M, Aoyama K, Nakaki T. Regulation of glutathione synthesis via interaction between glutamate transport-associated protein 3-18 (GTRAP3-18) and excitatory amino acid carrier-1 (EAAC1) at plasma membrane. Mol Pharmacol. 2007;72:1103–1110.

97 Watabe M, Aoyama K, Nakaki T. A dominant role of GTRAP3-18 in neuronal glutathione synthesis. J Neurosci. 2008;28:9404–9413.

98 Aebi S, Assereto R, Lauterburg BH. High-dose intravenous glutathione in man. Pharmacokinetics and effects on cyst(e)ine in plasma and urine. Eur J Clin Invest. 1991;21:103–110.

99 Cornford EM, Braun LD, Crane PD, Oldendorf WH. Blood-brain barrier restriction of peptides and the low uptake of enkephalins. Endocrinology. 1978;103:1297–1303.

100 Zeevalk GD, Manzino L, Sonsalla PK, Bernard LP. Characterization of intracellular elevation of glutathione (GSH) with glutathione monoethyl ester and GSH in brain and neuronal cultures: relevance to Parkinson's disease. Exp Neurol. 2007; 203:512–520.

101 Kannan R, Yi JR, Tang D, Li Y, Zlokovic BV, Kaplowitz N. Evidence for the existence of a sodium-dependent glutathione (GSH) transporter. Expression of bovine brain capillary mRNA and size fractions in Xenopus laevis oocytes and dissociation from gamma-glutamyltranspeptidase and facilitative GSH transporters. J Biol Chem. 1996;271:9754–9758.

102 Sechi G, Deledda MG, Bua G, Satta WM, Deiana GA, Pes GM, et al. Reduced intravenous glutathione in the treatment of early Parkinson's disease. Prog Neuropsychopharmacol Biol Psychiatry. 1996;20:1159–1170.

103 Tsan MF, White JE, Rosano CL. Modulation of endothelial GSH concentrations: effect of exogenous GSH and GSH monoethyl ester. J Appl Physiol. 1989;66:1029–1034.

104 Aschner M, Mullaney KJ, Wagoner D, Lash LH, Kimelberg HK. Intracellular glutathione (GSH) levels modulate mercuric chloride (MC)- and methylmercuric chloride (MeHgCl)-induced amino acid release from neonatal rat primary astrocytes cultures. Brain Res. 1994;664:133–140.

105 Pileblad E, Magnusson T. Increase in rat brain glutathione following intracerebroventricular administration of gamma-glutamylcysteine. Biochem Pharmacol. 1992;44:895–903.

106 Puka-Sundvall M, Eriksson P, Nilsson M, Sandberg M, Lehmann A. Neurotoxicity of cysteine: interaction with glutamate. Brain Res. 1995;705:65–70.

107 De Flora S, Izzotti A, D'Agostini F, Cesarone CF. Antioxidant activity and other mechanisms of thiols involved in chemoprevention of mutation and cancer. Am J Med. 1991;91:122S–130S.

108 Sekhon B, Sekhon C, Khan M, Patel SJ, Singh I, Singh AK. N-Acetyl cysteine protects against injury in a rat model of focal cerebral ischemia. Brain Res. 2003;971:1–8.

109 Hart AM, Terenghi G, Kellerth JO, Wiberg M. Sensory neuroprotection, mitochondrial preservation, and therapeutic potential of N-acetyl-cysteine after nerve injury. Neuroscience. 2004;125:91–101.

110 Aoyama K, Matsumura N, Watabe M, Nakaki T. Oxidative stress on EAAC1 is involved in MPTP-induced glutathione depletion and motor dysfunction. Eur J Neurosci. 2008;27:20–30.

111 Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med. 2004;10 Suppl:S18–S25.

112 Odetti P, Garibaldi S, Norese R, Angelini G, Marinelli L, Valentini S, et al. Lipoperoxidation is selectively involved in progressive supranuclear palsy. J Neuropathol Exp Neurol. 2000;59:393–397.

113 Zarkovic K. 4-hydroxynonenal and neurodegenerative diseases. Mol Aspects Med. 2003;24:293–303.
114 Christen Y. Oxidative stress and Alzheimer disease. Am J Clin Nutr. 2000;71:621S–629S.
115 Ramassamy C, Averill D, Beffert U, Theroux L, Lussier-Cacan S, Cohn JS, et al. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. Neurobiol Dis. 2000;7:23–37.
116 Bermejo P, Martin-Aragon S, Benedi J, Susin C, Felici E, Gil P, et al. Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from Mild Cognitive Impairment. Free Radic Res. 2008;42:162–170.
117 Lovell MA, Xie C, Markesbery WR. Decreased glutathione transferase activity in brain and ventricular fluid in Alzheimer's disease. Neurology. 1998;51:1562–1566.
118 Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, et al. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol. 1994;36:348–355.
119 Jenner P. Oxidative damage in neurodegenerative disease. Lancet. 1994;344:796–798.
120 Albers DS, Augood SJ. New insights into progressive supranuclear palsy. Trends Neurosci. 2001;24:347–353.
121 Fitzmaurice PS, Ang L, Guttman M, Rajput AH, Furukawa Y, Kish SJ. Nigral glutathione deficiency is not specific for idiopathic Parkinson's disease. Mov Disord. 2003;18:969–976.
122 Siderowf A, Stern M. Update on Parkinson disease. Ann Intern Med. 2003;138:651–658.
123 Kang Y, Viswanath V, Jha N, Qiao X, Mo JQ, Andersen JK. Brain gamma-glutamyl cysteine synthetase (GCS) mRNA expression patterns correlate with regional-specific enzyme activities and glutathione levels. J Neurosci Res. 1999;58:436–441.
124 Pileblad E, Magnusson T, Fornstedt B. Reduction of brain glutathione by L-buthionine sulfoximine potentiates the dopamine-depleting action of 6-hydroxydopamine in rat striatum. J Neurochem. 1989;52:978–980.
125 Wullner U, Loschmann PA, Schulz JB, Schmid A, Dringen R, Eblen F, et al. Glutathione depletion potentiates MPTP and MPP+ toxicity in nigral dopaminergic neurons. Neuroreport. 1996;7:921–923.
126 Trinh K, Moore K, Wes PD, Muchowski PJ, Dey J, Andrews L, et al. Induction of the phase II detoxification pathway suppresses neuron loss in Drosophila models of Parkinson's disease. J Neurosci. 2008;28:465–472.
127 Trotti D, Danbolt NC, Volterra A. Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration? Trends Pharmacol Sci. 1998;19:328–334.
128 Plaitakis A, Shashidharan P. Glutamate transport and metabolism in dopaminergic neurons of substantia nigra: implications for the pathogenesis of Parkinson's disease. J Neurol. 2000;247 Suppl 2:II25–II35.
129 Berman AE, Chan WY, Brennan AM, Swanson RA. Age-dependent dopaminergic cell loss and nitration of α-synuclein in the substantia nigra of EAAC1–/– mice. Soc Neurosci Abstr. 2007;33:370.3.
130 Schmidt N, Ferger B. Neurochemical findings in the MPTP model of Parkinson's disease. J Neural Transm. 2001;108:1263–1282.
131 Matsumura N, Kikuchi-Utsumi K, Nakaki T. Activities of 7-nitroindazole and 1-(2-(trifluoromethylphenyl)-imidazole independent of neuronal nitric-oxide synthase inhibition. J Pharmacol Exp Ther. 2008;325:357–362.
132 Przedborski S, Jackson-Lewis V, Yokoyama R, Shibata T, Dawson VL, Dawson TM. Role of neuronal nitric oxide in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. Proc Natl Acad Sci U S A. 1996;93:4565–4571.
133 Trotti D, Nussberger S, Volterra A, Hediger MA. Differential modulation of the uptake currents by redox interconversion of cysteine residues in the human neuronal glutamate transporter EAAC1. Eur J Neurosci. 1997;9:2207–2212.