Glutathione—a review on its role and significance in Parkinson's disease

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ABSTRACT Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting over a million people in the United States alone, and is characterized by rigidity, bradykinesia, resting tremor, and postural instability. Its main neuropathological feature is the loss of dopaminergic neurons of the substantia nigra pars compacta. However, the pathogenesis of this loss is not understood fully. One of the earliest biochemical changes seen in PD is a reduction in the levels of total glutathione, a key cellular antioxidant. Traditionally, it has been thought that this decrease in GSH levels is the consequence of increased oxidative stress, a process heavily implicated in PD pathogenesis. However, emerging evidence suggests that GSH depletion may itself play an active role in PD pathogenesis. This review aims to explore the contribution of GSH depletion to PD pathogenesis.—Martin, H. L., Teismann, P. Glutathione—a review on its role and significance in Parkinson's disease. *FASEB J.* 23, 3263–3272 (2009). www.fasebj.org

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PARKINSON'S DISEASE (PD) is a common neurodegenerative disease that affects over a million people in the United States alone (1). Clinically, it is characterized by rigidity, bradykinesia, resting tremor, and postural instability (2). The primary neuropathogical feature of PD is the loss of the dopaminergic neurons of the substantia nigra pars compacta (SN) that project to the striatum (2). Another major pathological feature of PD is the presence of Lewy bodies in the surviving neurons. These are cytoplasmic, proteinaceous inclusions rich in α-synuclein and ubiquitinated proteins (2). The pathogenesis of PD is poorly understood, and most cases are idiopathic in onset (2). However, a number of processes have been implicated in the degeneration of the dopaminergic neurons. These processes include oxidative stress, inhibition of mitochondrial complex I, ubiquitin-proteasome dysfunction, and inflammation (3–6). One of the earliest biochemical changes seen in PD patients is a decrease in reduced glutathione (GSH) levels; GSH is a major component of cellular antioxidant defenses (7–9). A decrease in GSH levels also occurs in incidental Lewy body disease (7), which is thought to be an asymptomatic precursor to PD (10). This suggests that GSH depletion in the SN may play a more active role in PD pathogenesis than previously thought. Indeed recent studies reveal that GSH depletion may be actively involved in complex I inhibition, disruption of the ubiquitin-proteasome system, and may have effects that affect the inflammatory processes seen in PD. This review aims to examine these links and determine how potentially important GSH depletion may be in PD pathogenesis.

GLUTATHIONE FUNCTIONS AND METABOLISM

Glutathione (γ-glutamylcysteinylglycine) is the most abundant nonprotein thiol in cells (9, 11). Glutathione protects cells against exogenous and endogenous toxins, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). Such radical species are removed *via* nonenzymatic reduction with GSH, whereas the removal of hydroperoxides requires enzymatic catalysis by glutathione peroxidase (**Fig. 1**) (9, 11–13). Both reactions lead to the generation of glutathione disulfide (GSSG, or oxidized glutathione), which is reduced back to GSH by glutathione reductase that uses NADPH from the pentose phosphate shunt (9). Glutathione also modifies protein sulfdryl groups by a number of reactions; reduction of protein sulfenic acids, formation of protein mixed disulfides and their subsequent reduction. Conjugation of GSH with electrophilic compounds, mediated by the glutathione-*S*-transferases (GSTs) and subsequent excretion of these conjugates from the cell, also serves to protect cells from toxins (9, 11–13).

When the redox state of a cell is altered, increased GSH usage occurs, and while the GSSG generated can be reduced back to GSH, the formation and export of GSH conjugates leads to GSH depletion. This depletion is attenuated by *de novo* synthesis of GSH (9, 13). Glutathione synthesis in neurons and the supporting glial cells, as in other cells, is a 2-step ATP-dependent
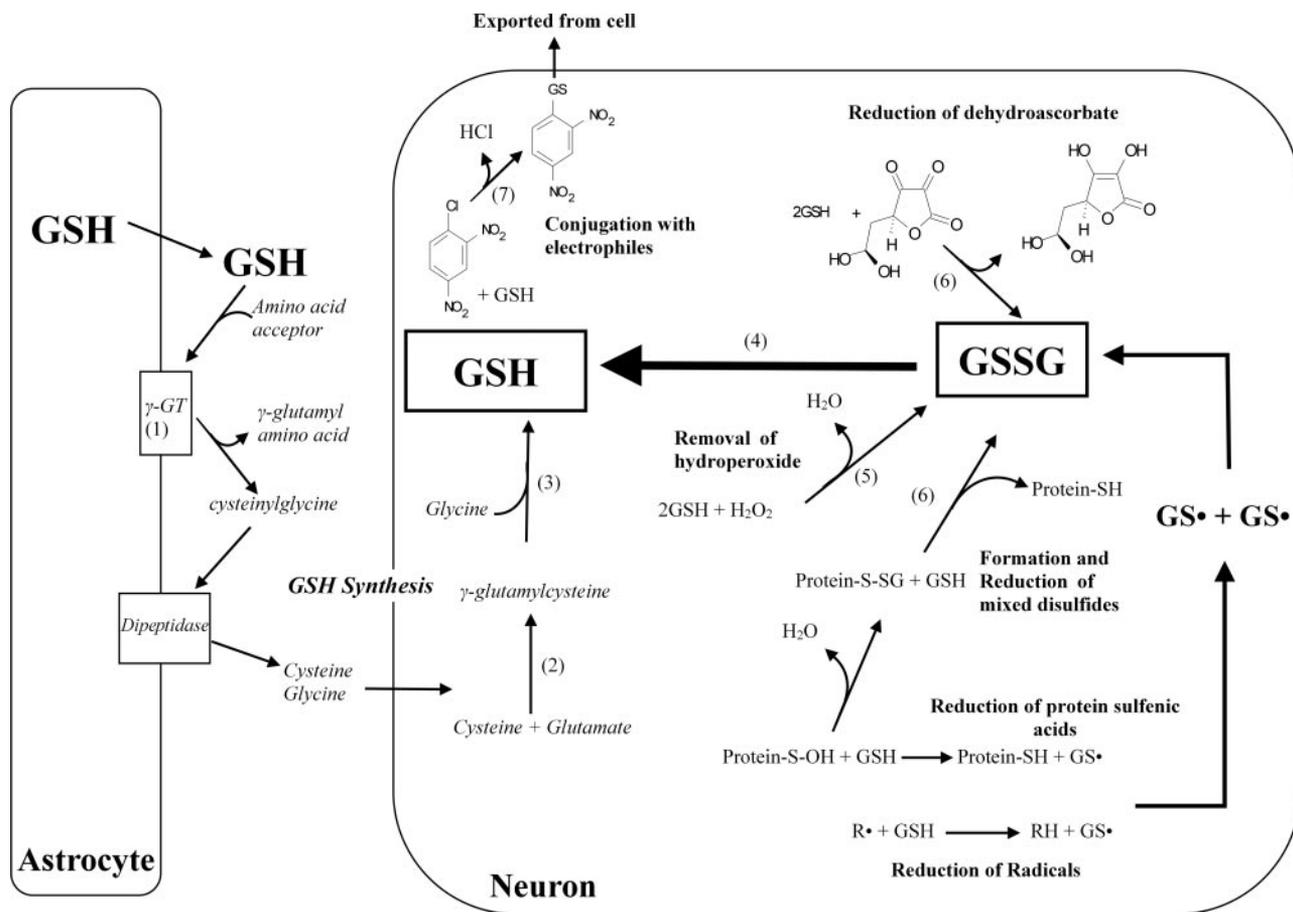
Figure 1. Glutathione synthesis and metabolism in the central nervous system. Both neurons and astrocytes have the capacity to synthesize GSH (shown in italics), but astrocytes also play important roles in substrate supply to neurons. Glutathione synthesized in astrocytes is exported in the extracellular space, where the ecto-enzyme γ-GT (*1*) transfers the glutamyl group onto an amino acid acceptor, creating cysteinylglycine, which is then broken down into its constituents, cysteine and glycine. Glutathione is synthesized in two steps: first, γ-glutamylcysteine is formed from cysteine and glutamate, catalyzed by GCL (*2*); then glycine is added by GSH synthase (*3*). Glutathione has many roles in detoxification and maintenance of redox equilibrium. The majority of these processes generate GSSG, which is reduced back to GSH by glutathione reductase and NADPH from the pentose phosphate shunt (*4*). (See refs. 9, 11–13). *1*) γ-GT; *2*) GCL; *3*) glutathione synthase; *4*) glutathione reductase; *5*) glutathione peroxidase; *6*) glutaredoxin; *7*) GST.

process (Fig. 1) (9, 11–13). The first of these steps, forming γ-glutamylcysteine from glutamate and cysteine, is rate limiting, as the critical factor is the supply of cysteine (11, 13). This step is also influenced by GSH levels, as L-glutamate:L-cysteine γ-ligase (GCL), the enzyme catalyzing this reaction, is nonallosterically inhibited by GSH in a negative feedback fashion. The second step, the addition of glycine to generate GSH, is catalyzed by glutathione synthase (11, 13). Although both neurons and glial cells can synthesize GSH, glial cells, specifically astrocytes, also have important roles to play in supplying GSH substrates to neurons. Astrocytes synthesize and export GSH, which can then undergo transpeptidation to cysteinylglycine and γ-glutamyl amino acid by the ecto-enzyme γ-glutamyl transpeptidase (γ-GT). The cysteinylglycine generated can then be utilized by neurons to manufacture GSH, probably undergoing dipeptide cleavage to its constituent amino acids first. This mechanism of substrate supply minimizes the neurotoxic effects of large amounts of extracellular cysteine, which can activate glutamate recep-

tors (11). A full discussion of the functions of GSH and its maintenance in neuronal cells is beyond the scope of this review, and the reader is referred to Zeevalk *et al.* (11) and Dringen (12) for further information.

CAUSES OF GLUTATHIONE DEPLETION

Glutathione is a major antioxidant that functions to maintain the redox equilibrium of a cell, which can be expressed as GSSG:2GSH (14). Oxidative stress results when this redox equilibrium is altered in favor of GSSG, which can either be due to a decrease in the reducing capacity of the cell or an increase in the reduction potential (15). The former is a result of decreased levels of cellular antioxidants, predominantly GSH, while the latter comes from increased generation of ROS and RNS. The sum of these changes is increased oxidative stress. Oxidative stress can damage most of the cellular macromolecules, evident from protein and DNA adduct formation and lipid peroxi-

dation. Such markers of oxidative stress are increased in PD patients, with raised levels of both 8-hydroxyguanine and 4-hydroxynonenal, indicative of oxidative DNA damage and lipid peroxidation, detected in the SN (16–17). Increased protein carbonyls are also seen in PD patients but not in incidental Lewy body disease, suggesting that oxidative protein damage is a late occurrence in PD pathogenesis (18).

ALTERATIONS IN THE SYNTHESIS AND METABOLISM OF GLUTATHIONE IN PD?

The GSH depletion seen in PD may result from a decrease in synthesis and recycling under normal redox conditions. To determine whether this is the case, it is necessary to assess the enzymes and substrates involved in GSH synthesis and metabolism. If alterations in GSH synthesis were the cause of the GSH depletion seen in PD, it would be anticipated that the activity of the rate-limiting enzyme in GSH synthesis, GCL, would be decreased. It has been reported that activity levels of GCL are reduced throughout the brain as a consequence of the aging process (19). This was attributed to a reduction in the levels of the light subunit of GCL (19) that modulates the affinity of the enzyme for substrates and inhibitors (13). This decrease is in agreement with the fall in GCL activity seen in immortalized N27 mesencephalic dopaminergic cells 6 and 12 h after administration of the parkinsonian toxin 1-methyl-4-phenylpyridinium (MPP^+). However, this decrease was transient, and the activity levels of GCL returned to control levels 24 h after MPP^+ treatment (20). The short-lived nature of this change is consistent with reports that the activity of GCL is unaffected in PD patients at postmortem (21). It appears unlikely that decreased GCL activity underpins GSH depletion in PD, although it may help explain why PD incidence increases with age (2). If it is true that GCL function in PD is normal, then a decrease in GSH would be anticipated to increase GCL activity due to the reduced amount of negative feedback (13); however, such an increase is not observed (20–21). This may be due to alterations in the cysteine supply that is critical for GSH synthesis (11). Indeed, circulating levels of cysteine decrease with age (22), and the incidence of PD increases with age (2), and such a decrease may contribute to the GSH depletion seen in PD. Also, the main uptake route for cysteine into neurons, the excitatory amino acid carrier 1 (EAAC1), is altered by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; metabolized to the bioactive MPP^+) treatment (23). Administration of MPTP caused EAAC1 translocation to the cell membrane of dopaminergic neurons. This finding suggests that dopaminergic neurons attempt to maintain GSH homeostasis as MPTP treatment reduces total GSH levels by ~30% (21, 23). However, nitration of EAAC1 was also increased by MPTP treatment, and this is thought to inactivate EAAC1, as the uptake of cysteine into midbrain slices treated with MPP^+ was reduced

(23). This study suggests that cysteine supply to neurons could be altered in PD; this finding is supported by increased activity and levels of γ-GT both in dopaminergic cells and in PD patients as an attempt to generate neuronal GSH (21, 24).

Glutathione depletion may also result from an increased efflux of GSSG and GSH conjugates (21). However, global GST activity remains unchanged, so at least enzymatic conjugation is not increased (21), and although MPP^+ treatment caused a 30% decrease in GSH in N27 cells, no increase in GSH efflux was detected (20). It is possible that nonenzymatic oxidation of GSH to GSSG is increased, and the most likely substrates for this would be ROS/RNS. The reduction of GSSG back to GSH, in addition to *de novo* synthesis, is important in maintaining cellular levels of GSH. Levels of glutathione reductase, the enzyme responsible for this reduction (9), are reported to be increased in PD patients (25). This finding suggests that levels of GSSG may be increased, and GSSG levels have been reported to be nonsignificantly increased in PD patients at postmortem (7). This increase in glutathione reductase levels again suggests that the cells of the SN are attempting to maintain GSH levels. These findings are in concurrence with those of Drechsel *et al.* (20), showing an increase in glutathione reductase activity levels in N27 cells after MPP^+ treatment. Initially, however, glutathione reductase activity was lowered transiently before it increased, in a similar fashion to GCL activity levels (20). This decrease in glutathione reductase activity (20) can alter the GSSG:2GSH ratio, leading toward a state of oxidative stress, which may be sufficient to start the downward spiral of neurodegeneration that continues even though the activity of the GSH synthesizing and metabolizing enzymes return to normal levels.

SOURCES OF OXIDATIVE AND NITROSATIVE STRESS IN PD

The possible decrease in GSSG reduction to GSH combined with a potential decrease in *de novo* synthesis of GSH could lead to the state of cellular oxidative stress seen in Parkinson's disease. However, the late onset of idiopathic PD (2) suggests that the oxidative load of the dopaminergic neurons may need to reach a threshold before GSH depletion becomes important in the spiral of neurodegeneration. As dopaminergic neurons normally have a high oxidative load, it may not require excessive additional ROS/RNS generation to have a negative effect on these cells (3). The sources and effects of oxidative stress in PD have been widely reviewed elsewhere (most recently in refs. 26, 27), but one reason why the dopaminergic neurons might be particularly vulnerable in PD is that the metabolism of dopamine (DA) generates ROS and hydrogen peroxide (H_2O_2) (28). Nonenzymatic autooxidation of DA forms a reactive quinone, while DA is metabolized enzymatically by monoamine oxidase and catechol-*O*-methyl

transferase, also generating H_2O_2 (28). It can also be metabolized by the peroxidase site of cyclooxygenase-2 (COX-2; also known as prostaglandin H_2 synthase), again generating reactive quinones and semiquinones (29, 30). The pharmacological or genetic ablation of COX-2 has been shown to provide neuroprotection in the MPTP mouse model of PD (31). The quinones and semiquinones formed interact with protein sulfdryl groups and GSH, thus decreasing levels of GSH (28). Detoxification of H_2O_2 requires glutathione peroxidise, and activity levels of this enzyme are mildly reduced in PD patients at postmortem (32), possibly because of the reduced GSH levels as glutathione peroxidise utilizes GSH as a cosubstrate. Hydrogen peroxide can also be converted into the more damaging hydroxyl radical in the presence of transition metals, and the levels of certain transition metals, including iron, zinc, manganese, and copper, have been shown to increase in rats after treatment with the parkinsonian toxin 6-hydroxydopamine (6-OHDA) (33). The most dramatic rise was in total iron content with an approximate increase of 2.5-fold (33) and in PD patients a rise in total iron, of a similar magnitude, was also observed (34). Thus, it can be seen that DA metabolism produces a variety of radical species that need GSH for detoxification. Consequentially, the basal demand for GSH is high in these dopaminergic neurons, so small alterations in GSH or ROS/RNS levels can have a big effect. Other important sources of ROS and RNS in PD include NADPH oxidase and nitric oxide synthase. Both of which can appear in microglia and whose genetic or pharmacological ablation provides protection against MPTP toxicity (35–40). This suggests that microglia could be a pivotal source of ROS/RNS important for GSH depletion and in PD pathogenesis. The relevance of microglia to PD pathogenesis is supported by the glial activation reaction that is seen in PD, and following MPTP administration (41–43). This glial reaction peaks before maximal dopaminergic neuron death occurs, indicating that it is not solely a response to the death of the dopaminergic neurons (38). Inhibition of microglia activation serves to attenuate MPTP neurotoxicity (44). The involvement of microglial activation in PD is not surprising considering that the SN has the highest density of microglia in the entire brain (45). The role of microglia in PD pathogenesis extends beyond ROS/RNS generation, as these cells are the immune sensors and effectors of the central nervous system (46) and therefore have important roles in the inflammatory processes seen in PD. The role of GSH in these inflammatory processes is explored in more detail later in this review.

In summary, GSH depletion and ROS/RNS generation are highly interlinked, and it is difficult to determine which comes first in PD pathogenesis. It is probable, however, that some degree of ROS/RNS generation occurs before GSH depletion. The precise source of these ROS/RNS remains elusive, although they probably come from multiple sources. The normally high oxidative load of dopaminergic cells may also make them more vulnerable to subtle increases in ROS/RNS. These increases might then be exacerbated by alterations in GSH synthesis and metabolism, especially alterations in cysteine supply. The resultant GSH depletion would then further increase the oxidative load on the dopaminergic neurons and affect the biological functioning of these cells.

GLUTATHIONE DEPLETION AND INHIBITION OF MITOCHONDRIAL COMPLEX I

Mitochondrial complex I is the first and most complicated complex of the electron transport chain (4). It oxidizes NADH and transfers the electrons to ubiquinone. Postmortem evidence from PD patients demonstrates a defect in complex I function in the SN when compared with age-matched controls (47). The role of a complex I defect in PD pathogenesis is supported further by the ability of MPP^+, 6-OHDA, and rotenone to induce dopaminergic neuron death, as these compounds are known to inhibit complex I (48, 49). It has been suggested that a prolonged yet mild inhibition of complex I would lead to increased ROS production and a subsequent decrease in GSH levels (4), implying that GSH depletion is secondary to complex I inhibition. However, MPTP and rotenone are also reported to reduce GSH levels by $\sim$30 and 50%, respectively (23, 50), and GSH depletion is seen in incidental Lewy body disease, a presymptomatic precursor to PD, in the absence of complex I inhibition (7). Consequently, it remains unclear whether GSH depletion or complex I inhibition comes first. However, a number of studies have shown that GSH depletion can impair complex I function and may even precede complex I inhibition. Hsu *et al.* (51) have demonstrated that reducing GSH by $\sim$50% in dopaminergic PC12 cells results in a 50% reduction in complex I activity as measured by reduction of 2,6-dichlorophenolindophenol. These cells were genetically engineered to permit inducible inhibition of GCL, and by using this method to reduce GSH levels, the changes seen in GSH and GSSG mimic those seen in PD patients (8, 52). These effects of GSH depletion on complex I activity were replicated in N27 cells using a pharmacological inhibitor of GCL, L-buthionine-*S*-sulfoximine (BSO), for 7 d to mimic more chronic GSH depletion as is the case in PD (53). Both these studies further explored this effect and implicated the complex I inhibition seen as being RNS dependent, which strongly suggests that *S*-nitrosation of complex I occurs in GSH-depleted conditions. This is in concurrence with Clementi *et al.* (54), who have shown that persistent inhibition of complex I occurs with prolonged exposure to nitric oxide and that this condition is accelerated by GSH depletion. This inhibition was reversible with the addition of reducing agents such as DTT, exogenous GSH, or the presence of light. This reversibility, especially the photolability, implies that a nitrosothiol group had been formed (54). Dahm *et al.* (55) have also demonstrated that

nitrosative stress leads to increased nitrosothiol group formation in mitochondrial proteins. They have proposed that this formation involves *S*-nitrosoglutathione, and these nitrosothiols would normally be removed by reduced GSH. Bharath and Andersen (56) have also shown an increase in nitrotyrosine immunoreactivity in complex I-enriched fractions from GSH-depleted cells. These studies indicate that GSH normally functions to protect complex I from nitrosative damage, so the GSH depletion seen in PD could lead to complex I inhibition.

Another mechanism by which GSH depletion is thought to have a negative effect on complex I activity is *via* the increase in γ-GT activity seen in PD (21, 24). It is hypothesized that such an increase would result in increased intraneuronal levels of cysteine which can react with DA to form 5-cysteinyl dopamine quinones, metabolites of which can inhibit complex I (57). However, inhibition of γ-GT exacerbates the GSH depletion-induced reduction in complex I activity that was alleviated by the addition of cysteine (24). These results suggest that the increases seen in γ-GT activity are a compensatory event attempting to increase GSH levels that would otherwise lead to a reduction in complex I activity (24).

It appears that these GSH depletion-induced reductions in complex I activity are primarily confined to dopaminergic cells, as Seaton *et al.* (58) did not find any in changes in complex I activity in the cerebral cortices of rats treated with BSO. Also, in contrast to dopaminergic cells, GSH depletion in glial cultures causes up-regulation of complex I activity and enhanced levels of mRNA of the ND6 subunit of complex I (59). This suggests that the ability of GSH depletion to inhibit complex I is specific to dopaminergic cells and

may, at least in part, account for their vulnerability in PD. If complex I inhibition is secondary to GSH depletion, it raises the question as to the mechanism by which GSH is depleted, as it is generally assumed that oxidative stress resulting from complex I inhibition and the downstream effects of this gives rise to the decreased GSH levels. As reviewed in the previous section, GSH depletion and oxidative stress are inextricably linked, and so the GSH depletion that affects complex I activity may result from multiple processes. Inhibition of complex I activity has several downstream effects, including increased ROS generation that compounds GSH depletion and a reduction in ATP generation by the electron transport chain. Both these effects affect the other pathogenic processes that result in PD.

GLUTATHIONE DEPLETION AND DYSFUNCTION OF THE UBIQUITIN-PROTEASOME SYSTEM (UPS)

The UPS targets misfolded and damaged proteins to the 26S proteasome for degradation by the addition of ubiquitin monomers (**Fig. 2**) (5). Evidence for UPS dysfunction in PD comes from the rare familial forms of the disease that can be linked to specific genes whose products are involved in the UPS–Parkin, for example, is an E3 ubiquitin ligase (5). Also, the Lewy bodies seen in PD contain high amounts of ubiquitinated proteins (2). Glutathione depletion may affect the UPS either in a direct manner or indirectly as a result of complex I inhibition and subsequent ATP depletion, as ubiquitination is an ATP-dependent process (5). In fact, GSH depletion directly affects the UPS at both the level of E1 ubiquitin ligase and at the level of proteasome. Jha *et al.*
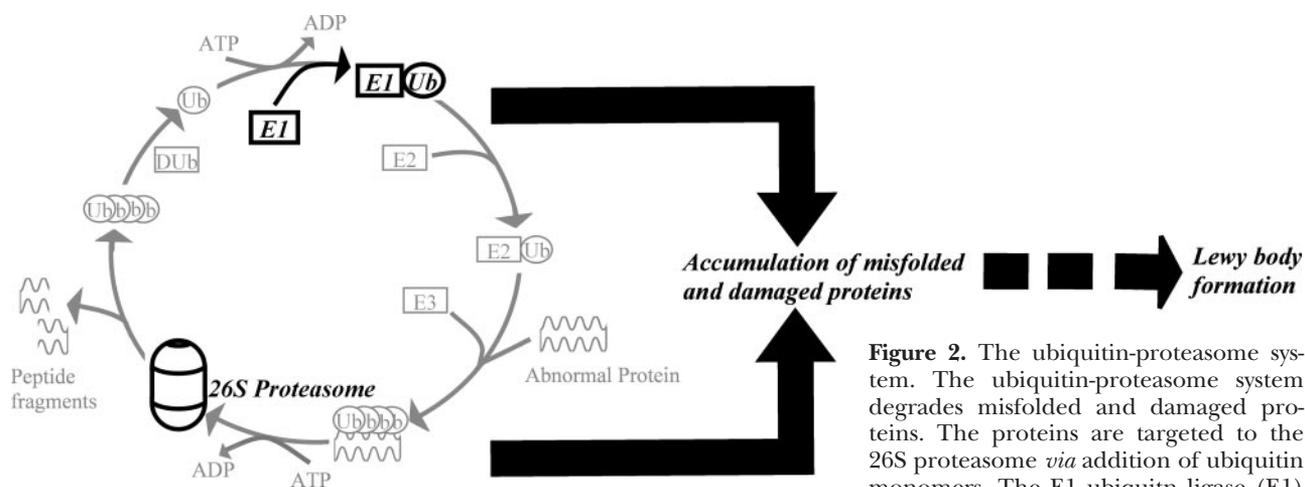

Figure 2. The ubiquitin-proteasome system. The ubiquitin-proteasome system degrades misfolded and damaged proteins. The proteins are targeted to the 26S proteasome *via* addition of ubiquitin monomers. The E1 ubiquitn ligase (E1) conjugates with an ubiquitin monomer in an ATP-dependent process. The ubquitin monomer is then transferred to E2 ubiquitin ligase (E2) before being attached to an abnormal protein by E3 ubiquitin ligase (E3). Once the abnormal protein has a polyubqiuitin chain, it is degraded by the 26S proteasome, which consists of a 20S catalytic subunit and two regulatory subunits, into small peptide fragments. The polyubiquitin chain is recycled by deubiquinating enzymes. The effects of GSH depletion on the ubiquitin-proteasome system are shown in black, with decreased ubiquitn tagging of misfolded and damaged proteins due to reduced E1 activity as well as reduced degradation of ubiquitin tagged proteins because of decreased proteasome activity. These reduction ubquitin-proteasome system activities lead to the buildup of misfolded and damaged proteins and Lewy body formation (5, 60–62). Ub, ubiquitin; Dub, deubiquinating enzyme.

(60) have shown that ubiquitination of proteins decreases in GSH-depleted PC12 cells. This is due to an inability of E1 to bind ubiquitin when GSH levels are <50%. Restoration of GSH to normal levels restores E1 ubiquitination. The reversibility of this event implies that it is due to a thiol oxidation event, as one of the major functions of GSH is to maintain thiol groups in a reduced, functional state (9, 60). It is known that E1 ubiquitin ligase contains a sulfhydryl group that functions to add ubiquitin to its targets, and this group also conjugates with GSH (61).

Glutathione depletion also has effects on the 26S proteasome itself. Caneda-Ferron *et al.* (62) have shown that GSH depletion precedes inhibition of chymotrypsin-like proteasome activity in SH-SY5Y cultures treated with 2 mM MPP$^+$ or 0.5 mM DA. Indeed, in the cultures treated with DA, this initially increased GSH levels, and proteasome inhibition did not occur in these cultures until GSH levels had dropped below 50% of the controls, providing strong evidence that GSH depletion needs to occur first for proteasome inhibition in this model. These compounds also inhibited activity in purified human erythrocyte 20S (the catalytic subunit of the 26S proteasome) extracts, indicating that they have a direct effect on the proteasome (62). It is probable that in whole cells the effects of MPP$^+$ and DA on the UPS are both direct and indirect *via* complex I inhibition and ATP depletion. The role of potential interplay between complex I inhibitors and the proteasome has been explored by Höglinger *et al.* (63) in rat primary mesencephalic cultures. They have demonstrated that administration of a proteasome inhibitor, epoxomicin, and complex I inhibitors, MPP$^+$ and rotenone, act synergistically to increase toxicity. A 1 μM dose of MPP$^+$ combined with 100 nM of epoxomicin gave approximately the same toxicity as a 10 μM dose of MPP$^+$ alone. This effect was specific to complex I inhibitors. Unfortunately, although this study assessed changes in ROS and ATP production induced by such combination treatments, alterations in GSH levels were not assessed. It would be interesting to see whether the effects reported were preceded by a reduction in GSH levels.

These studies, taken together, implicate GSH depletion in UPS dysfunction, with direct effects on E1 ubiquitin ligase and the proteasome as well as more indirect effects on UPS function due to inhibition of complex I and ATP depletion. The effects of GSH depletion on components of the UPS are unlikely to be the sole cause of UPS dysfunction. Misfolded and damaged proteins are also liable to contribute to the dysfunction, including α-synuclein, the major component of Lewy bodies (64–66). It is highly probable that some of this damage is oxidative, including proteins modified by lipid peroxidation products, such as 4-hydroxynoneal and acrolein, and those with oxidized thiol groups that would normally be maintained in a reduced state by GSH. Glutathione may, therefore, have a more important role in maintaining effective clearance of misfolded and damaged proteins than previously thought, and the GSH depletion seen in PD would negatively affect this process.

GLUTATHIONE DEPLETION AND INFLAMMATION

The effects of GSH depletion on the inflammatory processes seen in PD have not been well studied, but evidence suggests that GSH and cytokine regulation are highly interlinked. Cellular redox status plays an important role in the regulation of interleukin (IL)-1, IL-6, and tumor necrosis factor-α transcription, and in the regulation of the signaling pathways triggered by these cytokines (67). These cytokines play a role in PD pathogenesis, as evidenced by raised levels of these proinflammatory cytokines in the cerebrospinal fluid, peripheral blood, and brains of PD patients (68–71). Yet the role of GSH in proinflammatory signaling is complex and not fully understood. Evidence suggests that GSH depletion may actually suppress immune responses (72–75). Examination of IL-1 signaling illustrates the complex nature of the influence of GSH on proinflammatory signaling. IL-1 signaling has three main stages: complex formation, activation of nuclear factor κB (NF-κB), and NF-κB nuclear translocation leading to gene transcription (67). All of these stages are inhibited by thiol-oxidizing compounds (76), the effects of which would normally be reversed by GSH. The relationship between NF-κB activation and GSH is not solely one of inhibition by thiol oxidizers. Indeed NF-κB is activated in conditions of oxidative stress (76), and this activation is inhibited by overexpression of glutathione peroxidases, indicating an important role for GSH. Glutathione also regulates NF-κB-mediated signaling, as glutathionylation of the p50 subunit prevents DNA binding (76). This suggests that the precise redox state is crucial for NF-κB activation, and so GSH depletion is likely to have mixed effects on this process. The complexity of redox state influences, and therefore GSH levels, on inflammatory mediators is highlighted by a report that the inflammatory response induced by lipopolysaccharide, in primary mesencephalic cultures, prevented GSH depletion-induced cell death (77). However, in this study and in alveolar macrophages, IL-1β and GSH depletion acted synergistically to increase cell death (77, 78). Thus, it appears that the effects of GSH depletion on inflammatory processes depend on cell type and the spectrum of inflammatory mediators produced.

Glutathione depletion can also affect the inflammatory processes occurring in PD by affecting the c-Jun N-terminal kinase (JNK) pathway, which has many roles in inflammation and immunity (reviewed in ref. 79). The JNK pathway can be activated by upstream effectors, including apoptosis signal-regulating kinase (ASK1) which normally associates with glutaredoxin. However, in a GSH-depleted environment, glutaredoxin dissociates from ASK1, which then activates JNK (11, 80). Indeed, both JNK2 and JNK3 null mice showed signif-

icant protection against MPTP-induced dopaminergic neuron loss in the SN, while the greatest protection was seen in the double-knockout animals (81). Activation of JNK can lead to COX-2 induction (81), which, as discussed above, can oxidize dopamine in its peroxidase site, generating reactive quinones. Cyclooxygenase-2 is a major source of prostaglandins, notably prostaglandin E_2, but also 15-deoxy-Δ-12,14-prostaglandin-J_2 *via* further metabolic steps. 15-deoxy-Δ-12,14-prostaglandin-J_2 can up-regulate COX-2 in a GSH-dependent manner (82). Pretreatment with BSO increased the up-regulation of COX-2 by 15-deoxy-Δ-12,14-prostaglandin-J_2 in human mesangial cells, while the GSH precursor *N*-acetylcysteine (NAC) decreased COX-2 up-regulation. It appears also that COX-2 activation is enhanced in GSH-depleted conditions. Whether this situation extends into PD pathogenesis remains to be seen, but long-term users of nonsteroidal antiinflammatory drugs that inhibit COX-2 have a reduced risk of developing PD (82), which suggests that COX-2 is important.

These studies suggest that GSH depletion may modulate the inflammation seen in PD; however, this area requires further exploration. It will be of interest, for instance, to investigate the effects of GSH depletion on IL-1 signaling in neuronal tissues, as this may differ from the effects seen in alveolar macrophages. Also, examination of the role of other JNK-activated inflammatory pathways, such as cytokine production (79), may provide useful insights into PD pathogenesis.

TREATMENT STRATEGIES

It has become clear that decreased GSH levels are linked to a significant number of the cellular processes known to be affected in PD (**Fig. 3**). This finding, combined with the fact that GSH depletion occurs early in PD, suggests that such depletion may be a critical factor in dopaminergic neurodegeneration and that replenishment of GSH may provide an option for patient treatment. However, it needs to be ascertained whether GSH replenishment would be an effective treatment at the stage of degeneration seen when PD patients are diagnosed, as this is often later than the stage when interventions are given in PD models. Administration of a precursor may provide a better
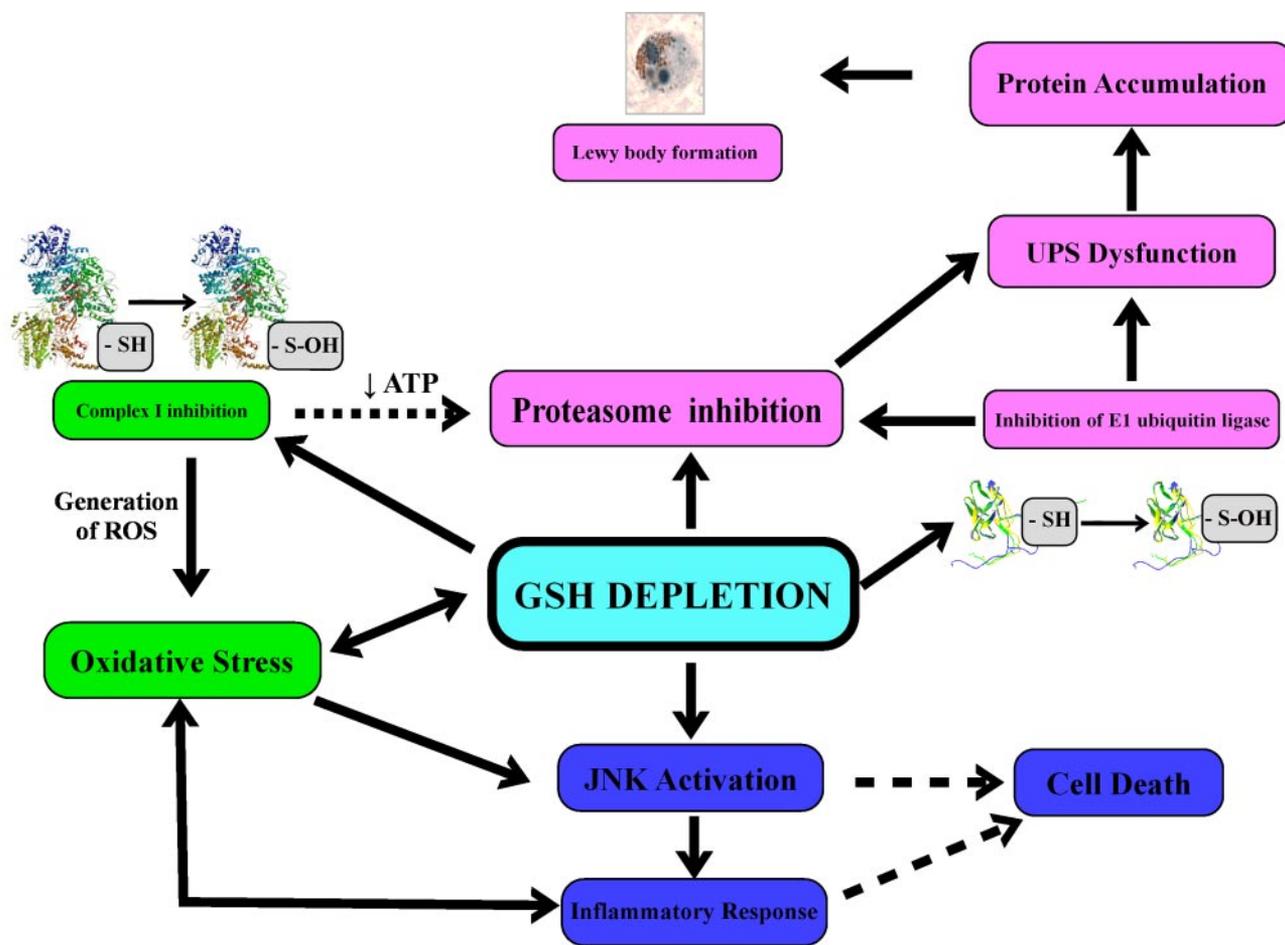

Figure 3. Effects of glutathione depletion. Glutathione depletion can inhibit complex I, E1 ubiquitin ligase (E1), and proteasome activity. It can also exacerbate oxidative stress and activate the JNK pathway, leading to an inflammatory response. These effects have the potential to cause dopaminergic neuron death and accumulation of protein into Lewy bodies. These effects suggest that GSH may have an important role to play in the pathogenesis of Parkinson's disease (32, 92–93). Dashed lines indicate potential links.

treatment, because GSH itself does not penetrate neurons, since they do not possess a GSH transporter (11). This is supported by the failure of subcutaneous GSH ethyl ester administration to rats to increase brain GSH levels (83). The most common GSH precursor, *N*-acetylcysteine (NAC), does not cross the blood-brain barrier in significant amounts (84). However, intraperitoneal administration of NAC to rats and gerbils has provided protection against oxidative damage to brain proteins and lipids (85, 86). Recent work by Zeevalk *et al.* (87) suggests that administration of γ-glutamylcysteine and cysteinylglycine can alleviate GSH depletion in mesencephalic cultures and provide neuroprotection against MPP$^+$ and oxidative stress. They have also demonstrated that these dipeptides can be utilized when attached to nanoparticles formed from human serum albumin, giving a potential administration route as dipeptide access to the brain is highly regulated by the blood-brain barrier. Indeed intraperitoneal administration to mice of γ-glutamylcysteine ethyl ester, another dipeptide precursor of GSH in a lipid soluble form that can penetrate the blood-brain barrier, provided a degree of protection from MPTP-induced loss of dopaminergic neurons (88). Despite these promising results, only one small, 9-patient study was reported by Sechi *et al.* (89). Intravenous administration of GSH showed promising results with the clinical disability of patients reduced by ~40%. The biochemical mechanisms of these effects are unclear, especially as these patients are likely to have already suffered significant dopaminergic neuron loss to present with clinical symptoms. It can be seen that replenishing GSH may have therapeutic benefits, but delivery to the dopaminergic neurons is very challenging. However, a brain-penetrable version of NAC, *N*-acetylcysteine amide (also known as AD4), has recently been developed (90). It has been shown to protect PC12 cells from rotenone-induced toxicity, and this protection was extended to the *in vivo* situation (91). These results suggest that a PD treatment that replenishes GSH levels is a clinical possibility, and to this end it will be interesting to see the outcomes of randomized, blinded placebo-controlled studies with GSH and its precursors.

CONCLUSIONS

Although GSH depletion has long been known to be an early change in PD, only recently have the biochemical effects of such depletion become apparent, notably the inhibition of complex I activity and UPS. Despite the advances in our knowledge of the role of GSH in PD pathogenesis, GSH depletion and oxidative stress in this process remain inextricably linked, with one leading to the other and *vice versa*. This means identifying the primary pathogenic event in PD remains as elusive as ever. If the cause of the GSH depletion could be delineated, this finding may provide an alternative route to maintaining GSH levels in PD patients, alleviating the neurodegenerative spiral that occurs in a GSH-depleted environment. However, the importance of GSH in the maintenance of reduced thiol groups has become clear as the inhibition of complex I and UPS dysfunction can be induced, at least in part, by oxidative and nitrosative modification of thiol groups. Such knowledge provides a potential therapeutic target, but further work must determine whether alleviation of GSH depletion slows the degeneration seen in PD. The recent advances in our understanding of the role of GSH in PD could provide exciting and promising potential for new treatments for PD patients. However, the benefits of GSH treatment for patients remain unclear and will require further studies. $\boxed{\text{FJ}}$

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