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The Redox Pathway of *S*-Nitrosoglutathione, Glutathione and Nitric Oxide in Cell to Neuron Communications

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Recent results demonstrated that *S*-nitrosoglutathione (GSNO) and nitric oxide (•NO) protect brain dopamine neurons from hydroxyl radical (•OH)-induced oxidative stress *in vivo* because they are potent antioxidants. GSNO and •NO terminate oxidant stress in the brain by (i) inhibiting iron-stimulated hydroxyl radicals formation or the Fenton reaction, (ii) terminating lipid peroxidation, (iii) augmenting the antioxidative potency of glutathione (GSH), (iv) mediating neuroprotective action of brain-derived neurotrophin (BDNF), and (v) inhibiting cysteinyl proteases. In fact, GSNO – *S*-nitrosylated GSH – is approximately 100 times more potent than the classical antioxidant GSH. In addition, *S*-nitrosylation of cysteine residues by GSNO inactivates caspase-3 and HIV-1 protease, and prevents apoptosis and neurotoxicity. GSNO-induced antiplatelet aggregation is also mediated by *S*-nitrosylation of clotting factor XIII. Thus the elucidation of chemical reactions involved in this GSNO pathway ($GSH \rightarrow GS^{\bullet} + {}^{\bullet}NO \rightarrow [GSNO] \rightarrow GSSG + {}^{\bullet}NO \rightarrow GSH$) is necessary for understanding the biology of •NO, especially its beneficial antioxidative and neuroprotective effects in the CNS. GSNO is most likely generated in the endothelial and astroglial cells during oxidative stress because these cells contain mM GSH and nitric oxide synthase. Furthermore, the transfer of GSH and •NO to neurons via this GSNO pathway may facilitate cell to neuron communications, including not only the activation of guanylyl cyclase, but also the nitrosylation of iron complexes, iron containing enzymes, and cysteinyl proteases. GSNO annihilates free radicals and promotes neuroprotection via its c-GMP-independent nitrosylation actions. This putative pathway of GSNO/GSH/•NO may provide new molecular insights for the redox cycling of GSH and GSSG in the CNS.

Keywords: Apoptosis, Brain dopamine neurons, caspases, GSNO/GSH, HIV-1 protease, nitrosylation by nitric oxide

INTRODUCTION

The discovery of nitric oxide (•NO) as the endothelial derived relaxing factor (EDRF) has been rewarded with the aware of the 1998 Nobel Prize for medicine. •NO has also been suggested to be a potent antioxidant,[1] which completely inhibits the generation of cytotoxic hydroxyl radical

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(•OH)[2] and brain lipid peroxidation.[3] Among free radicals, •NO is less reactive than most of the reactive oxygen species (i.e., superoxide anion (O_2^{-}•), •OH, peroxyl lipid radicals (LOO•) and semiquinone radicals) and thiyl radicals (i.e., cysteinyl radical (Cys•) and glutathionyl radical (GS•)). Based on its unique chemistry, •NO can nitrosylate iron complexes and annihilate reactive free radicals including lipid radicals. In fact, •NO may be listed as a neuroprotective antioxidant in the brain.[4]

The biological half-life of •NO is in seconds, which can be prolonged to last for minutes or hours after •NO reacts with GSH and converts to *S*-nitrosoglutathione (GSNO). GSNO is a potent inhibitor of platelet aggregation but a weak vasorelaxant because it is a weak activator for guanylyl cyclase.[5,6] GSNO more than •NO may induce *S*-transnitrosylation of reactive cysteine residues of peptides and proteins,[7,8] which can inhibit enzymatic activities such as clotting factor XIII to stop platelet aggregation.[5,9] It has been suggested that •NO may participate in c-GMP-dependent cell to cell communication.[10] Our group has proposed that GSNO may participate in the c-GMP-independent cell to neuron communications for a much longer duration and at sites further away than those of •NO. Independent of the activation of guanylyl cyclase, both GSNO and •NO could provide antioxidative cellular defense not only in the cells that generate •NO but also in neighboring neurons via this cell to neuron communication process. Significantly, GSNO is more potent than GSH in suppressing iron-evoked oxidant stress.[11] Moreover, the free radical reaction of •NO with O_2^{-}• yields peroxynitrite ($ONOO^{-}$),[12] which generates weak pro-oxidative effects that are completely neutralized by GSNO.[11]

Based on a discovery that GSNO and •NO protect brain dopamine neurons against oxidative damage *in vivo*,[2,11,13] this mini-review discusses the chemistry and biological effects of a possible antioxidative pathway of GSNO, GSH and •NO in the brain. Cumulative evidence has substantiated that GSNO may be a critical part of the GSH cellular defense system to protect cardiovascular[14,15] and central nervous system against oxidative stress.[2,11,13,16] This notion of •NO as a neuroprotective antioxidant[4] has been supported further by recent data that GSNO and •NO prevent proteolysis and apoptosis caused by cysteinyl proteases, including caspase-3[17] and HIV-1 protease.[16,18]

THE CHEMISTRY OF THE GSNO, GSH AND •NO PATHWAY IN THE CNS

The Formation of GSNO in Endothelial and Astroglial Cells

GSNO has been identified in brain tissues that contain nitric oxide synthase (NOS) and high mM concentrations of GSH.[19] Normally, glial GSH cannot diffuse through the cell membrane and migrate to neighboring neurons, which do not synthesize GSH. Interestingly, both GSH (mM) and GSNO (μM) provide antioxidative protection of brain tissue against oxidative stress.[11] At normal situations, not much GSNO is generated since there is little •NO to react with a large quantity of GSH. In responding to oxidative stress, GSH scavenges reactive oxygen species and converts itself to GSSG or oxidized GSH via an intermediate of GS• (reaction (1)). Moreover, stress also induces NOS leading to a delayed and sustained increase of •NO.[20,21] •NO scavenges peroxyl and alkoxyl lipid radicals (reaction (2)–(4))[2,3,22–24] and terminates the propagation of lipid peroxidation in the brain.[2,11,13] •NO can also scavenge toxic GS• which leads to the generation of GSNO (reaction (5)) in astroglial and endothelial cells during or after oxidative stress. This free radical to free radical reaction can take place only at their generating sites such as endothelial and astroglial cells in the brain (Figure 1).

$$GSH \rightarrow GS^{\bullet} \rightarrow GSSG \quad (1)$$

$$L^{\bullet} + {}^{\bullet}NO \rightarrow LNO \quad (2)$$

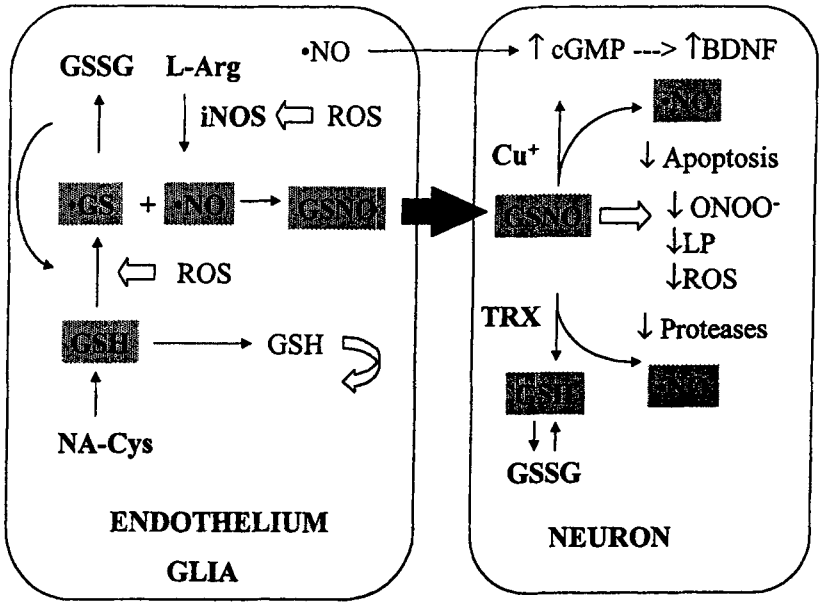

FIGURE 1 The antioxidative GSNO pathway: A possible role in cell to neuron communications. BDNF: Brain-derived neurotrophin; GSNO: *S*-nitrosoglutathione; NOS: Nitric oxide synthase; NACys: *N*-Acetylcysteine; LP: Lipid peroxidation; $ONOO^-$: Peroxynitrite; ROS: Reactive oxygen species or free radicals; TRX: thioredoxin.

$$LOO^\bullet + {}^\bullet NO \rightarrow LOONO \quad (3)$$

$$LO^\bullet + {}^\bullet NO \rightarrow LONO \quad (4)$$

$$GS^\bullet + {}^\bullet NO \rightarrow GSNO \quad (5)$$

GSNO: A Carrier for Transporting GSH and •NO

In contrast with GSH, GSNO may be able to diffuse across the cell membrane and thus serve as the transfer carrier for both GSH and •NO generated by astroglial and endothelial cells. Owing to its relatively long half-life, GSNO may become the •NO reservoir in the brain, and release •NO only when it reacts with either thioredoxin or Cu^+ in neighboring brain neurons or cells (reaction (6), Figure 1).[25,26] Because GSNO is approximately 100 times more potent than the classical antioxidant GSH in the suppression of •OH generation and brain lipid peroxidation,[11] this GSNO pathway may become an important part of the antioxidative redox pathway of GSH/GSSG. GSNO may be the key component of the cytoprotective principle of GSH not only in the supporting brain cells (i.e., endothelial and astroglial cells) but also in neurons. Furthermore, recently, it has been suggested that •NO mediates anti-apoptotic and neuroprotective effects of BDNF.[27,28] This putative GSNO pathway (reaction (6)) may, therefore, play a key role in not only the cell to neuron communication but also the endogenous GSH antioxidative defense system in protecting neurons and cells against oxidative stress caused by oxidants.

$$GSH + \text{oxidant} \rightarrow GS^\bullet + {}^\bullet NO \rightarrow GSNO \rightarrow {}^\bullet NO + GSSG \rightarrow GSH \quad (6)$$

BIOLOGY OF THE GSNO, GSH AND •NO PATHWAY IN THE BRAIN

Antioxidative Properties: GSNO > GSH

GSH, at mM concentrations, acts as a neuroprotective antioxidant, which inhibits •OH

generation and lipid peroxidation evoked by ferrous citrate in brain tissue.[11] GSH can also remove the unpaired electron from free radicals to form GSSG through an intermediate thiyl radical of GS•. GSSG is then reduced back to GSH by glutathione reductase. At μM concentrations, GSH is a weak antioxidant and thus the ratio of GSSG/GSH has been used as an index of oxidative stress in the brain.[29] In addition, toxic GS• may be nitrosylated by •NO and converted to GSNO (reaction (5), Figure 1). Since NOS is induced by oxidative stress, more GSNO could be generated under the stress. Moreover, GSNO, at μM concentrations, produces greater antioxidative action than GSH by approximately two orders of magnitude in suppressing iron-induced generation of •OH and peroxidation of brain lipids.[11] Photodegraded and •NO-depleted old GSNO solution is devoid of such antioxidative actions. Furthermore, GSNO, at μM concentrations, rather than GSH provides antioxidative protection against lipid peroxidation caused by $ONOO^-$[11] and hemoglobin.[30]

These atypical antioxidative properties of GSNO are mediated by the release of •NO instead of nitrosyl cations (NO^+). It is impossible for NO^+ to terminate free radical chain reactions via scavenging lipid radicals (L•, LO• and LOO•) to nonradicals (LNO, LONO and LOONO; see reactions (2)–(5)).[2,3,22–24] Moreover, •NO but not NO^+ can nitrosylate iron complexes and thus suppress generation of •OH[2] and terminate free radical chain reactions.[3] These atypical antioxidant properties of freshly prepared GSNO have been recently demonstrated in the brain *in vivo*[11] and *in vitro*.[30]

Cyto- and Neuroprotective Properties of GSNO and •NO

Controversy in the Experiments using •NO Donors

Recent *in vivo* studies indicate that GSNO and •NO protect brain dopamine neurons against oxidative injury caused by •OH radicals generated by iron complexes.[2,11] As discussed above, GSNO is approximately 100 times more potent than GSH against iron-induced oxidative stress. In addition, through the increase of cyclic GMP and *S*-nitrosylation of cysteinyl proteases, •NO may also augment anti-apoptotic effect of BDNF and yield neuroprotection.[21,27,28,31] These findings have substantiated a hypothesis that •NO is a neuroprotective antioxidant[4] that is completely at odds with a controversial hypothesis that •NO mediates neurotoxicity in the brain.[32] This controversial issue on, •NO and peroxynitrite ($ONOO^-$) has been recently discussed.[33,34]

Similar to ferrous citrate iron complex, the commonly used •NO donor, sodium nitroprusside ($Na_2[NO–Fe–(CN)_5]$) causes degeneration of the nigrostriatal dopamine neurons.[13,35] Hydroxyl radical but not •NO mediates this particular neurotoxic effect of sodium nitroprusside. After a brief release of •NO, •OH are persistently generated by redox cycling of the iron moieties of sodium nitroprusside (i.e., $[(CN)_5–Fe^{3+}]^{2-}$ and $[(CN)_4–Fe^{2+}]^{2-}$). These nitroprusside-derived iron complexes are completely different from the commonly used sham control agent $K_3–[Fe^{3+}–(CN)_6]$, which is a stable iron complex. Moreover, •NO-depleted sodium nitroprusside, which can no longer release •NO, generates more •OH[35] and is more toxic than the freshly prepared sodium nitroprusside in cell cultures (A. Khaldi, P. Rauhala and C.C. Chiueh, unpublished observation). In fact, sodium nitroprusside-induced oxidative stress is completely blocked by •NO, deferoxamine and oxyhemoglobin.[35] Apparently, oxyhemoglobin is not a selective •NO scavenger; it also scavenges oxygen, carbon dioxide, carbon monoxide, and cyanide. Other non-iron containing •NO donors may cause cytotoxicity only after a significant accumulation of nitrites and nitrates in the cell culture medium, which could lead to nitration of tyrosine containing proteins and even cell death. Toxic metabolites other than nitrites and nitrates may be formed after •NO donors release its molecular •NO.

Moreover, emerging evidence indicates that nitration of tyrosine has been misused as an index for protein oxidation caused by $ONOO^-$.[34,36] Therefore, cautions should be made to avoid the possibility of misinterpretation of experimental results of studies, using •NO donors without doing proper control experiments.

Inhibition of Fenton Reaction by GSNO, GSH and •NO

Besides the protection of brain dopamine neurons,[11] it has been demonstrated that GSNO protects myocardium, hepatic cells and endothelium against oxidative stress.[14,37–41] Most of the •NO donors excepting sodium nitroprusside inhibit iron-catalyzed Fenton reaction or the generation of •OH from hydrogen peroxide.[2] Due to the unique action of nitrosylation of iron complex, GSNO is approximately 100 times more potent than GSH in the suppression of •OH generation stimulated by bidentate and tridentate iron complexes of ferrous citrate.[11] This mechanism may explain why •NO protects cells and neurons from oxidative injury evoked by hydrogen peroxide.[37,38,40,42,43] However, these unique antioxidative and cytoprotective properties of •NO have been ignored for a long time (Table I).

Termination of Brain Lipid Peroxidation by GSNO, GSH and •NO

•NO also terminates lipid peroxidation in many preparations.[1–4,11,15,22–24,44] •NO scavenges those short-lived, highly reactive free radicals such as $O_2^{-\bullet}$, •OH, LOO• and GS•, yielding nitrogen species including nitrites, nitrates, LOONO, $ONOO^-$ and GSNO (Table I). GSNO is 100-fold more potent than GSH in suppression of brain lipid peroxidation; freshly prepared GSNO completely inhibits a weak peroxidative effect of $ONOO^-$ in the brain.[11] Freshly prepared GSNO releases both •NO and GSH in the presence of thioredoxin[26] and thus GSNO produces both GSH- and •NO-like antioxidative activities. It protects against oxidative stress in several preparations such as brain neurons, myocardium, hepatic cells, endothelial cells and intestinal epithelial cells.[2,11,13–15,21,24,27,28,30,31,41,42,44–47]

TABLE I Antioxidative mechanisms for GSNO and •NO via nitrosylation

Nitrosylation actions	Functional outcomes
(1) Iron complexes	
• Small molecular weight (ferrous citrate)	↓ •OH ↓ Lipid peroxidation (initation)
• Large molecular weight (hemoglobin)	↓ •OH ↓ Lipid peroxidation (initiation)
• Cytochrome p450 2E1	↓ Enzymatic activities
(2) Lipid radicals	↓ Lipid peroxidation (propagation)
(3) Caspases	↓ Proteolysis ↓ Apoptosis ↓ Cyto- and neurotoxicity
(4) HIV-1 protease	↓ Proteolysis ↓ Apoptosis ↓ Cyto- and neurotoxicity ↓ Viral proliferation?
(5) Clotting factor XIII	↓ Platelet aggregation
(6) Glutathionyl radical (GS•)	↑ GSNO ↑ Cyto- and neuroprotection

GSNO could diffuse far away from its generating sources, to provide cyto- and neuro-protection via its potent antioxidative properties produced by •NO and GSH (Figure 1).[2,11,13] Thus antioxidative properties of GSNO at μM concentrations are mediated by a novel nitrosylation reaction of •NO, which may contribute to its neuroprotective action.[2,11] These novel neuroprotective properties of GSNO may have physiological significance during oxidative stress when GSH levels decline from mM to μM concentrations and no longer can produce antioxidative actions.

Inactivation of Caspases by GSNO via S-Nitrosylation of Cysteine Residues

•NO Prevents Caspases -induced Apoptosis by S-Nitrosylation

It has been suggested that the caspase family (up to 14 subtypes of cysteinyl aspartate-specific

proteinases) plays a critical role in the regulation of apoptosis (programmed cell death) in nematodes and mammals. During oxidative stress, cysteinyl proteases can induce cyto- and neurotoxicity via activation of proteolytic and apoptotic cascades.[48] Moreover, emerging evidence indicates that *S*-nitrosylation of caspases inactivates these cysteinyl proteases,[17,39,49,50] which in turn may prevent proteolytic activation of factors that trigger apoptotic cell death.[28,47,51] Therefore, GSNO and •NO inactivate cysteinyl proteases via *S*-nitrosylation of reactive cysteine residues that could terminate the apoptotic cascads.

S-Nitrosylation of Cysteinyl Thioferrous Complexes (Peptidyl Cys-S-Fe^{2+}) of Cysteinyl Protease (Caspase-3 and HIV-1 Protease)

The *S*-nitrosylation process of the cysteine residues of caspases may be accelerated during oxidative stress because oxidants generate more GSNO and •NO. This in turn leads to the formation of *S*-nitrosylated cysteinyl proteases, which renders them incapable of generating reactive oxygen species via reactive cysteinyl thioferrous complex.[16] Based on a longer half-life of GSNO and the NO^+-mediated transnitrosylation between GSH and cysteine,[7] GSNO may be more potent than •NO in the inactivation of proteases via *S*-nitrosylation of the cysteine residues. One of the cysteinyl proteases – caspase-3 (CPP 32. Yama, apopain) – may regulate apoptosis during brain development since larger brain mass is found in the caspase-3 knockout mice. Interestingly, •NO and GSNO inactivate caspase-3 and related apoptosis via *S*-nitrosylation.[16,17,47,49,50]

We have proposed that the cysteine residues of peptides (i.e., clotting factor XIII, caspase-3 and HIV-1 protease) may react with ferrous ion to form reactive thioferrous complex (see reactions (7)–(9)), leading to the generation of reactive oxygen species, proteolysis and apoptotic cell death.[16] Conversely, if protease-induced apoptosis was involved in the degenerative brain disorders such as brain atrophy and dementia complexes, then GSNO/•NO would provide neuroprotection. In theory, GSNO should prevent and block the redox cycling of cysteinyl thioferrous complex (reactions (7)–(9)) via nitrosylation of either the thiol group or the thioferro group (reactions (10)–(14)). This iron-mediated free radical hypothesis of cysteinyl protease has now been substantiated in cell cultures, using protease generated by HIV-1 virus.[52] GSNO and deferoxamine prevent the formation of reactive thioferrous complexes at cysteine residues (i.e., Cys 67 and 95) of HIV-1 protease and thus inhibit its cyto- and neurotoxicity.[18]

$$\text{Peptidyl Cys-SH} \rightarrow H^+ + \text{Peptidyl Cys-S}^-$$
$$\text{Peptidyl Cys-S}^- + Fe^{3+} \rightarrow \text{Peptidyl Cys-S-Fe}^{2+} \quad (7)$$

$$\text{Peptidyl Cys-S-Fe}^{2+} + H_2O_2 \rightarrow \text{Peptidyl Cys-S-Fe}^{3+} + {}^{\bullet}OH$$
$$\text{Peptidyl Cys-S-Fe}^{3+} + \text{Peptidyl Cys-SH} \rightarrow \text{Peptidyl Cys-S-Fe}^{2+} + \text{Peptidyl Cys-S}^{\bullet} \quad (8)$$

$$\text{Peptidyl Cys-S-Fe}^{2+} + O_2 \rightarrow \text{Peptidyl Cys-S-Fe}^{2+}(O_2) \rightarrow \text{Peptidyl Cys-S-Fe}^{3+}(O_2^{\bullet -}) \rightarrow \text{Peptidyl Cys-S-Fe}^{3+} + O_2^{\bullet -} \quad (9)$$

$$\text{Peptidyl Cys-S}^{\bullet} + GSH \rightarrow \text{Peptidyl Cys-SH} + GS^{\bullet}$$
$$GS^{\bullet} + {}^{\bullet}NO \rightarrow GSNO \quad (10)$$

$$\text{Peptidyl Cys-SH} + GSNO \rightarrow \text{Peptidyl Cys-S-NO} + GSH \quad (11)$$

$$\text{Peptidyl Cys-S}^- + [NO^+] \rightarrow \text{Peptidyl Cys-S-NO} \quad (12)$$

$$\text{Peptidyl Cys-S}^{\bullet} + {}^{\bullet}NO \rightarrow \text{Peptidyl Cys-S-NO} \quad (13)$$

$$\text{Peptidyl Cys-S-Fe}^{3+} + {}^{\bullet}NO \rightarrow \text{Peptidyl Cys-S-Fe}^{3+}(NO)_2 \quad (14)$$

(Peptides: HIV-1 protease, caspases, clotting factor XIII)

CONCLUDING REMARKS

Beneficial Effects of GSNO via Nitrosylation in the Brain

In this putative redox pathway of GSNO, GSNO may become a carrier to transport both GSH and •NO to neurons far away from the generating cells, e.g. the endothelial and astroglial cells. GSNO is less reactive than •NO in the activation of guanylyl cyclase, leading to vasodilation.[6] Hence, it may reach distant neurons or other cells to exert its biological effects for prolonged duration. Independent of activation of guanylyl cyclase, GSNO could produce antiplatelet aggregation,[5] antiapoptosis,[18] antioxidation,[11,30] and associated cyto- and neuroprotection.[3,11] Most of the beneficial effects of GSNO in the CNS are mediated by the nitrosylation of peptidyl cysteinyl thiols and/or iron complexes that are independent of the activation of guanylyl cyclase (Table II).

c-GMP-independent Antioxidative Properties of GSNO and •NO

Both •NO and GSNO inhibit iron complexes-induced •OH generation and lipid peroxidation in the brain via a novel nitrosylation mechanism. These antioxidative properties of •NO and GSNO are significantly greater than GSH because •NO and GSNO can induce nitrosylation of iron complexes, alkoxyl and peroxyl lipid radicals.[2,11] Thus, •NO and GSNO effectively terminate the lipid peroxidation chain reactions at both the initiation and propagation steps caused by bidentate and tridentate complexes of ferrous citrate. Through a similar nitrosylation mechanism, GSNO and •NO can nitrosylate large molecular-weight iron complexes such as cytochrome P450-2E1 and hemoglobin, thus modulating their enzymatic functions or minimizing their pro-oxidative activities.[30,45] Recent data indicate that GSNO may also nitrosylate highly reactive cysteinyl thioferrous complex, which in turn inactivates cysteinyl proteases such as caspase-3 and HIV-1 protease and prevents apoptotic cell death.[16,18,52] Therefore, GSNO and •NO could protect against apoptosis evoked by caspase-3, FAS, JNK2 and TNF_α.[16,17,27,28,39,46,47,49–51,53] Furthermore, GSNO may inactivate HIV-1 protease and associated neurotoxicity via a *S*-nitrosylation mechanism.[16,18] Acting through a similar nitrosylation procedure, GSNO inactivates clotting factor XIII. This leads to antiplatelet aggregation and thus may prevent stroke and provide cardioprotection.[5,9,15] It is concluded that these beneficial antioxidative effects of GSNO do not require the activation of guanylyl cyclase.

TABLE II Beneficial effects of GSNO and •NO in the CNS

(1) Increases in cerebral blood flow (•NO > GSNO)
(2) Antiplatelet aggregation (GSNO > •NO)
(3) Anti-apoptosis (GSNO > •NO)
↑ BDNF
↓ Caspases
↓ HIV-1 protease
(4) Antioxidation (GSNO ≥ •NO > GSH)
↓ •OH generation
↓ Redox cycling of iron complexes
(5) Termination of lipid peroxidation (GSNO ≥ •NO > GSH)
↓ Chain reactions of lipid peroxidation
(6) Cyto- and neuroprotection (GSNO ≥ •NO > GSH)
↓ Iron- or •OH-induced neurotoxicity
↓ $ONOO^-$ -induced oxidative stress
↓ Proteolysis evoked by proteases (caspases and HIV-1 protease)

GSNO versus $ONOO^-$ in the Brain

Intracellular pH is normally below 7.4 and acidosis is the hallmark of brain injury. Under this condition, more GSNO than $ONOO^-$ can be generated in cells with mM GSH and NOS. •NO could scavenge $O_2^{-\bullet}$, generating a weak pro-oxidant of $ONOO^-$,[12] which can be completely neutralized by GSNO.[11] Negative *in vivo* results are obtained after infusing saturated solution of $ONOO^-$ into the rat brain[11] because carbon dioxide at physiological concentrations also blocks the pro-oxidative effects of $ONOO^-$ but not the formation of nitrotyrosine.[34,36] Finally,

infusion of GSNO or •NO into the substantia nigra protects dopaminergic nigral neurons from oxidative stress caused by •OH generated by iron complex.[11,12] These antioxidative, neuroprotective properties of GSNO, but not the prooxidative actions of $ONOO^-$, have now been replicated in the brain *in vivo*.

•NO Perspectives: Is GSNO an Endogenous HIV-1 Protease Inhibitor?

In perspective, nitrosylation of peptidyl cysteinyl residues and iron complexes by GSNO induces important biological functions in the brain such as protection of neurons and other cells from oxidative injury caused by iron complexes, peroxide, lipopolysaccharide, caspases, and even HIV-1 protease. This putative redox pathway of GSNO and •NO may be a part of the antioxidative, defensive system of GSH in the brain.[11] GSNO, therefore, plays an important role in cell to neuron communications via a novel mechanism of nitrosylation that is independent of the activation of guanylyl cyclase. Based on the new discovery that *S*-nitrosylation by GSNO inactivates HIV-1 protease and its cyto- and neurotoxicity, we have proposed that GSNO/•NO may be an endogenous protease inhibitor.[16,18,52] Its possible inhibitory role in the regulation of viral proliferation is currently under investigation. Elucidation of this novel antioxidative and neuroprotective mechanism of GSNO could stimulate the development of new neuroprotective agents for the treatment and prevention of oxidant-induced degenerative brain disorders such as Parkinson's disease, Alzheimer's dementia and AIDS dementia complex.

Functional Adaptation in Mice Lacking eNOS or nNOS

There are at least three subtypes of NOS. The distribution of neuronal NOS (nNOS) is not only in neurons but also in skeletal muscle.[54] Moreover, spinal motor neurons contain endothelial NOS (eNOS; J. Beckman, personal communications). Interestingly, transgenic knockout of eNOS leads to increase of nNOS in blood vessels and vice versa.[55,56] Apparently, there is a redundant •NO-mediated vasodilation for blood pressure regulation and blood flow control. After knocking out eNOS, nNOS is upregulated and thus provides adequate levels of •NO for maintaining vasodilation evoked by acetylcholine.[56] These compensatory changes could maintain homeostasis of the •NO-mediated physiological and biological functions including antioxidation, vasodilation, antiplatelet aggregation, and reproduction. However, without the understanding of mechanisms underlying these compensatory changes in transgenic mice lacking NOS, it is impossible to correctly interpret the biological functions of •NO in the brain *in vivo*.

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