

Chapter 16

Critical Roles of Oxidative Signals in Age-Related Decline of Cerebellar Synaptic Plasticity

Sho Kakizawa and Nozomu Mori

Abstract A strong correlation between increasing age and the accumulation of oxidative modifications of functional molecules, such as proteins and lipids, has been observed in biological systems, including the central nervous system. Thus, oxidative signals, especially reactive oxygen species (ROS), are thought to be primary factors affecting age-related decline in brain functions. However, the molecular mechanism of the inhibitory action of oxidative signals is not yet fully understood. In this review article, we introduce our novel hypothesis on the molecular mechanism of aging in the nervous system: oxidative signals impair neuronal function through the inhibition of protein S-nitrosylation by nitric oxide (NO). This idea is based on the fact that the thiol group in cysteine residues is the common target of NO and oxidative signals. Actually, S-nitrosylation-dependent synaptic plasticity in the cerebellar cortex as well as NO-induced S-nitrosylation of cerebellar proteins are abolished by ROS treatment and aging. Furthermore, the functions of some proteins involved in synaptic plasticity, such as NSF, stargazin and ryanodine receptor 1, are demonstrated to be regulated in dependent on S-nitrosylation. Taken together, these studies open a new avenue in the study of the molecular mechanism of brain aging.

Keywords Aging • Brain • S-nitrosylation • Oxidative stress • Posttetanic potentiation • Long term potentiation • Cerebellum • Purkinje cell • Parallel fiber

S. Kakizawa

Department of Biological Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan

Department of Anatomy and Neurobiology, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

e-mail: sho-kaki@pharm.kyoto-u.ac.jp

N. Mori (✉)

Department of Anatomy and Neurobiology, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

e-mail: morinosm@nagasaki-u.ac.jp

16.1 Introduction

Aging is associated with a general decline in physiological function in biological systems, including those in the nervous system, such as impairments of learning and memory. In fact, cells in all regions of the nervous system are affected by aging, as indicated by the decline of sensory, motor and cognitive functions with time (Barnes 1988, 2003; Finch 2003; Hofer et al. 2003; Landfield 1988). These nervous system dysfunctions are thought to occur when cells fail to respond adaptively to age-related increases in oxidative, metabolic and ionic stress. Among these stressors, the “oxidative stress” hypothesis of aging is widely accepted due to the strong correlation between increasing age and the accumulation of oxidative damage of cellular macromolecules observed in a wide range of studies (for a review, see Beckman and Ames 1998). In this hypothesis, oxidative stress is thought to induce damage to the cell via the oxidation of cellular components such as membrane lipids, proteins and DNA. For example, enhanced lipid peroxidation and protein oxidation are observed in aged rat brains (Calabrese et al. 2004; Cini and Moretti 1995; Devi and Kiran 2004; Forster et al. 1996; Murray and Lynch 1998). Correspondingly, several studies have shown that behavioral deficits of aged animals are associated with increases in oxidative stress (Butterfield et al. 2006; Cantuti-Castelvetri et al. 2000; Fukui et al. 2001).

Oxidative stress refers to the imbalance between the cellular production of reactive oxygen species (ROS), such as superoxide, hydroxyl radical and hydrogen peroxide (H_2O_2), for example, and the antioxidant mechanisms that remove them (Halliwell 1992). In fact, many studies indicate that the redox environment of the brain can be altered in favor of oxidation by an increased production of ROS and/or by a decreased activity of antioxidant defenses. However, the mechanisms through which oxidative signals induce decline in neuronal functions are not yet fully understood. This decline occurs, at least partly, due to the inconsistency of the effects of oxidative signals on the nervous system. For example, the effects of oxidative signals on synaptic plasticity in studies where ROS were exogenously applied to hippocampal slices induced paradoxical effects (Klann and Thiels 1999; Serrano and Klann 2004). In some studies, ROS are suggested to be essential for long-term potentiation (LTP) in hippocampal slices (Kamsler and Segal 2003a; Knapp and Klann 2002). Conversely, inhibitory effects of ROS on the synaptic plasticity are also reported in other studies (Auerbach and Segal 1997; Kamsler and Segal 2003a, b; Pellmar et al. 1991; Watson et al. 2002). In these studies, different protocols were applied for the induction of synaptic plasticity, and the species and ages of the animals studied were diverse. In addition, the target molecules or signaling pathways of oxidation in the course of the induction of the synaptic plasticity were not identified in these experiments. Therefore, the identification of the target molecules of oxidation in synaptic plasticity could provide critical insight concerning how oxidative stress results in deficits in synaptic plasticity and brain function in aged animals.

16.2 Effects of Oxidative Signals on NO-Dependent LTP in the Cerebellum

Among the excitatory synapses in the central nervous system, the parallel fiber to Purkinje cell synapse (PF synapse) in the cerebellar cortex is a good model for examining molecular mechanisms in synaptic plasticity (Ito 2006; Jörntell 2014). The cerebellum, a structure located at the back of the brain stem, consists of two parts: the cerebellar cortex and cerebellar nuclei. The cerebellar cortex consists of three layers: the molecular layer, the Purkinje cell layer and the granule cell layer (granular layer), from the surface to the inner portion. Purkinje cells (PCs), the sole output of the cerebellar cortex, receive two types of glutamatergic inputs. These inputs are climbing fibers (CF) from the inferior olive, and PF, the axon of cerebellar granule cells (Fig. 16.1). In the mature cerebellum, a single CF makes

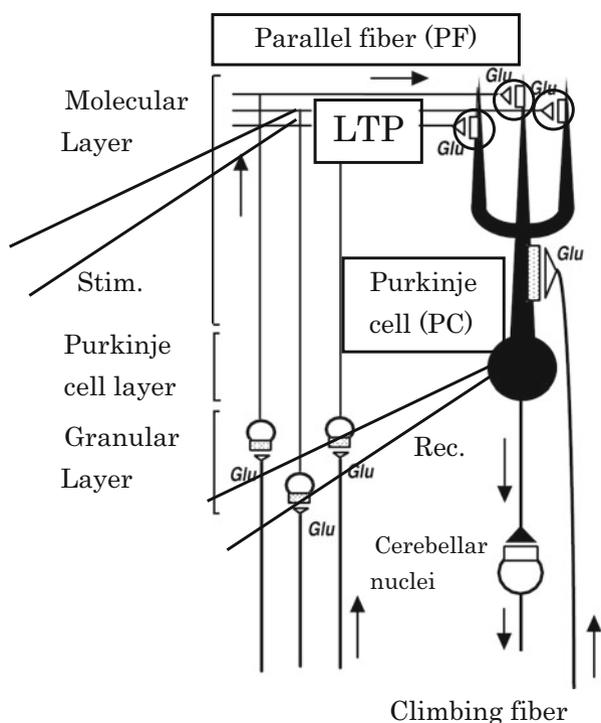


Fig. 16.1 Excitatory synaptic inputs to cerebellar Purkinje cell. The cerebellar cortex consists of three layers: the molecular layer, Purkinje cell layer and granular layer (also called granule cell layer). Purkinje cells (PCs), the sole output from the cerebellar cortex, receives two types of excitatory inputs. One input is the climbing fiber (CF), which originates from the inferior olive, and the other is the parallel fiber (PF), the axon of the granule cell. From the presynaptic terminals of CF and PF, the neurotransmitter glutamate (Glu) is released. At the PF-to-PC synapse, a form of synaptic plasticity, long-term potentiation (LTP), is induced by the repetitive stimulation of PF. This LTP is dependent on NO/S-nitrosylation signals (see text for detail)

an excitatory synapse onto the proximal dendritic region of the PC. On the other hand, each PC is innervated by hundreds thousands of PFs making synapses on the distal dendritic region. At the PF synapse, various types of synaptic plasticity have been characterized so far, including postsynaptic forms of LTP and long-term depression (LTD) as well as a presynaptically expressed LTP (Evans 2007; Ito 2006; Jörntell 2014). The LTD at the PF synapse is one of the most extensively studied plasticities in the central nervous system, and its involvement in motor learning tasks, such as eyeblink conditioning and vestibulo-ocular reflex (VOR), is indicated in multiple studies (Ito 2002, 2013). On the other hand, the functional roles of postsynaptic and presynaptic LTP at PF are not yet fully understood, although the possible involvement of postsynaptic LTP in fear conditioning has been suggested (Sacchetti et al. 2004).

Among them, the postsynaptically expressed LTP at the PF synapse is demonstrated to be dependent on NO signals (Kakegawa and Yuzaki 2005; Lev-Ram et al. 2002; Namiki et al. 2005). Nitric oxide is a gaseous messenger in biological systems and is produced from L-arginine by three distinct NO synthases (NOSs) (Alderton et al. 2001; Stuehr et al. 2004). Two of these NO synthases, neuronal (nNOS) and endothelial (eNOS), are calcium dependent, whereas inducible NOS (iNOS) is calcium independent. This NO-dependent LTP at the PF synapse (NO-LTP) is shown to be induced by the repetitive stimulation of PF (Kakegawa and Yuzaki 2005; Lev-Ram et al. 2002; Namiki et al. 2005). In addition, granule cells, the origin of PF, express high levels of nNOS (also called NOS1), whereas the immunohistochemical signal of nNOS is not observed in PCs. These observations suggest that NO, which is essential for the induction of the LTP at the PF synapse, derives from PF. Nitric oxide has two downstream signaling pathways: the activation of soluble guanylyl cyclase (sGC) and S-nitrosylation (the term “S-nitrosation” is also used for this modification of proteins Iyer et al. 2014) (Fig. 16.2). The activation of sGC induces the elevation of cyclic guanosine monophosphate (cGMP) in cytoplasm and the subsequent activation of protein kinase G, which regulates the functions of various proteins through protein phosphorylation. On the other hand, S-nitrosylation of cysteine residues in various proteins results in the modification of protein functions including those of enzymes and ionic channels (Calabrese et al. 2007; Hess et al. 2005; Jaffrey et al. 2001; Nakamura and Lipton

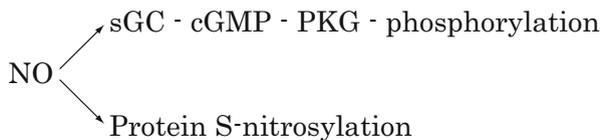


Fig. 16.2 The two downstream signaling pathways of nitric oxide. Nitric oxide (NO) has two downstream signaling pathways. One pathway involves the activation of soluble guanylyl cyclase (sGC) and results in the elevation of cyclic GMP (cGMP) levels in the cytoplasm (*upper*). cGMP subsequently activates protein kinase G (PKG), which phosphorylates a wide range of proteins. The other pathway involves protein S-nitrosylation (*lower*). The functions of some protein are known to be regulated by S-nitrosylation

2007). The NO-LTP at the PF synapse is not impaired by 1H-[1,2,4] Oxadiazolo [4,3-a] quinoxalin-1-one (ODQ), an inhibitor of sGC, but is inhibited by ascorbic acid, a reductant which reduces S-nitrosylated thiol, when applied to the PC using a micropipette (Kakizawa et al. 2012b; Namiki et al. 2005). These results indicate that the S-nitrosylation of the proteins in PCs is essential for the induction of PF-LTP. Moreover, the LTP is also abolished by the preincubation of the cerebellar slices with the N-ethyl-maleimide (NEM), a membrane-permeable thiol blocker, or glutathione (reduced form; GSH) and N-acetyl-cysteine (NAC), membrane impermeable thiol donors (Kakizawa et al. 2012a). These results indicate critical roles of thiol groups in cysteine residues in NO-LTP. Furthermore, because GSH and NAC are membrane impermeable, the results also indicate that NO released from PF into the synaptic cleft significantly contributes to the induction of the LTP. Taken together, the NO-LTP is indicated to be dependent on the S-nitrosylation of proteins in PCs.

Thiol groups in cysteine residues are the targets of S-nitrosylation by NO as well as the targets of oxidation and the subsequent formation of disulfide bonds by oxidative signals including ROS. In addition, the accumulation of protein oxidation is reported by many studies, as described above. Therefore, it is highly possible that oxidative signals inhibit NO-induced protein S-nitrosylation through the formation of disulfide bonds. When a thiol group is in a reduced state (R-SH), it has the potential to be S-nitrosylated by NO signals. However, the same thiol is difficult to S-nitrosylate when it is already oxidized and involved in a disulfide bond (R-S-S-R') because the disulfide bond is very stable in general (Fig. 16.3).

If this hypothesis is true, protein S-nitrosylation as well as S-nitrosylation-dependent biological events could be inhibited by endogenous and exogenous oxidative signals. Kakizawa et al. examined this hypothesis and experimentally demonstrated the inhibitory effects of oxidative signals on the S-nitrosylation of cerebellar proteins and NO-LTP which is indicated to be dependent on protein S-nitrosylation in PCs (Kakizawa et al. 2012a). They first demonstrated that NO-LTP, induced by burst stimulation of PF, was blocked by the pretreatment of

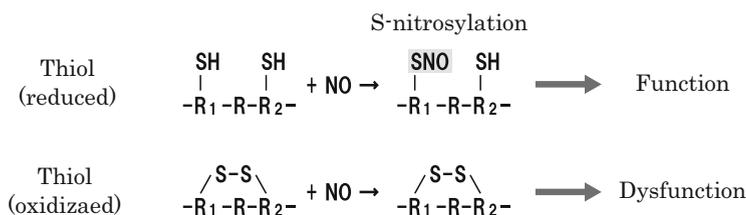


Fig. 16.3 Inhibition of protein S-nitrosylation by oxidative signals through the formation of a disulfide bond. Thiol groups in cysteine residue are targets of both S-nitrosylation by nitric oxide (NO) and oxidation (formation of a disulfide bond) by oxidative signals. When a thiol is in a reduced state (*upper*; R₁-SH), the group can be S-nitrosylated by NO (R₁-SNO) and exert its biological function. Conversely, when the same thiol is already involved in a disulfide bond (*lower*; R₁-S-S-R₂), NO cannot S-nitrosylate the group and induce its biological function because the disulfide bond is very stable and difficult to break

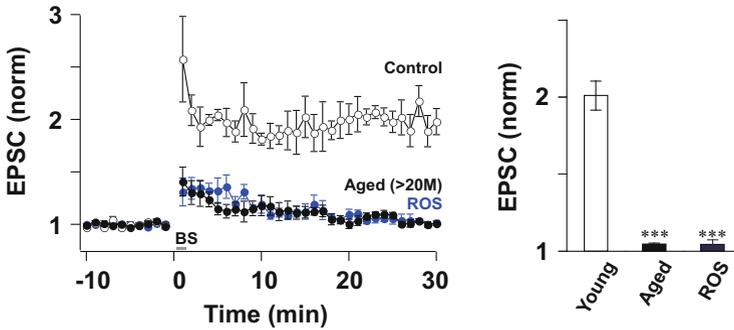


Fig. 16.4 Inhibitory effects of ROS and aging on NO-dependent LTP at the PF synapse. When the cerebellar slices from young mice (1-month-old) are not pretreated with ROS (Control), burst stimulation (BS) to parallel fibers (PF) induces long-term potentiation (LTP) at the PF synapse. Conversely, when slices from young mice are pretreated with ROS (ROS), or when cerebellar slices are obtained from aged mice (older than 20 months old (Aged >20 M)), the LTP is almost completely abolished. (*Left*) Normalized amplitude of excitatory postsynaptic current (EPSC (norm)) at the PF synapse before and after the BS. The value is normalized with the averaged value over 1–10 min before the BS, and results are expressed as the Mean \pm S.E.M. (*Right*). The averaged amplitudes of PF-EPSC during the 21–30 min after the BS. Mean \pm S.E.M. *** $p < 0.001$, compared with the value for the control

the cerebellar slices from young 1-month-old mice with oxidative agents (e.g., hydrogen peroxide) or a thiol blocker (NEM, for example) (Fig. 16.4). The pretreatments did not affect basic electrophysiological properties (e.g., input-output relations and paired-pulse ratio) of the excitatory postsynaptic current of PF (PF-EPSC). In addition, the application of the oxidative reagent itself did not induce LTP at the PF synapse, and the results exclude the possibility that NO-LTP is occluded in the cerebellar slices pretreated with the oxidizing reagents. Therefore, the oxidative signals were revealed to specifically inhibit the signaling pathways essential for the induction of NO-LTP at the PF synapse.

In addition to the inhibitory effects of oxidative reagents, which are exogenous oxidative signals, the possible involvement of endogenous oxidative signals in NO-LTP inhibition was also indicated in that study (Kakizawa et al. 2012a). NO-LTP was impaired in the cerebellar slices from aged mice older than 20 months, whereas no significant changes in basic electrophysiological properties of PF-EPSC were observed. These results suggest the inhibition of NO-LTP by endogenous oxidative signals in aged mice (Fig. 16.4).

Do oxidative signals inhibit NO-LTP through the inhibition of protein S-nitrosylation? To examine this issue, the S-nitrosylation levels of cerebellar proteins were quantitatively estimated by biochemical analysis, biotin-switch assay in which S-nitrosylated thiols are displaced with biotin labels (Jaffrey et al. 2001) (Fig. 16.5). Incubation of the cerebellar slices from young mice with NO donor, NOC7, resulted in the increase in S-nitrosylation levels of the cerebellar proteins. On the other hand, pretreatment of the cerebellar slices with oxidative

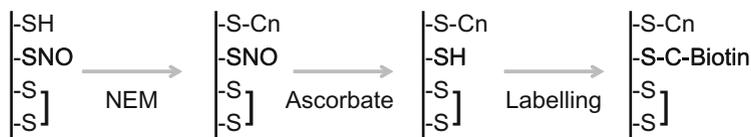


Fig. 16.5 The biotin-switch assay. S-nitrosylated thiol was labeled and detected in a biotin-switch assay. First, reduced thiols ($-\text{SH}$) are specifically blocked by N-ethylmaleimide (NEM). Then, S-nitrosylated thiol ($-\text{SNO}$) was reduced by ascorbic acid (ascorbate), and subsequently labeled by biotin ($-\text{S-C-Biotin}$). The biotinylated thiol was detected using streptavidin conjugated with horseradish peroxidase (HRP)

reagents, such as H_2O_2 , severely impaired the NO-induced elevation of the protein S-nitrosylation levels (Kakizawa et al. 2012a) (Fig. 16.6). The possible involvement of endogenous oxidative signals in the inhibition of protein S-nitrosylation is also suggested. In the cerebellar slices from mice older than 20 months old, the elevation of protein S-nitrosylation levels induced by NO-donor application is severely impaired. Again, the result supports the idea that endogenous oxidation signals also inhibit protein S-nitrosylation (Fig. 16.6). Taken together, these results strongly indicate that the inhibitory action of oxidizing signals on the induction of the NO-LTP is mediated by the impairment of protein S-nitrosylation induced by acute NO signals.

16.3 Target Proteins of S-Nitrosylation in the Central Nervous System

In the notion that oxidative signals affect biological events through the inhibition of NO-induced protein S-nitrosylation, oxidative signals could impair the protein S-nitrosylation in a “competitive” manner because thiol groups are targets of S-nitrosylation as well as oxidation. Therefore, it is quite informative to specify S-nitrosylated /oxidized proteins to reveal the molecular mechanisms of the inhibitory actions of the oxidative signals on the biological systems. For a long time, S-nitrosylation site specification has been laboriously tackled on a protein-by-protein basis (Hess et al. 2005). Moreover, a high-throughput proteomic approach that enables the simultaneous identification of S-nitrosylated-Cys sites and their cognate proteins in complex biological mixtures has only recently been developed (Hao et al. 2006). This approach, termed SNOSID (SNO Site Identification), is a modification of the biotin-switch technique of S-nitrosylated cysteines (Jaffrey et al. 2001), including the biotinylation of protein SNO-Cys residues, trypsinolysis, affinity purification of biotinylated-peptides, and amino acid sequencing by liquid chromatography tandem mass spectrometry (MS) (Hao et al. 2006). Studies using these techniques have identified several proteins which undergo S-nitrosylation, and some of these proteins are indicated to be involved in neuronal functions, including synaptic plasticity, in the central nervous system. These proteins could be

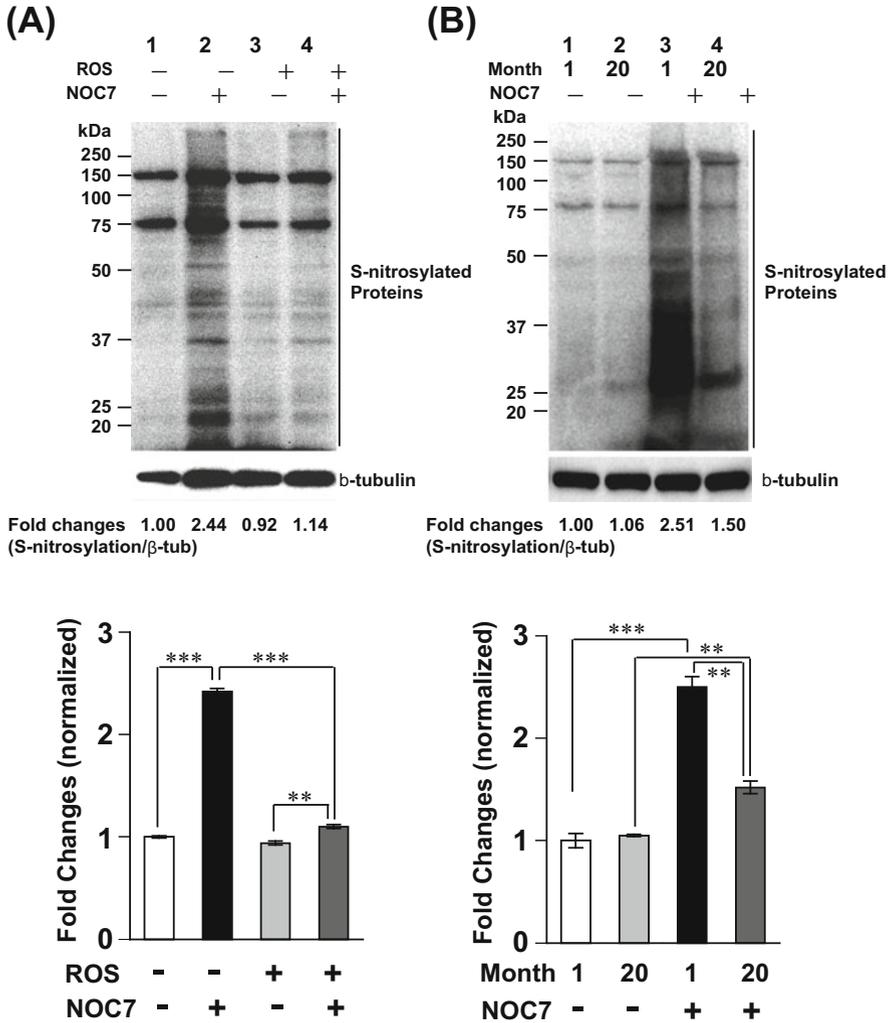


Fig. 16.6 Inhibition of NO-induced protein S-nitrosylation by pretreatment with ROS or aging. The overall protein S-nitrosylation in cerebellar slices treated with NOC7 was resolved using a biotin-switch assay (see Fig. 16.5). (a) (upper) Effects of H₂O₂ on NO-induced protein S-nitrosylation in the cerebellar slices from 1-month-old mice. Lane 1, vehicle-pretreated cerebellar slice; Lane 2, cerebellar slice treated with NOC7, an NO donor; Lane 3, H₂O₂-pretreated cerebellar slice; Lane 4, NOC7-treated cerebellar slice after the pretreatment with H₂O₂. The molecular weights of marker proteins are given to the left. The values shown at the bottom are overall protein S-nitrosylation levels, calibrated with β -tubulin levels and then normalized with the value for the vehicle-pretreated group (lane 1). (lower) Summary of the fold changes in S-nitrosylation levels. Mean \pm S.E.M. (b) (upper) NO-induced protein S-nitrosylation in the cerebellar slices from young (1-month-old) or aged (older than 20-month-old) mice. Lane 1, cerebellar slice from a young mouse treated with vehicle; Lane 2, cerebellar slice from an aged mouse treated with vehicle; Lane 3, cerebellar slice from a young mouse treated with NOC7; Lane 4, cerebellar slice from an aged mouse treated with NOC7. Molecular weights of marker proteins

potential candidates of the targets of the oxidative signals, and the oxidation of these target-proteins is expected to inhibit S-nitrosylation-dependent biological events (e.g., NO-LTP) through the inhibition of the S-nitrosylation of the proteins. In this section, we highlight representative proteins that are demonstrated to undergo S-nitrosylation and play key roles in the modulation of neuronal functions in a manner dependent on protein S-nitrosylation.

NSF

N-ethylmaleimide sensitive factor (NSF) is thought to be a target for NO-mediated S-nitrosylation. In fact, its name originates from its sensitivity to N-ethylmaleimide, which is known to alkylate the sulfhydryl groups of cysteine residues in proteins (Block et al. 1988). Actually, NO-mediated S-nitrosylation of NSF was identified as a regulator of the exocytosis of endothelial granules (Matsushita et al. 2003).

The essential role of NSF S-nitrosylation in the regulation of synaptic plasticity is also suggested (Huang et al. 2005). NSF is revealed to regulate excitatory synaptic transmission by stabilizing or recycling alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA-R) subunit GluR2 (Osten et al. 1998; Song et al. 1998) and disassemble the GluR2/PICK1 protein complex that regulates cerebellar LTD (Steinberg et al. 2004; Xia et al. 2000). Huang et al. (2005) reported that NSF is physiologically S-nitrosylated by endogenous, neuronally derived NO (Huang et al. 2005). The S-nitrosylation of NSF enhances its binding to the GluR2 subunit, and the surface expression of GluR2 is regulated by NO and NSF-GluR2 interactions. These results strongly suggest that the S-nitrosylation of NSF is a physiological mediator for the surface expression of GluR2 during the induction of synaptic plasticity.

16.3.1 Stargazin

Stargazin has also emerged as a principal regulator of AMPAR surface expression. Selvakumar et al. (2009) demonstrated that stargazin is physiologically S-nitrosylated, resulting in increased surface expression of the receptor in HEK 293 cells and primary neurons (Selvakumar et al. 2009). The S-nitrosylation of stargazin enhances its binding to the AMPAR subunit GluR1, resulting in increased surface expression of the AMPAR.



Fig. 16.6 (continued) and the values shown at the *bottom* are the same as **(a)**. (*lower*) Summary of the fold changes in S-nitrosylation levels. Mean \pm S.E.M. $**p < 0.01$ and $***p < 0.001$ indicate significant differences different

16.3.2 Type 1 Ryanodine Receptors

Ryanodine receptors (RyRs) located in the sarcoplasmic / endoplasmic reticulum (SR/ER) membrane are required for the intracellular Ca^{2+} release that is involved in a wide range of cellular functions. In addition to Ca^{2+} -induced Ca^{2+} release (CICR) in cardiac cells and voltage-induced Ca^{2+} release in skeletal muscle cells, NO-induced Ca^{2+} release (NICR) was recently identified in neuronal cells such as cerebellar Purkinje cells and cortical neuronal cells (Kakizawa et al. 2012b).

Thus far, three isoforms of the RyRs have been identified and cloned: type 1 RyR (RyR1) was first detected in skeletal muscle (Takeshima et al. 1989), type 2 RyR (RyR2) was first found in cardiac muscles (Nakai et al. 1990) and type 3 RyR (RyR3) was found in the brain (Giannini et al. 1992; Hakamata et al. 1992). They are encoded by three distinct genes and share ~70 % sequence homology (Rossi and Sorrentino 2002). Because the RyR has approximately 100 cysteine residues per subunit and almost half of the thiol groups are kept in a reduced state under the resting condition, RyR is thought to be one of the substrate proteins for S-nitrosylation. Actually, the open probability of RyR1 measured in lipid bilayers is increased by NO donors (Eu et al. 2000; Stoyanovsky et al. 1997; Sun et al. 2003; Xu et al. 1998), and this response is accompanied by an increase in the S-nitrosylation of the Ca^{2+} release channels.

S-nitrosylation-dependent activation of RyR1 is also observed in neuronal cells. In cerebellar Purkinje cells, which express significantly higher levels of RyR1, the application of an NO donor induced prominent increases in Ca^{2+} levels. Because this NO-induced Ca^{2+} increase is impaired by dantrolene, an inhibitor of RyR1, or the systemic deletion of the *RyR1* gene, the Ca^{2+} increase is revealed to be due to Ca^{2+} release from the ER and mediated by RyR (Kakizawa et al. 2012b). In addition, the NO-induced Ca^{2+} release is not inhibited by ODQ, a sGC inhibitor, but is inhibited by ascorbic acid. These results indicate that the NO-induced Ca^{2+} release is indicated to be dependent on protein S-nitrosylation. Furthermore, a single-site C3635A-mutation in rabbit RyR1 abolished NO-induced Ca^{2+} release and S-nitrosylation of the mutated channels expressed in HEK 293 cells. Taken together, S-nitrosylation of RyR1 is revealed to be essential for the activation of the channel and the resultant cellular response, NO-induced Ca^{2+} release (Fig. 16.7). Thus far, the possible involvement of NICR in NO-LTP and NO-induced neuronal cell death is suggested by pharmacological studies (Kakizawa et al. 2012b).

16.4 Perspectives

In this review article, we propose a possible molecular mechanism of the inhibitory action of oxidative signals on biological events. Specifically, we propose that the inhibitory action of an oxidizing signal could be mediated through the impairment of protein S-nitrosylation. Because thiol groups in cysteine residues are the targets of S-nitrosylation by NO as well as the targets of oxidation and the subsequent formation of disulfide bond, NO and oxidative signals, such as H_2O_2 , could

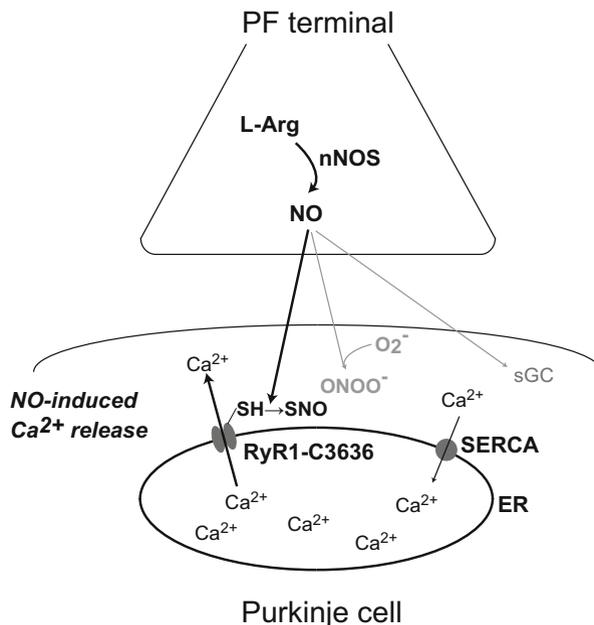


Fig. 16.7 A schematic diagram of signaling pathways for nitric oxide-induced Ca^{2+} release (NICR) in cerebellar Purkinje cells. Nitric oxide (NO) is produced at the parallel-fiber terminal in response to neuronal activity and diffuses into the cerebellar Purkinje cell. Then, type I ryanodine receptors (RyR1) located in the endoplasmic reticulum (ER) membrane are S-nitrosylated and activated, resulting in Ca^{2+} release from the ER. Cyclic GMP and peroxynitrite produced by the reaction of NO with superoxide are unlikely to be involved in NICR. *nNOS* neuronal NO synthase, *SERCA* sarco/endoplasmic reticulum Ca^{2+} ATPase

compete the binding to thiol groups. When a thiol is in a reduced state (R-SH), NO could S-nitrosylate the thiol (formation of R-SNO) and induce its effects. On the other hand, when a thiol is already involved in a disulfide bond (R-S-S-R'), NO can no longer modify the groups because the disulfide bond is very stable. In accordance with this notion, in cerebellar slices pretreated with an oxidizing reagent (e.g., H_2O_2), NO-induced S-nitrosylation of cerebellar proteins is severely impaired. Furthermore, the treatment also inhibits S-nitrosylation-dependent LTP at the PF-PC synapse (NO-LTP).

These findings indicate that the S-nitrosylation of functional protein(s) is essential for the induction of the NO-LTP. As described above, some functional proteins such as NSF, stargazin and RyR1 are candidates for S-nitrosylation and oxidation (formation of disulfide bond) because these proteins are indicated to be involved in synaptic plasticity and the functions of these molecules are regulated in a manner dependent on S-nitrosylation. The inhibitory actions of oxidative signals on the molecular functions of these proteins are expected to be clarified in future studies.

However, it should also be noted that the target cysteines of S-nitrosylation and oxidation are not necessarily the same. For example, phosphatase with sequence homology to tensin (PTEN) is known to be oxidized by high concentrations of H_2O_2

(>0.5 mM), which results in disulfide bond formation between Cys-71 and Cys-124 (Lee et al. 2002). On the other hand, the protein is selectively S-nitrosylated by low concentrations of NO donor (1–10 μ M SNO) at a specific cysteine residue, Cys-83, and the nitrosylation of the cysteine is reported to regulate the enzymatic activity of the protein (Numajiri et al. 2011). Whether NO-dependent-S-nitrosylation is abolished by oxidative signals is probably influenced by the 3-D topology of the protein as well as the concentration of NO and oxidative signals.

In this review article, we discussed a possible molecular mechanism for age-related decline in brain function, focusing on S-nitrosylation-dependent LTP (NO-LTP) at the PF synapse in the cerebellum as a model. In addition to NSF, stargazin and RyR1, which have been already demonstrated to have S-nitrosylation-dependent functions involved in synaptic plasticity, it is also possible that molecules involved in the structural plasticity of dendritic spines in hippocampal and cortical regions could be S-nitrosylated or oxidized. The identification of the candidate molecules involved in S-nitrosylation-dependent structural plasticity and the observation of an inhibitory action of oxidative signals on plasticity may further clarify the molecular mechanisms of aging in brain systems (Fig. 16.8).

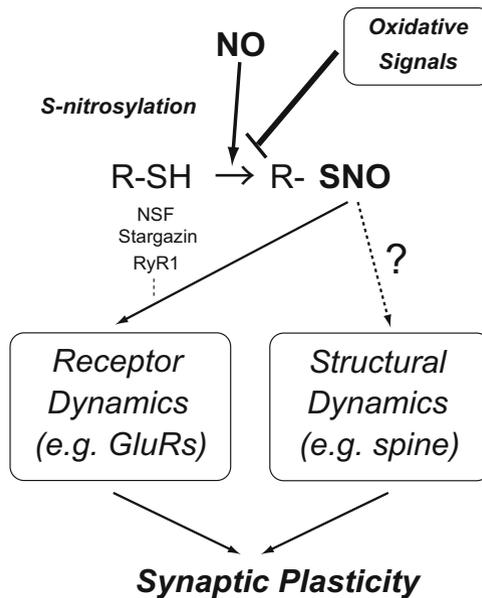


Fig. 16.8 A schematic diagram of possible mechanisms through which oxidative signals inhibit synaptic plasticity. A thiol group in a reduced state (R-SH) is S-nitrosylated (R-SNO) by nitric oxide (NO). S-nitrosylation of some molecules, including N-ethylmaleimide sensitive factor (NSF), stargazin and type 1 ryanodine receptor (RyR1), regulates the dynamics of postsynaptic receptors, e.g., glutamate receptors (GluRs), and subsequently induces synaptic plasticity. It is also possible that molecules involved in the structural dynamics of dendritic spines are S-nitrosylated and induce synaptic plasticity, although the candidate molecule(s) has yet to be identified. On the other hand, oxidative signals could inhibit S-nitrosylation through the mechanism shown in Fig. 16.3 and could therefore abolish synaptic plasticity

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