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Protein oxidation inhibits NO-mediated signaling pathway for synaptic plasticity

Sho Kakizawa[a,b,*], Masahiko Shibazaki[a], Nozomu Mori[a,*]

[a] Department of Anatomy and Neurobiology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
[b] Department of Biological Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

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Abstract

Oxidative stress is a primary factor inducing brain dysfunction in aged animals. However, how oxidation affects brain function is not fully understood. Here we show that oxidation inhibits signaling pathways essential for synaptic plasticities in the cerebellum. We first revealed that nitric oxide (NO)-dependent plasticities at the parallel fiber-Purkinje cell synapse (PF synapse) were impaired in the cerebellar slices from aged mice, suggesting a possible inhibitory action of protein oxidation by endogenous reactive oxygen species. PF-synaptic plasticities were also blocked in the cerebellar slices from young mice preincubated with oxidizing agents or thiol blocker. Because the treatment of the slices with the oxidizing agent did not affect basic electrophysiological properties of excitatory postsynaptic current of PF (PF-EPSC) and did not occlude the synaptic plasticities, oxidation was revealed to specifically inhibit signaling pathways essential for PF-synaptic plasticities. Finally, biochemical analysis confirmed the idea that inhibitory action of protein oxidation on the PF-synaptic plasticities was mediated by impairment of nitric oxide-induced protein S-nitrosylation. Therefore, oxidation was revealed to inhibit the S-nitrosylation-dependent signaling pathway essential for synaptic plasticity in a "competitive" manner.

Keywords: Aging; S-nitrosylation; Oxidative agent; Posttetanic potentiation; Long term potentiation; Cerebellum; Purkinje cell; Parallel fiber

1. Introduction

Aging is associated with a general decline in physiological function in biological systems including the nervous system (Barnes, 1988, 2003; Finch, 2003; Landfield, 1988; Mattson and Magnus, 2006). With the passage of time, the redox environment of the brain can be altered in favor of oxidation by an increased production of reactive oxygen species (ROS) or by a decreased activity of antioxidant defenses. This condition, known as oxidative stress or oxidative damage, is thought to be a general contributing factor to aging in the nervous systems (Beckman and Ames, 1998; Finkel and Holbrook, 2000; Harman, 1956). Actually, numerous studies have demonstrated various correlations between age and the accumulation of oxidative damage to cellular macromolecules (Floyd and Hensley, 2002; Stadtman, 2001). For example, enhanced lipid peroxidation (Calabrese et al., 2004; Devi and Kiran, 2004; Gupta et al., 1991; Murray and Lynch, 1998; O'Donnell and Lynch, 1998) and protein oxidation (Cini and Moretti, 1995; Forster et al., 1996; Sohal et al., 1994; Sultana et al., 2009; Vaishnav et al., 2007) are observed in the brains of aged rodents. Furthermore, 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; Carney et al., 1991; Forster et al., 1996; Fukui et al., 2001). Although these associations between oxidative damage and brain dysfunction cannot establish a causal link between the 2, they do support the idea that oxidation is involved in age-related brain dysfunction (Droge and Schipper, 2007; Serrano and Klann, 2004).

One type of cellular process strongly affected by oxidation is synaptic plasticity, a cellular process proposed as a biological substrate for learning and memory (Bliss and Collingridge, 1993; Ito, 2001; Lynch, 2004; Malenka and

* Corresponding authors at: Department of Biological Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501 Japan. Tel.: +81 75 753 4552 (S. Kakizawa) or Department of Anatomy and Neurobiology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki 852-8523, Japan. Tel.: +81 95 819 7019 (N. Mori).
E-mail addresses: sho-kaki@pharm.kyoto-u.ac.jp (S. Kakizawa) or morinosm@nagasaki-u.ac.jp (N. Mori).

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Nicoll, 1999). However, examination of the effects of oxidation on synaptic plasticity in studies where ROS (e.g., hydrogen peroxide) was applied exogenously to hippocampal slices resulted in paradoxical effects (Klann and Thiels, 1999; Serrano and Klann, 2004). Some studies suggest that ROS are essential for long term potentiation (LTP) in hippocampal slices (Kamsler and Segal, 2003a; Knapp and Klann, 2002), whereas inhibitory effects of ROS are reported in other studies (Auerbach and Segal, 1997; Kamsler and Segal, 2003a, 2003b; Pellmar et al., 1991; Watson et al., 2002). Different protocols were used for induction of synaptic plasticity and species or age of animals were different among these studies, and target signaling pathways of oxidation during the induction of synaptic plasticity were unclear in these experiments. Therefore, the identification of the target signaling systems of oxidation in synaptic plasticity would provide critical insight concerning how oxidative stress results in deficits in synaptic plasticity and brain function in aged animals.

Parallel fiber-Purkinje cell synapse (PF synapse) in the cerebellum is a good model for examining molecular mechanisms in synaptic plasticity in the central nervous system (Hansel et al., 2001; Ito, 2006). Purkinje cells (PCs), solely output from the cerebellar cortex, receive 2 types of excitatory inputs: climbing fiber (CF) from inferior olive and PF, the axon of granular cells in the cerebellar cortex. Various types of synaptic plasticity were reported to date at the PF synapse (Evans, 2007; Ito, 2006; Jorntell and Hansel, 2006). Among them, the synaptic potentiation induced by repetitive activity of PF are known to be dependent on nitric oxide (NO)-mediated signaling pathways (Kakegawa and Yuzaki, 2005; Lev-Ram et al., 2002; Namiki et al., 2005). Nitric oxide exerts its effects via 2 pathways. One pathway is mediated by soluble guanylyl cyclase (sGC). Activation of sGC induces increased cytosolic cyclic guanosine monophosphate (GMP) level and activates protein kinase G. Another pathway is mediated by S-nitrosylation of cysteine residues in various proteins. S-nitrosylation of proteins resulted in modification of function of proteins including ion channels and enzymes (Calabrese et al., 2007; Hess et al., 2005; Jaffrey et al., 2001; Nakamura and Lipton, 2007). Because the potentiation of the PF synapse is dependent on NO but not sensitive to 1H-[1,2,4] Oxadiazolo[4,3-a] quinoxaline-1-1 (ODQ), a specific antagonist for sGC, S-nitrosylation-mediated pathway is indicated to be involved in the potentiation (Lev-Ram et al., 2002; Namiki et al., 2005). Oxidizing agents including endogenous ROS also modify cysteine residues and exert its action on proteins (Ansari et al., 2006; Forman et al., 2008; Mikkelsen and Wardman, 2003; Suzuki et al., 2010). Therefore, NO and oxidizing agents competitively share cysteine residues to exert their action, and it is highly possible that oxidizing agents affect S-nitrosylation-mediated signaling pathways essential for the potentiation of the PF synapse and impair or occlude the potentiation.

In the present study, we first demonstrated that posttetanic potentiation (PTP) and LTP at the PF synapse were severely impaired in the cerebellar slices from aged (20- to 24-month-old) mice, suggesting involvement of endogenous ROS in age-dependent decline in PF-PTP and PF-LTP. This hypothesis was confirmed by subsequent experiments demonstrating application of oxidizing agents also inhibited the induction of PF-PTP and PF-LTP in the cerebellar slices from young (4–6 weeks old) mice. Because both PF-PTP and PF-LTP is suggested to be dependent on S-nitrosylation, we propose that oxidation by ROS induces its inhibitory effects on synaptic plasticity via modification of cysteine residues whose S-nitrosylation by acute NO signal is essential for the induction of the plasticities, at least at PF synapses in the cerebellum.

2. Methods

2.1. Slice preparation

All experiments were carried out according to the guidelines established by the Animal Welfare Committee of Nagasaki University.

Wild-type C57BL/6 mice at 4 to 6 weeks or 20 to 24 months of age were sacrificed by cervical dislocation under deep anesthesia with diethyl ether. The cerebellum was excised, and parasagittal cerebellar slices (250 μm thick) were prepared from the vermis (Edwards et al., 1989; Kakizawa et al., 2000, 2005). Whole-cell recordings were obtained from visually identified PCs under an upright microscope (BX51WI, Olympus, Tokyo, Japan) using a 40× water-immersion objective at room temperature (23–25 °C). The resistances of patch pipettes were 2.0–3.5 MΩ when filled with an intracellular solution composed of (in mM), 130 K-gluconate, 10 KCl, 10 NaCl, 1 ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 4 adenosine triphosphate (ATP)-Mg, 0.4 guanosine triphosphate (GTP)-Na and 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.3; adjusted with potassium hydroxide [KOH]). The standard bathing solution was composed of (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 20 glucose, bubbled with 95% CO_2 and 5% CO_2. An antagonist for the type-A γ-aminobutyric acid receptor ($GABA_A$ receptor), bicuculline (10 μM), was always added to block inhibitory postsynaptic currents.

2.2. Electrophysiology

For the focal stimulation of PF, a stimulation pipette (5–10 μm tip diameter) was filled with the standard bathing solution and used in applying square pulses (0.1 ms in duration, 0–20 V in amplitude) in the molecular layer at the middle 1-third from the pial surface. The intensity of each stimulus was adjusted to evoke excitatory postsynaptic currents of PF (PF-EPSCs) with amplitudes of 70–150 pA. Ionic current was recorded from PCs with a patch-clamp amplifier (EPC-9, HEKA, Lambrecht/Pfalz, Germany) at a holding potential of −90 mV or −80 mV, after the com-