

# Chapter 32

## Nitric Oxide

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**Abstract** Nitric oxide (NO) is a gaseous molecule with chemical formula NO. In biological systems, NO is produced from L-arginine by three distinct NO synthases (NOSs), two of which, neuronal (nNOS) and endothelial (eNOS), are calcium dependent, whereas inducible NOS (iNOS) is calcium independent. In the cerebellum, expressions of all types of NOS are observed in physiological and/or pathological conditions. Although recognized originally as the endothelium-derived relaxing factor (EDRF), it is now well recognized that NO is involved in a wide range of neurobiological functions such as neurogenesis, synaptic plasticity, cerebellar-dependent learning, neuroprotection and neuronal-cell death.

**Keywords** Nitric oxide • cGMP • Soluble guanylyl cyclase • S-nitrosylation • Nitric oxide synthase • Granule cell • Synaptic plasticity • Parallel fiber • Purkinje cell • Motor learning

### 32.1 Nitric Oxide Synthase

Nitric oxide synthase (NOS) catalyzes the conversion of L-Arginine to NO and L-citrulline, in the presence of nicotinamide adenine dinucleotide phosphate (NADPH), O<sub>2</sub> and various cofactors (Abbott and Nahm 2004). NOS exist in three major isoforms. These are named type I/neuronal NOS (NOS1/nNOS), type II/inducible NOS (NOS2/iNOS) and type III/endothelial NOS (NOS3/eNOS) (Alderton et al. 2001; Stuehr et al. 2004). Two of them, nNOS and eNOS, are constitutively expressed mainly in the nervous system and the vascular endothelium, respectively, and synthesize NO in a calcium-dependent manner under basal conditions and upon stimulation. By contrast, iNOS is induced when stimulated by microbial endotoxins or certain proinflammatory cytokines and produces NO in a calcium-independent manner.

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Among the three enzymatic isoforms of NOSs, nNOS and eNOS are expressed in the cerebellum in physiological condition, while iNOS starts to appear in pathologic states. High levels of nNOS are detected in cerebellar granule, basket and stellate cells. Glial cells also express NOS. Specifically, iNOS has been detected in microglia and astrocytes upon stimulation by cytokines and other compounds (Schilling et al. 1994; Stojkovic et al. 1998). Mutant mice lacking each NOS isoform as well as triple-knockout mice have been developed (Tsutsui et al. 2010).

## 32.2 Signal Transduction

The effects of NO on cellular functions are mediated by two pathways (Fig. 32.1). One is activation of soluble guanylyl cyclase (sGC), resulting in the production of cyclic guanosine monophosphate (cGMP). Another pathway for NO signal transduction is mediated by reversible post-translational modification of proteins, S-nitrosylation of thiol groups in cysteine (a term “S-nitrosation” is also used for this modification, Iyer et al. 2014). Although another type of post-translational modification of proteins, tyrosine nitration, is well known, this is an irreversible modification, and is not direct reaction of NO, but reaction of peroxynitrite ( $\text{ONOO}^-$ ) produced from NO and superoxide ( $\text{O}_2^-$ ) (Pacher et al. 2007; Ischiropoulos 2009).

The activation of sGC by NO results in surge of cellular cGMP, which is the main cellular transducer of NO signals whose concentration and kinetics are affected by phosphodiesterases, a catalytic enzyme for cGMP. In addition to regulating various channel proteins by direct binding, cGMP activates protein kinases G (PKGs) regulating functions of a wide range of other proteins through phosphorylation (Fig. 32.1, upper) (Friebe and Koesling 2003; Garthwaite 2010).

S-nitrosylation is a covalent addition of an NO group to a cysteine thiol/sulfhydryl (-SH), which results in formation of an S-nitrosothiol derivative (-SNO). S-nitrosylation is now well established as a major source of NO bioactivity, and proteins shown to be modified *in situ* by S-nitrosylation participate in a wide range of biological processes (Fig. 32.1, lower) (Foster et al. 2009; Shahani and Sawa 2011).



**Fig. 32.1** Two pathways for NO signaling. NO signal is mediated by activation of soluble guanylyl cyclase (sGC) and resulting increase in cyclic GMP (cGMP) level (*upper*), or by S-nitrosylation (also called S-nitrosation) of thiol groups in cysteine residue (*lower*). PKG, protein kinase G

## 32.3 Physiological and Pathophysiological Functions

### 32.3.1 Granule Cell Neurogenesis

In mammals, NO has been shown to down-regulate adult neurogenesis, occurring in granule cells in hippocampal dentate gyrus, for example.

In the cerebellum, negative regulation of cerebellar granule cells precursor proliferation by NO is indicated from several lines of studies *in vitro*. In neonatal rat cerebellar slices, NOS inhibition maintains an age-dependent higher proliferation rate among neuronal precursors localized in external granular layer. In primary cultures of dissociated cerebellar granule cells, NOS inhibition increases precursor proliferation (Contestabile 2012).

### 32.3.2 Synaptic Plasticity in the Cerebellar Cortex

Purkinje cell (PC), a principle neuron in the cerebellar cortex, receives two types of excitatory inputs: climbing fibers (CF), originate from inferior olive, and parallel fibers (PF), axons of granule cells. Both CF-PC synapse and PF-PC synapse show bidirectional plasticity, long-term depression (LTD) and long-term potentiation (LTP). However, neither LTD nor LTP at CF-PC synapse show NO-dependency, consistent with the negative staining of these architectures with nNOS antibody. On the other hand, many studies indicate that both LTD and LTP at PF-PC synapse are dependent on NO signals.

A currently accepted model for induction of the LTD is that NO produced by PF activity diffuses into PC where it stimulates guanylyl cyclase and activates PKG, increasing phosphorylation level of AMPA receptors which results in their de-clustering and endocytotic recycling, thus lowering excitatory response to glutamate (Ito 2002).

In addition to LTD, involvements of NO signals in LTP at PF-PC synapse are indicated. However, in contrast to the LTD, the LTP is not inhibited by a selective inhibitor of sGC activation by NO (ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) that abolishes the LTD (Lev-Ram et al. 2002). Instead, the action of NO is mediated by S-nitrosylation of N-ethyl maleimide sensitive factor (NSF) and type 1 ryanodine receptor (RyR1), a Ca<sup>2+</sup> release channel expressed in the membrane of sarco/endoplasmic reticulum, intracellular Ca<sup>2+</sup> store (Kakegawa and Yuzaki 2005; Kakizawa et al. 2012). S-nitrosylation of NSF results in the insertion of AMPA receptor to plasma membrane and increases the response to glutamate. S-nitrosylation of RyR1 elicits NO-induced Ca<sup>2+</sup> release from the intracellular store, which is essential for the induction of the LTP.

### 32.3.3 *Involvement in Cerebellar-Dependent Learning*

The LTD at CF-PC synapse is implicated to be involved in specific forms of motor learning such as adaptation of vestibule-ocular reflex (VOR), for example. Because NO signal is indicated to be necessary for the LTD, it is reasonable to speculate that NO signals are involved in the cerebellar-dependent motor learning such as VOR (Yuzaki 2013; Ito et al. 2014).

Actually, several lines of studies indicate possible involvement of NO signals in the motor learning. NOS inhibitors or NO scavengers, which block cerebellar LTD, impair some forms of motor learning, such as adaptation of the horizontal VOR, smooth pursuit eye movement, coordinated locomotion and eyeblink conditioning. Moreover, adaptation of optokinetic response eye movements is impaired in nNOS-knockout mice.

### 32.3.4 *Neurotoxic and Neuroprotective Roles of NO in the Cerebellum*

Numerous studies implicate that NO is neuroprotective, facilitating normal neuronal function, or neurotoxic, contributing to neuronal damage or death (Abbott and Nahm 2004).

NO reacts with superoxide to form peroxynitrite (ONOO<sup>-</sup>), a strong oxidizing agent inducing various neurotoxic effects through tyrosine nitration.

On the other hand, NO is also indicated to function as an antioxidant by scavenging oxygen free radicals. For example, NO is demonstrated to protect against CNS lesions induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an inhibitor of complex I in mitochondria inducing overproduction of reactive oxygen species.

## References

- Abbott LC, Nahm SS (2004) Neuronal nitric oxide synthase expression in cerebellar mutant mice. *Cerebellum* 3:141–151
- Alderton WK, Cooper CE, Knowles RG (2001) Nitric oxide synthases: structure, function and inhibition. *Biochem J* 357:593–615
- Contestabile A (2012) Role of nitric oxide in cerebellar development and function: focus on granule neurons. *Cerebellum* 11:50–61
- Foster MW, Hess DT, Stamler JS (2009) Protein S-nitrosylation in health and disease: a current perspective. *Trends Mol Med* 15:391–404
- Friebe A, Koesling D (2003) Regulation of nitric oxide-sensitive guanylyl cyclase. *Circ Res* 93:96–105
- Garthwaite J (2010) New insight into the functioning of nitric oxide-receptive guanylyl cyclase: physiological and pharmacological implications. *Mol Cell Biochem* 334:221–232

- Ischiropoulos H (2009) Protein tyrosine nitration – an update. *Arch Biochem Biophys* 484:117–121
- Ito M (2002) The molecular organization of cerebellar long-term depression. *Nat Rev Neurosci* 3:896–902
- Ito M, Yamaguchi K, Nagao S et al (2014) Long-term depression as a model of cerebellar plasticity. *Prog Brain Res* 210:1–30
- Iyer AK, Rojanasakul Y, Azad N (2014) Nitrosothiol signaling and protein nitrosation in cell death. *Nitric Oxide* 42C:9–18
- Kakegawa W, Yuzaki M (2005) A mechanism underlying AMPA receptor trafficking during cerebellar long-term potentiation. *Proc Natl Acad Sci U S A* 102:17846–17851
- Kakizawa S, Yamazawa T, Chen Y et al (2012) Nitric oxide-induced calcium release via ryanodine receptors regulates neuronal function. *EMBO J* 31:417–428
- Lev-Ram V, Wong ST, Storm DR et al (2002) A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. *Proc Natl Acad Sci U S A* 99:8389–8393
- Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87:315–424
- Schilling K, Schmidt HH, Baader SL (1994) Nitric oxide synthase expression reveals compartments of cerebellar granule cells and suggests a role for mossy fibers in their development. *Neurosci* 59:893–903
- Shahani N, Sawa A (2011) Nitric oxide signaling and nitrosative stress in neurons: role for S-nitrosylation. *Antioxid Redox Signal* 14:1493–1504
- Stojkovic T, Colin C, Le Saux F et al (1998) Specific pattern of nitric oxide synthase expression in glial cells after hippocampal injury. *Glia* 22:329–337
- Stuehr DJ, Santolini J, Wang ZQ et al (2004) Update on mechanism and catalytic regulation in the NO synthases. *J Biol Chem* 279:36167–36170
- Tsutsui M, Shimokawa H, Otsuji Y et al (2010) Pathophysiological relevance of NO signaling in the cardiovascular system: novel insight from mice lacking all NO synthases. *Pharmacol Ther* 128:499–508
- Yuzaki M (2013) Cerebellar LTD vs. motor learning—lessons learned from studying GluD2. *Neural Netw* 47:36–41