NO/cGMP-Dependent Modulation of Synaptic Transmission

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Abstract Nitric oxide (NO) is a multifunctional messenger in the CNS that can signal both in antero- and retrograde directions across synapses. Many effects of NO are mediated through its canonical receptor, the soluble guanylyl cyclase, and the second messenger cyclic guanosine-3′,5′-monophosphate (cGMP). An increase of cGMP can also arise independently of NO via activation of membrane-bound particulate guanylyl cyclases by natriuretic peptides. The classical targets of cGMP are cGMP-dependent protein kinases (cGKs), cyclic nucleotide hydrolysing phosphodiesterases, and cyclic nucleotide-gated (CNG) cation channels. The NO/cGMP/cGK signalling cascade has been linked to the modulation of transmitter release and synaptic plasticity by numerous pharmacological and genetic studies. This review focuses on the role of NO as a retrograde messenger in long-term potentiation of transmitter release in the hippocampus. Presynaptic mechanisms of NO/cGMP/cGK signalling will be discussed with recently identified potential downstream components such as CaMKII, the vasodilator-stimulated phosphoprotein, and regulators of

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G protein signalling. NO has further been suggested to increase transmitter release through presynaptic clustering of α-synuclein. Alternative modes of NO/cGMP signalling resulting in inhibition of transmitter release and long-term depression of synaptic activity will also be addressed, as well as anterograde NO signalling in the cerebellum. Finally, emerging evidence for cGMP signalling through CNG channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels will be discussed.

1 Introduction

The gas nitric oxide (NO) is well suited to serve as a messenger molecule because of its capacity for rapid diffusion in both aqueous and lipid environments. A myriad of reports document a broad range of NO effects in the CNS, such as the modulation of neuronal development, nociception, apoptosis, synaptic plasticity, and complex behavioral responses (for review see Prast and Philippu 2001; Hofmann et al. 2003). Nearly 20 years ago, Garthwaite and co-workers (1988) recognized for the first time the potential of NO as a neuromodulator. They observed that activation of Ca^{2+}-permeable ionotropic glutamate receptors in cultured cerebellar granule cells triggers the release of a messenger similar to endothelium-derived relaxing factor, which is identical to NO (Ignarro et al. 1987; Furchgott 1996). Based on these results, they put forward the truly inspiring hypothesis that this factor may provide a universal link from postsynaptic activity to functional modifications of neighbouring presynaptic terminals and glial cells. Ironically, the concept of retrograde signalling via NO has been proven in many regions of the brain, but does not hold true for glutamatergic parallel fiber synapses in the cerebellum (see Section 3.2.2). This chapter aims to summarize the current knowledge about NO-dependent mechanisms implicated in the modulation of synaptic transmission. First, the cellular and molecular components involved in NO signalling in the brain will be described. Then, we will provide a synopsis of NO/cGMP-dependent effects on synaptic transmission and corresponding signal transduction based on observations from electrophysiological studies and studies with radioactive or fluorescent markers for presynaptic function. With regard to the general topic of this volume, which is the control of transmitter release, the focus will be set on presynaptic signalling mechanisms of NO and its intracellular second messenger cyclic guanosine-3′,5′-monophosphate (cGMP).

2 Overview of the NO/cGMP Signalling System and Its Expression in the CNS

2.1 Sources of NO and cGMP

NO is produced by a complex reaction via oxidative release from L-arginine giving rise to L-citrulline. Three mammalian isozymes catalyzing this reaction have been identified in various cell types, the constitutively expressed neuronal and endothelial NO synthase (nNOS/NOS1 and eNOS/NOS3), and the inducible NO synthase (iNOS /NOS2). The nNOS is ubiquitously and abundantly expressed throughout the CNS and represents the principal source of NO in many neuronal populations (Bredt and Snyder 1990; Dawson and Dawson 1996; Prast and Philippu 2001). It is a Ca^{2+}/calmodulin-regulated enzyme, which can be activated by Ca^{2+} influx via N-methyl-D-aspartate (NMDA) receptors (Garthwaite et al. 1988). This functional relationship is thought to be especially effective, because the scaffolding molecule PSD-95 keeps the nNOS protein in close proximity to NMDA receptors (Christopherson et al. 1999; Valtschanoff and Weinberg 2001). The eNOS, another Ca^{2+}/calmodulin-dependent NOS initially detected in endothelial cells, has also been reported to be expressed in hippocampal pyramidal cells and neurons of other brain regions (Dinerman et al. 1994; O'Dell et al. 1994). However, this observation has been challenged by others (Stanarius et al. 1997; Demas et al. 1999; Blackshaw et al. 2003). It is now suggested that eNOS expression is confined to endothelial cells, and that NO released within the cerebral vasculature is able to signal to axons located in close proximity (Garthwaite et al. 2006). In contrast to the two constitutive forms of NOS, the iNOS is normally not detectable in the CNS, but upregulated following toxic or inflammatory stimuli.

In many cases, NO acts in the brain by increasing the concentration of cGMP, which is achieved through activation of the canonical NO receptor, the soluble guanylyl cyclase (sGC) (Figure 1) (Friebe and Koesling 2003). The NO-sensitive sGC is a heme-containing enzyme consisting of two different subunits, α and β. Heterodimers of the composition $\alpha_1\beta_1$ and $\alpha_2\beta_1$ have been shown to be functional enzymes, while the role of a putative β_2 subunit remains unclear (for review see Koesling et al. 2005). The $\alpha_1\beta_1$ isoform is ubiquitously expressed in the CNS, whereas expression of $\alpha_2\beta_1$ may be restricted to specific areas in the brain (Mergia et al. 2003; Ding et al. 2004; Russwurm and Koesling 2004). Taken together, it appears that principally every neuron in the CNS can generate NO and/or cGMP. Note that, due to the diffusible nature of NO, the sites of NO and cGMP synthesis must not necessarily overlap. A useful tool to analyze and compare the expression patterns of components of the NO/cGMP signalling pathway in the brain is the recently released Allen Brain Atlas (http://www.brainatlas.org/aba/), which contains the mRNA expression profiles of virtually all mouse genes in the brain (Lein et al. 2006).

It is important to note that many cell types possess alternative NO-independent mechanisms to generate cGMP (Figure 1), namely through activation of membrane-

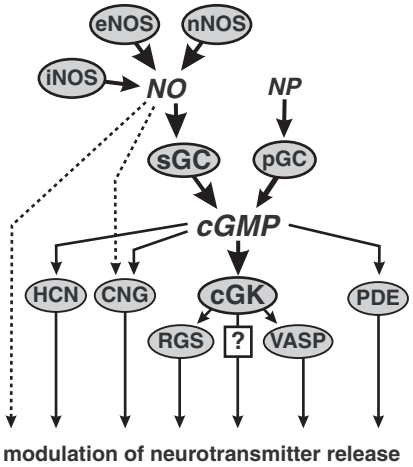

Fig. 1 NO signalling pathways. Nitric oxide (NO) can be generated by endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). NO synthase and activates the cytosolic soluble guanylyl cyclase (sGC), leading to a rise of intracellular cGMP, which in turn activates cGMP-dependent protein kinase (cGK). An increase of cGMP can also be induced by natriuretic peptides (NP) through activation of membrane-bound particulate guanylyl cyclases (pGC). cGK substrates that may be functionally important in the brain include the vasodilator-stimulated phosphoprotein (VASP protein) and regulators of G protein signalling (RGS proteins). The question mark indicates unknown cGK substrates. cGMP can also signal via cGK-independent mechanisms, e.g., by activating cyclic nucleotide-gated (CNG) channels, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, or by modulating various phosphodiesterases (PDE). Note that the mechanisms that modulate transmitter release downstream of cGMP receptors are not well understood. There have been proposed signalling mechanisms of NO not related to the cGMP pathway (dotted lines). For example, NO may trigger Ca^{2+} entry by S-nitrosylation of a CNG channel subunit.

bound particulate guanylyl cyclases (pGCs), e.g., GC-A (NPR-A) and GC-B (NPR-B), by natriuretic peptides (NPs) (for review see Kuhn 2004). NPs comprise a family of three homologous members, atrial (ANP), brain (BNP), and C-type (CNP) natriuretic peptide. Despite the fact that BNP and CNP as well as their receptors (GC-A and GC-B) are found in the brain (Sudoh et al. 1988, 1990; Komatsu et al. 1991; Herman et al. 1993; DiCicco-Bloom et al. 2004), their function in the CNS remains largely elusive and, therefore, NPs and pGCs will not be discussed further in the following sections. Recent findings suggest a modulation of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and ionotropic γ-amino butyric acid ($GABA_A$) receptors by NPs in retinal neurons (Tian and Yang 2006; Yu et al. 2006).

2.2 cGMP Receptors

Extensive analysis of the cGMP system has led to the identification of several effectors for this intracellular second messenger. The classical cGMP receptors include

cGMP-dependent protein kinases (cGKs), cyclic nucleotide-gated (CNG) cation channels, members of the family of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, and of the family of cyclic nucleotide hydrolysing phosphodiesterases (PDEs). We will not discuss mechanisms related to cGMP-mediated cross-activation of cAMP-dependent protein kinase.

2.2.1 cGMP-Dependent Protein Kinases (cGKs)

A major role in the signal transduction of NO and cGMP in the nervous system has been assigned to cGKs (for review see Feil R et al. 2005b; Hofmann et al. 2006). cGKs belong to a family of serine/threonine kinases found in diverse eukaryotic organisms and various tissues, including the nervous system (Muller 1997; Wang and Robinson 1997; Francis and Corbin 1999; Pfeifer et al. 1999). Mammals possess two cGK genes, *prkg1* and *prkg2*, that encode cGKI and cGKII, respectively. The cGKI gene encodes two isoforms, cGKIα and cGKIβ. These isoforms are identical except for their N-termini (≈90–100 amino acid residues), which are encoded by two alternatively used 5′-exons of the cGKI gene. In vitro, cGKs are activated at submicromolar to micromolar concentrations of cGMP, the cGKIα isoform being ≈10-fold more sensitive to activation by cGMP than cGKIβ (Ruth et al. 1991). Both the cGKI and cGKII are homodimers of subunits with a molecular weight of ≈75 kDa and ≈85 kDa, respectively. Each subunit is composed of three functional domains. The *N-terminal domain* comprises regions that control dimerization, targeting, and the activity of the catalytic center. In contrast to the cytosolic cGKI, the N-terminus of the cGKII is myristoylated, thereby anchoring the enzyme to the plasma membrane. The *regulatory domain* contains two tandem cGMP-binding sites that bind cGMP with high and low affinity and interact allosterically. Occupation of both binding sites induces a large change in secondary structure (Landgraf et al. 1990) to yield a more elongated protein molecule (Wall et al. 2003). The *catalytic domain* bears the kinase activity and contains the MgATP- and peptide-binding pockets. Binding of cGMP to both sites in the regulatory domain releases the inhibition of the catalytic center by an N-terminal autoinhibitory/pseudosubstrate site and allows the phosphorylation of serine/threonine residues in target proteins and in the N-terminal autophosphorylation site.

Both cGKs are expressed in the nervous system, while the cGKI appears to be the dominating isoform (http://www.brainatlas.org/aba/). Correlating with the maximum of enzymatic cGK activity in the brain, the cGKIα isoform is highly expressed in cerebellar Purkinje cells (Hofmann and Sold 1972; Lohmann et al. 1981; Geiselhoringer et al. 2004; Feil S et al. 2005). Significant levels of cGKIα have also been observed in nociceptive neurons of the dorsal root ganglia (Qian et al. 1996; Schmidt et al. 2002; Tegeder et al. 2004). Other brain regions with cGKI expression include the dorsomedial nucleus of the hypothalamus, the suprachiasmatic nucleus, the hippocampus, the amygdala, the olfactory bulb, and the cerebral cortex (El-Husseini et al. 1999; Kleppisch et al. 1999; Revermann et al. 2002; Feil S et al. 2005). Noteworthy, cGKIβ appears to be the prevailing isozyme in these

latter regions (Geiselhoringer et al. 2004; Feil S et al. 2005). For the cGKII, low to moderate levels of expression have been reported in the hippocampus, the olfactory tubercle, the suprachiasmatic nucleus, and the thalamus (El-Husseini et al. 1995, 1999; Kleppisch et al. 1999; De Vente et al. 2001; Oster et al. 2003).

Several substrates of cGKs have been identified unequivocally in non-neuronal tissues (Hofmann et al. 2006), while evidence for functional cGK substrates in the nervous system is lagging behind. Table 1 summarizes proteins phosphorylated by

Table 1 cGK Substrates with Possible **Funtion** in the Nervous System

Substrate	Possible Function of Phosphorylation in the CNS	Brain Region Experimental System	References
G-substrate	Protein phosphatase inhibition Initiation of cerebellar LTD	Cerebellum	Aswad and Greengard 1981; Ajima and Ito 1995; Hall et al. 1999; Endo et al. 2003
DARPP-32	Protein phosphatase inhibition Modulation of signallingr pathways	Substantia nigra	Tsou et al. 1993; Nishi et al. 2005
IP_3 receptor type I	Stimulation of Ca^{2+} release from IP_3 sensitive stores	Cerebellum	Haug et al. 1999; Wagner et al. 2003
VASP	Regulation of actin dynamics Vesicle trafficking Aggregation of vesicle proteins	Cerebellum, Hippocampus	Hauser et al. 1999; Arancio et al. 2001; Wang et al. 2005
RhoA	Regulation of actin dynamics	Hippocampus	Wang et al. 2005[#]
	Vesicle trafficking	Rat C6 glioma cells	Zhuang et al. 2004
	Aggregation of vesicle proteins	Vascular smooth muscle	Sauzeau et al. 2000
		NIH 3T3 cells	Ellerbroek et al. 2003
Septin-3	Vesicle trafficking	Hippocampus	Xue et al. 2000, 2004
		COS7 cells	
PDE5	Enhanced cGMP degradation	Cerebellar Purkinje cells	Shimizu-Albergine et al. 2003
	Accelerated termination of cGMP signal	Smooth muscle	Rybalkin et al. 2002

(*Continued*)

Table 1 Continued

Substrate	Possible Function of Phosphorylation in the CNS	Brain Region Experimental System	References
RGS3 and RGS4	Termination of GPCR activation	Astrocytes	Pedram et al. 2000
RGS2	Termination of GPCR activation	Hippocampus	Oliveira-Dos-Santos et al. 2000; Han et al. 2006[§]
	Downregulation of G protein-mediated presynaptic inhibition	Vascular smooth muscle	Tang et al. 2003
	Increase in transmitter release		
ADP ribosyl cyclase	Decreased transmitter release	Hippocampus	Fossier et al. 1999; Reyes-Harde et al. 1999a,b
	Initiation of hippocampal LTD		
BK_{Ca}	Increased open probability	Pituitary nerve terminals	Klyachko et al. 2001
	Enhances afterhyperpolarisation	Smooth muscle	Sausbier et al. 2000

Substrates were suggested according to *in vivo* or *in vitro* phosphorylation studies or based on functional studies. Note that most of the proteins were described as substrates of cGKI; cGKII substrates in the brain are poorly characterized.
[#] Wang et al. (2005) suggest that RhoA, though involved in synaptic plasticity, is not a downstream target of cGK. RhoA has been identified as a cGK substrate in other tissues.
[§] Studies in the hippocampus solely support a possible function of RGS2 in synaptic transmission, but do not prove its phosphorylation by cGK. RGS2 has been identified as a cGK substrate in other tissues.

cGKs in neurons and known substrates of cGKs that are expressed in the brain and known to modulate transmitter release. In general, cGK substrates identified so far include ion channels, G proteins and associated regulators, and cytoskeleton-associated proteins.

2.2.2 Cyclic Nucleotide-Gated (CNG) Channels

Cytosolic cGMP can activate CNG channels. These ion channels are not only an important part of the signal transduction pathway in the visual and olfactory system (Biel et al. 1999; Hofmann et al. 2003), but are also expressed in other neurons where they may be involved in the modulation of various functions by NO. The CNG channel family comprises six homologous subunits. Based on phylogenetic relationship, these proteins are classified as A subunits (CNGA1-A4) and B subunits (CNGB1 and CNGB3) (Biel et al. 1999; Bradley et al. 2001). Native CNG channels

are heterotetramers composed of A and B subunits, and their sensitivity for cGMP largely depends on the individual subunit composition. For instance, the cGMP-gated rod channel consists of CNGA1 (Kaupp et al. 1989) and a long splice variant of CNGB1 (CNGB1a) (Korschen et al. 1995). Activation of CNG channels causes depolarization and, thus, facilitates excitation of neurons. In addition, CNG channels have been suggested to permit Ca^{2+} influx relevant for synaptic function (Broillet and Firestein 1997; Parent et al. 1998).

2.2.3 Phosphodiesterases (PDEs)

Cyclic nucleotide signalling is controlled not only at the level of cAMP and cGMP synthesis, but also by the rate of cyclic nucleotide degradation via PDEs (Sonnenburg and Beavo 1994). The PDE superfamily comprises 11 subfamilies with 21 genes that are transcribed into perhaps more than 50 enzyme species by alternative splicing, and virtually all PDEs are expressed in the CNS (Menniti et al. 2006). The families are classified according to substrate specificity, mechanism of regulation, sensitivity to inhibitors, and amino-acid sequence. Cyclic GMP is degraded by a number of cGMP-specific PDEs as well as by dual-substrate PDEs that hydrolyse both cAMP and cGMP. Importantly, cGMP can also inhibit or activate specific PDE subtypes via binding to their regulatory domains. Thereby, cGMP can modulate its own level and/or that of cAMP. For instance, cGMP can increase cAMP levels via binding to the cGMP-inhibited cAMP-PDE, PDE3A; it can lower cyclic nucleotide levels by binding to the cGMP-stimulated cAMP/cGMP-PDE, PDE2A, or by binding to the cGMP-specific PDE5A, the target of sildenafil. Thus, the cGMP-regulated PDEs are important cGMP receptors helping to shape the spatiotemporal profile of cyclic nucleotide signals. Particularly, they might represent “switches” that transform cGMP signals into cAMP signals, allowing cyclic nucleotide cross-talk in the brain. Another interesting mode of PDE regulation is represented by the dual-substrate PDE1 family. This family has the unique feature of being activated by the binding of Ca^{2+}/calmodulin, providing a mechanism for cross-talk between the Ca^{2+} and cyclic nucleotide signalling pathways.

2.3 cGMP-Independent NO Signalling

It is important to note that not all effects of NO on synaptic transmission can be accounted for by cGMP-dependent signalling, i.e., NO can also signal through alternative mechanisms independent of cGMP. Owing to the limited space and the general scope of this chapter, we will not cover these mechanisms in detail but just discuss them briefly. First, NO can give rise to free radical species that, in turn, may affect cellular functions through various mechanisms (for review see Stamler et al. 1997). Second, NO can modulate proteins controlling neuronal functions by stimulating their ADP ribosylation (Schuman et al. 1994; Zhang et al. 1994; Sullivan et al.

1997). This mechanism may account partially for the stimulatory action of NO on transmitter release in hippocampal synapses (Schuman et al. 1994). Last but not least, NO may modulate cellular functions by S-nitrosylation of various proteins. For example, S-nitrosylation of nuclear proteins associated with cAMP response element (CRE) binding protein is involved in regulation of its DNA binding and, hence, CRE-mediated gene expression (Riccio et al. 2006). S-nitrosylation has also been reported to modify the function of various ion channels (Broillet and Firestein 1996, 1997; Xu et al. 1998; Jaffrey et al. 2001).

3 NO and cGMP as Modulators of Synaptic Transmission

3.1 Effects of NO/cGMP on Transmitter Release

The proposal that NO might act presynaptically to modify transmitter release (Garthwaite et al. 1988) prompted a plethora of investigations into the effects of NO donors and inhibitors of NOS and sGC on transmitter release. Studies were performed in vitro, e.g., on brain slices, synaptosomes, or cultured neurons, or in vivo using microdialysis or the push-pull superfusion technique. The effects of NO donors suggest that the release of several transmitters, including acetylcholine, catecholamines, and excitatory and inhibitory amino acids, may be influenced by endogenous NO. NO donors also inhibit the uptake of various radiolabeled transmitters (Pogun et al. 1994). However, these NO donor studies usually lacked important controls for potential unspecific effects of NO carrier molecules (Guevara-Guzman et al. 1994). Other experiments more convincingly show that authentic NO (Guevara-Guzman et al. 1994) as well as endogenous NO that is "tonically" present in unstimulated tissue (Prast and Philippu 1992; Bugnon et al. 1994) or "phasically" generated by NMDA receptor activation (Dickie et al. 1992; Hanbauer et al. 1992; Sorkin 1993; Montague et al. 1994) modulates transmitter release. Recent in vitro studies suggest that tonic NO might come from eNOS in blood vessels, while the phasic NO signals are produced by nNOS in neurons (Garthwaite et al. 2006; Hopper and Garthwaite 2006). The concept of vessel-to-neuron signalling by NO is supported by the finding that mice lacking eNOS in the cerebral vasculature exhibit various neuronal phenotypes, including altered transmitter release or turnover (Kano et al. 1998; Frisch et al. 2000).

It appears that NO, predominantly via cGMP-dependent mechanisms, regulates primarily the release of glutamate, which then modulates the release of various other transmitters in several brain regions, such as the hippocampus, the striatum, the hypothalamus, and the locus coeruleus (Prast and Philippu 2001). Depending on the brain region and NO concentration, NO can both stimulate and inhibit the release of a particular transmitter. Based on the observations of many groups, Prast and Philippu (2001) have proposed the following model for the modulation of acetylcholine release by endogenous NO: NO stimulates cGMP synthesis within

glutamatergic neurons. Depending on the NO concentration, glutamate release is either enhanced (high NO) or decreased (low NO), resulting in either enhanced or decreased activation of postsynaptic NMDA or AMPA/kainate receptors on cholinergic neurons followed by enhanced or decreased acetylcholine release. NO may influence cholinergic transmission not only via excitatory glutamatergic neurons, but also via effects on inhibitory GABAergic neurons. The release of catecholamines, histamine, and serotonin may be modulated in a similar manner.

Basically, then, NO seems to be able to modulate vesicular release of transmitter in either direction, or not at all, depending on the coincident level of presynaptic activity and NO concentration. The concept of an activity-dependent retrograde NO signal that is generated in the postsynapse and then diffuses into the presynapse to regulate transmitter release has been investigated extensively, due to its possible involvement in neuronal excitability and memory processes (see Section 3.2.1).

Although many studies have indicated that NO/cGMP can modulate transmitter release (Arancio et al. 1995; Meffert et al. 1996; Sporns and Jenkinson 1997; Klyachko et al. 2001), the molecular mechanisms and functional relevance of presynaptic NO/cGMP signalling are not well understood. It is generally accepted that activity-dependent changes in synaptic strength, such as long-term potentiation (LTP) and long-term depression (LTD), underlie learning and memory (Pastalkova et al. 2006; Whitlock et al. 2006). However, there are ongoing debates whether a given form of synaptic plasticity in a given brain region results from presynaptic and/or postsynaptic alterations, as will be exemplified below for the hippocampus. Conflicting evidence for presynaptic changes has been obtained largely from the classical electrophysiological approach of quantal analysis of pairs of monosynaptically connected neurons, vesicular antibody uptake, and postsynaptic drug-infusion studies (Stanton et al. 2003 and refs. therein). A more direct visualization of presynaptic vesicle release has been achieved with the fluorescent styryl dye FM1-43 (Betz and Bewick 1992; Ryan et al. 1993). FM1-43 is taken up into synaptic vesicles in an activity-dependent manner as a result of endocytosis after transmitter release. Subsequent synaptic stimulation evokes the release of the dye by exocytosis, which is visualized as a destaining whose rate is a direct measure of presynaptic release efficacy. FM1-43 imaging of cultured neurons and acute brain slices demonstrated that certain forms of LTP or LTD in the hippocampus depend, at least in part, on increased or decreased transmitter release from the presynaptic neuron. Interestingly, retrograde NO signalling appears to contribute to both presynaptic LTP and LTD of transmitter release.

A model of retrograde NO signalling in a glutamatergic synapse is presented in Figure 2. The pathway comprises (1) an action potential-driven release of glutamate from presynaptic axon terminals, (2) activation of postsynaptic NMDA receptors, which trigger the synthesis of NO, (3) diffusion of NO to a presynaptic site, where (4) it stimulates cGMP production and activation of cGK leading to (5) increase (as illustrated in Figure 2) or decrease (not shown in Figure 2) of transmitter release (Sporns and Jenkinson 1997; Stanton et al. 2001, 2003, 2005). In line with such a model, increases in cGMP have been implicated in the induction of both hippocampal LTP (Arancio et al. 2001) and LTD (Gage et al. 1997; Reyes-Harde et al. 1999a).

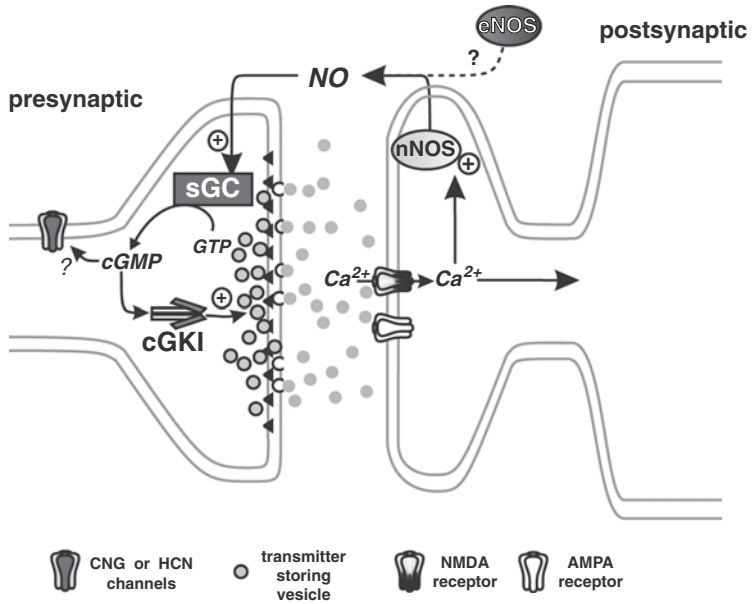

Fig. 2 Retrograde NO signalling in a glutamatergic synapse. Nitric oxide (NO) generated postsynaptically by Ca^{2+}/calmodulin-activated neuronal NO synthase (nNOS) and/or derived from endothelial NO synthase (eNOS) in nearby vessels diffuses to the presynaptic terminal, where it activates the soluble guanylyl cyclase (sGC). The resulting increase of the intracellular second messenger cGMP activates various receptors, including the cGMP-dependent protein kinase I (cGKI). Through phosphorylation of its substrates, cGKI leads to an increase in presynaptic transmitter release. This action is thought to involve clustering of vesicular proteins and other proteins of the docking/fusion machinery (black triangles) at the release sites. In addition, cGMP may modulate transmitter release by activating presynaptic ion channels regulated by cyclic nucleotides (CNG and HCN channels).

However, the mechanisms or mediators controlling the switch from one to the other effect dominating the net change remain to be identified (see also Section 3.2.1). The dual role of the NO/cGMP/cGK pathway in both LTP and LTD may also explain some contradictory reports on the role of this pathway in synaptic plasticity.

What are the molecular mechanisms of presynaptic NO/cGMP signalling that alter transmitter release? In some systems, NO-induced transmitter release occurs independently of an increase in presynaptic Ca^{2+} (Schuman et al. 1994; Stewart et al. 1996), perhaps by modulating the interaction of components of the vesicle docking/fusion machinery (Meffert et al. 1996). On the other hand, it has been reported that one action of presynaptic cGMP required for LTD induction is the stimulation of presynaptic ryanodine receptor-mediated Ca^{2+} release (Reyes and Stanton 1996; Reyes-Harde et al. 1999a), but how an *increase* in presynaptic Ca^{2+} results in long-term *reduction* of release probability is not clear. An alternative mechanism to increase Ca^{2+} is the stimulation of presynaptic Ca^{2+} channels. In the brain stem, for

instance, the NO/cGMP/cGK pathway might facilitate glutamate release through an enhancement of presynaptic N-type Ca^{2+} channel activity (Huang et al. 2003).

In posterior pituitary nerve terminals, NO stimulates Ca^{2+}-activated K^+ (BK) channel activity via a cGMP/cGK-dependent mechanism (Klyachko et al. 2001). Opening of BK channels results in K^+ efflux and membrane hyperpolarization. The enhancement of BK channel activity by cGMP was greatest at depolarized potentials, so it will manifest during and immediately after action potentials and, thus, increase the magnitude of the spike afterhyperpolarization, which in turn promotes Na^+ channel recovery from inactivation. This mechanism would reduce action potential failures and allow more Ca^{2+} to enter followed by increased transmitter release. The stimulation of presynaptic K^+ channels by the NO/cGMP/cGK cascade could generate a short-term, use-dependent enhancement of release at nerve terminals and may also operate at a certain stage of LTP induction in the hippocampus (Klyachko et al. 2001).

After exocytosis, synaptic vesicles must be retrieved and refilled with transmitters to supply the needs of an active neuron. Work by Micheva and colleagues (2001, 2003) showed that retrograde signalling via NO promotes synaptic vesicle recycling in CNS neurons, especially at synapses with high levels of recycling, i.e., in an activity-dependent manner. The authors monitored exo- and endocytosis in cultured hippocampal neurons using a pH-sensitive green fluorescent protein (GFP) reporter protein and the fluorescent dye FM4-64. Based on fluorescence imaging and pharmacological analysis they proposed that NMDA receptor-dependent postsynaptic production of NO accelerates vesicle endocytosis by increasing the level of presynaptic phosphatidylinositol 4,5-bisphosphate (PIP_2) via a cGMP-dependent mechanism. PIP_2 has been extensively implicated in vesicle cycling (Martin 2001; Osborne et al. 2001), but how NO/cGMP leads to an increase in PIP_2 at the synapse is not known. Studies in non-neuronal cells suggest that the NO/cGMP/cGK pathway can inhibit the PIP_2-hydrolysing phospholipase C (Clementi et al. 1995; Xia et al. 2001).

Recent results support the view that microstructural changes occur already at early stages of long-lasting synaptic plasticity (Engert and Bonhoeffer 1999; Maletic-Savatic et al. 1999). For instance, glutamate-induced potentiation of synaptic transmission in cultured hippocampal neurons is accompanied by a rapid increase in clusters of presynaptic synaptophysin and postsynaptic GluR1 protein (Antonova et al. 2001). A recent study by Wang and colleagues (2005) suggests that NO/cGMP/cGKI signalling, actin, and the small GTPase RhoA play important roles in potentiation in cultured hippocampal neurons. Surprisingly, cGMP and cGKI appear to act directly in both the presynaptic and postsynaptic neurons, where they contribute to an increased aggregation of synaptic proteins, perhaps via phosphorylation of the cGKI substrate vasodilator-stimulated phosphoprotein (VASP) and regulation of the actin cytoskeleton. The structural changes promoted by the NO/cGMP/cGKI pathway may not only affect transmitter release and strengthen existing synapses, but may also trigger the activity-dependent formation of new synapses (cf. Wang et al. 2005). These mechanisms will be discussed in more detail later in the context of hippocampal LTP (see Section 3.2.1).

3.2 Long-term Potentiation (LTP) and Long-Term Depression (LTD) as Model Systems to Study NO/cGMP Signalling in Central Synapses

Long-lasting activity-dependent changes of synaptic transmission in a neural network are thought to serve as cellular mechanisms for learning and memory (Hebb 1949). Two prominent forms of synaptic plasticity, LTP and LTD, are indeed associated with different types of learning (Rogan et al. 1997; Manahan-Vaughan and Braunewell 1999; Ito 2002; Whitlock et al. 2006) and have become well-established model systems for studying the modulation of synaptic transmission in various brain regions (Malenka and Bear 2004). It is now generally acknowledged that both pre- and postsynaptic processes can contribute to the changes in glutamatergic synaptic transmission seen after induction of LTP and LTD (for review see Bear and Malenka 1994; Larkman and Jack 1995; Lisman and Raghavachari 2006). Postsynaptic mechanisms ultimately lead to changes in the density and/or function of AMPA type glutamate receptors. For example, LTP is associated with the insertion of AMPA receptors directly into the synaptic region and/or into the extrasynaptic membrane with subsequent diffusional redistribution to the synaptic region. Conversely, internalization of AMPA receptors from these sites plays an important role in LTD. Presynaptic mechanisms lead to a modification of glutamate release, e.g., by changing the mode of vesicle fusion and/or the number of vesicles released in response to afferent stimulation. In addition, aligning presynaptic release sites with postsynaptic receptors may enhance synaptic transmission (Antonova et al. 2001; Wang et al. 2005). NO and cGMP have been reported to signal through many of these mechanisms (see also Section 3.1). The subsequent section is dedicated to the function of NO as a retrograde messenger, i.e., its presynaptic signalling.

3.2.1 NO as a Retrograde Messenger in Hippocampal LTP

Considerable efforts have been made to elucidate the role of NO for synaptic plasticity in various brain regions (for review see Larkman and Jack 1995; Huang 1997; Daniel et al. 1998; Hawkins et al. 1998; Feil R et al. 2005b). The following discussion focuses on findings about the role of NO for LTP in Schaffer collateral/CA1 synapses of the hippocampus, a NMDA receptor-dependent form of synaptic plasticity characterized in great detail (for review see Malenka and Bear 2004; Lisman and Raghavachari 2006). In spite of some controversial findings, it is accepted that NO (1) is involved in LTP in the Schaffer collateral pathway and (2) acts as a retrograde messenger (Garthwaite and Boulton 1995; Arancio et al. 1996; Huang 1997; Hawkins et al. 1998). Both nNOS and eNOS have been detected in postsynaptic pyramidal neurons (Dinerman et al. 1994; O'Dell et al. 1994; Brenman et al. 1996) and various NOS inhibitors suppress LTP in the CA1 region (Schuman and Madison 1991; Arancio et al. 1996). Analysis of mice lacking nNOS and/or eNOS revealed the functional contribution of either NOS isoform to

hippocampal LTP (Table 2). Mice deficient in either nNOS or eNOS appear to be capable of normal LTP in the CA1 region (O'Dell et al. 1994; Son et al. 1996), while a strong impairment was observed in compound nNOS/eNOS knockout mice (Son et al. 1996). Other groups have shown that the lack of eNOS alone can result in reduced LTP in the Schaffer collateral pathway (Wilson et al. 1999) and other regions (Haul et al. 1999; Doreulee et al. 2001). A major role for this isoform has also been suggested by Kantor et al. (1996), who reported that disrupting the localization of eNOS to the membrane causes impairment of LTP, and that this effect could be overcome by expressing a chimeric form of eNOS constitutively targeted to the membrane. On the other hand, the function of nNOS has been underscored by a recent report that selective inhibitors of this isoform reduce hippocampal LTP (Hopper and Garthwaite 2006). It was further suggested that phasic and tonic NO signals are needed for LTP, and that these signals are derived from nNOS in hippocampal pyramidal cells in response to neural activity and eNOS expressed in nearby vascular endothelial cells, respectively (Garthwaite et al. 2006; Hopper and Garthwaite 2006). The findings discussed so far demonstrate the ability of NO to support LTP, but they do not discriminate between possible sites of its action. The observation that bath application of hemoglobin, a membrane-impermeable scavenger of NO, blocks LTP (O'Dell et al. 1991) suggests that NO travels extracellularly through the synaptic cleft. Additional evidence for a presynaptic action of NO comes from studies of miniature excitatory postsynaptic currents (EPSC) (O'Dell et al. 1991) and NMDA receptor-dependent LTP in cultured hippocampal neurons (Arancio et al. 1995, 1996, 2001; Wang et al. 2005). NO increases the frequency of spontaneous miniature EPSCs. Oxymyoglobin, another membrane-impermeable NO scavenger, is able to suppress LTP between pairs of cultured neurons following a tetanus, but its extracellular application has no effect on LTP induced by a weak tetanus in conjunction with photolytic uncaging of NO in the presynaptic cell. These data can be compiled into a scheme with NO as a retrograde messenger in hippocampal LTP (Figure 2). The following section will review efforts made to unravel presynaptic signalling mechanisms of NO involved in the strengthening of synaptic transmission.

Role of the Canonical NO/cGMP/cGK Pathway in Retrograde Signalling

There are some reports that NO can support LTP through cGMP-independent mechanisms, e.g., by stimulating the ADP ribosylation of proteins regulating synaptic transmission (Schuman et al. 1994; Zhang et al. 1994; Sullivan et al. 1997). Also, direct modulation of a CNG channel subunit by NO may cause a Ca^{2+} influx with functional impact on synaptic function (Broillet and Firestein 1997; Parent et al. 1998). But it is no big surprise that the bulk of pharmacological studies as well as the phenotypes of transgenic mouse models (Table 2) support the conclusion that NO signals through its canonical pathway via the sGC, cGMP, and cGKs. LTP-inducing stimuli elicit an increase of cGMP in the hippocampus that is sensitive to NOS inhibitors and NO scavengers (Chetkovich et al. 1993). Moreover,

Table 2 Phenotypes Related to Synaptic Plasticity in Transgenic Mice with Genetic Alterations of NO/cGMP Signalling

Gene	Mouse Model	Phenotypes	References
nNOSα	Null mutation*	Reduced LTD in cerebellar parallel fiber synapses	Lev-Ram et al. 1997b
		Normal LTP in Schaffer collateral pathway	O'Dell et al. 1994
eNOS	Null mutation	Defective LTP in Schaffer collateral pathway	Wilson et al. 1999
		Defective LTP mossy fiber pathway	Doreulee et al. 2001
		Defective LTP in cerebral cortex	Haul et al. 1999
eNOS + nNOSα	Null mutation#	Defective LTP in Schaffer collateral pathway Normal LTP in stratum oriens	Son et al. 1996
sGC α1	Null mutation	Reduced LTP in visual cortex	Mergia et al. 2006
sGC α2	Null mutation	Reduced LTP in visual cortex	Mergia et al. 2006
cGKI	Null mutation	Normal LTP in Schaffer collateral pathway of young mice (4 weeks old)	Kleppisch et al. 1999
cGKII	Null mutation	Normal LTP in Schaffer collateral pathway of adult mice (12 weeks old)	Kleppisch et al. 1999
cGKI + cGKII	Null mutation	Normal LTP in Schaffer collateral pathway of young mice (4 weeks old)	Kleppisch et al. 1999
cGKI	Hippocampus-specific mutation	Reduced L-LTP in Schaffer collateral pathway of adult mice (12 weeks old)	Kleppisch et al. 2003
cGKI	Purkinje cell-specific mutation	Reduced LTD at the parallel fiber-Purkinje cell synapse	Feil R et al. 2003

*The nNOS knockout mice reported were created by a deletion of exon 2 eliminating the splice variant nNOSα, which accounts for about 95% of the catalytic activity in the whole brain. nNOS mutants retain significant NOS activity in the brain, perhaps reflecting the function of the remaining isoform, eNOS. However, this interpretation is complicated by the expression of two alternative splice variants, nNOSβ and nNOSγ, lacking exon 2 in these mice (Eliasson et al. 1997).

the membrane-permeable cGMP analogue dibutyryl cGMP partially restores LTP blocked by a NOS inhibitor (Haley et al. 1992), and various sGC inhibitors suppress LTP (Zhuo et al. 1994; Arancio et al. 1995; Boulton et al. 1995). Evidence for the presynaptic localization of sGC came from studies of LTP between pairs of cultured hippocampal neurons (Arancio et al. 1995). Importantly, cGMP produces activity-dependent LTP when it is injected into the presynaptic neuron, but not when it is injected into the postsynaptic neuron. Results from quantal analysis together with the observation that 8-Br-cGMP mimicks the NO effect on the frequency of spontaneous miniature EPSCs support the conclusion that cGMP-dependent potentiation of synaptic transmission is due to an increase in presynaptic transmitter release.

As described above, the cGKs are major receptors of cGMP in the CNS. The first pharmacological evidence for a role of cGK in hippocampal LTP came from a study of Zhuo and coworkers (1994). These authors observed an enhancement of LTP following a weak tetanic stimulation in the presence of cGK activators, and, conversely, a suppression of LTP in the presence of cGK inhibitors. However, due to the inadequate selectivity of the pharmacological tools, the functional relevance of individual cGK isoforms was not clear. Two pieces of evidence hint at a predominant function of cGKI. First, the expression level of cGKII in the hippocampus appears substantially lower than that of cGKI (El-Husseini et al. 1995; Kleppisch et al. 1999; De Vente et al. 2001). Second, in line with the idea of NO as a retrograde messenger, cGKI has been detected in presynaptic terminals colocalized with the vesicle protein synaptophysin (Arancio et al. 2001). The concept of presynaptic NO/cGMP signalling via cGKI was further supported by studies of NMDA receptor-dependent LTP between pairs of cultured hippocampal neurons (Arancio et al. 2001): infusion of purified cGKI protein into the presynaptic neuron facilitated LTP in response to a weak tetanus, while a cGK inhibitor injected into the presynaptic neuron blocked LTP. Both substances failed to affect LTP when applied postsynaptically, underlining that cGK functions as a presynaptic target of NO/cGMP (Arancio et al. 2001). Apparently at odds with these findings, conventional knockout mice lacking cGKI, cGKII, or both (double knockout mice) are capable of normal LTP in the Schaffer collateral pathway (Kleppisch et al. 1999; Table 2). This may be due to the fact that null mutants lack the cGKs during their entire ontogenesis and in all tissues, so that other phenotypes or functional compensation could occlude potential defects of LTP. Notably, the analysis of synaptic plasticity in the conventional cGKI knockout was limited to juvenile mice (up to $\sim$4 weeks of age) due to their premature death caused by cardiovascular and gastrointestinal defects (Pfeifer et al. 1998). Hippocampus-specific cGKI knockout ($cGKI^{hko}$) mice, which exhibit a normal life expectancy, also show normal hippocampal LTP in response to a single theta burst stimulation (Kleppisch et al. 2003). NO and cGMP have been shown to support late phase LTP (L-LTP) in the CA1 region (Lu et al. 1999; Lu and Hawkins 2002), a protein synthesis-dependent form of LTP that requires multiple strong tetanic stimulation of Schaffer collaterals (Frey et al. 1988; Barco et al. 2002). The following findings in $cGKI^{hko}$ mice support the view that cGKI may act as a downstream target of NO/cGMP in L-LTP (Kleppisch et al. 2003). First, LTP following multiple episodes of strong theta burst stimulation is decreased in adult $cGKI^{hko}$ mice ($\sim$12

weeks of age). Second, the protein synthesis inhibitor anisomycin has no effect on LTP in $cGKI^{hko}$ mice and decreases LTP in control mice to the level observed in the mutants. It remains unresolved whether this cGKI-dependent form of L-LTP is related to presynpatic and/or postsynaptic changes in protein synthesis.

Signal Transduction Mechanisms Downstream of cGK

What are the functionally relevant substrates and downstream effectors of cGK involved in LTP? Studies in cultured hippocampal neurons have shed light on potential presynaptic effector molecules of the NO/cGMP/cGK signalling cascade, such as the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Ninan and Arancio 2004), the VASP protein (Wang et al. 2005), and α-synuclein (Liu et al. 2004). Some regulators of G protein signalling (RGS proteins) and a few other synaptic proteins thought to modulate transmitter release are candidate substrates for cGKs in the brain (see Table 1). Differently from the VASP protein and RGS proteins, CaMKII and α-synuclein have not been shown to be phosphorylated directly by cGK.

The CaMKII is expressed in hippocampal neurons and plays an important role in synaptic plasticity. Primary interest was focussed on the postsynaptic function of the enzyme (for review see Lisman et al. 2002). A recent study with cultured hippocampal neurons has elucidated the presynaptic function of CaMKII in synaptic plasticity in the context of NO/cGMP/cGK signalling (Ninan and Arancio 2004). Presynaptic injection of the membrane-impermeable CaMKII inhibitor peptide 281-309 blocks long-lasting potentiation of synaptic transmission induced by combining a weak tetanus with brief superfusion of either the NO donor DEA/NO or the cGK activator 8-pCPT-cGMP. The CaMKII inhibitor peptide also suppresses the increase in the number of active presynaptic boutons occurring normally under these conditions. Thus, CaMKII appears to be essential for potentiation of synaptic transmission induced by retrograde NO/cGMP/cGK signalling. Additional findings show that the enzyme can also be activated independent of NO/cGMP/cGK signalling, e.g., by increased presynaptic Ca^{2+} entry, and that this is sufficient to induce long-lasting potentiation. However, few data support a function of CaMKII in transmitter release. A study in conditional knockout mice lacking the CaMKII α subunit selectively in presynaptic CA3 neurons of the Schaffer collateral pathway even suggests that the enzyme *inhibits* the activity-dependent increase of transmitter release (Hinds et al. 2003). Future studies are needed to improve our understanding of the functional relationship between NO/cGMP/cGK signalling, CaMKII activation, and presynaptic transmitter release.

Two additional putative downstream components of presynaptic NO/cGMP/cGK signalling in the course of synaptic plasticity are VASP and the small GTP binding protein RhoA (Wang et al. 2005). VASP has been detected in the brain and shown to serve as an endogenous substrate of cGK in hippocampal neurons (Hauser et al. 1999; Arancio et al. 2001). It is also well known that VASP and related proteins are crucial in the regulation of actin dynamics, which have been linked to synaptic plasticity (for review see Lisman 2003; Lisman and Raghavachari 2006).

Activity patterns leading to LTP are associated with actin filament reorganization in dendritic spines (Fukazawa et al. 2003), and actin filament reorganization is essential to enhance AMPA receptor-mediated transmission during LTP (for review see Lisman and Raghavachari 2006). How is this related to presynaptic NO/cGMP/cGK signalling? It has emerged that similar cytoskeletal changes occur in an activity-dependent manner at the presynaptic site and support a rapid increase in clusters of the vesicular proteins synaptophysin and synapsin I (Antonova et al. 2001; Wang et al. 2005). Evidence from studies in cultured hippocampal neurons further suggests that this presynaptic process can be induced through retrograde NO/cGMP/cGK signalling with cGK-dependent VASP phosphorylation as a critical step (Wang et al. 2005). Elements of the NO signalling cascade, including sGC, cGKI, and VASP, are expressed in presynaptic terminals, where cGKI colocalizes with synaptophysin. Finally, VASP is phosphorylated at the site preferentially phosphorylated by cGK (Ser-239) during synaptic potentiation (Arancio et al. 2001). Hence, cGK, via phosphorylation of VASP, appears to modulate presynaptic cytoskeletal structures linked to vesicle function and transmitter release. Unexpectedly, mice lacking VASP or related proteins do not show severe phenotypes related to synaptic plasticity (Hauser et al. 1999; Reinhard et al. 2001).

Another interesting substrate of cGK is the small GTP binding protein RhoA (Sauzeau et al. 2000; Ellerbroek et al. 2003). Its cGK-dependent phosphorylation, similar to that of VASP, may contribute to the regulation of actin filament dynamics in presynaptic terminals. The finding that the general inhibitor of Rho GTPases, *Clostridium difficile* toxin B, and the inhibitor of RhoA-dependent kinase Y27632 reduce glutamate-induced synaptic potentiation in cultured hippocampal neurons (Wang et al. 2005) would indeed support this idea. However, toxin B has no effect in the presence of the cGK activator 8p-CPT-cGMP, which argues against a role of RhoA as a downstream target of cGK.

It has been reported recently that α-synuclein, a protein linked to neurodegeneration, contributes to synaptic potentiation by increasing transmitter release and that activation of the NO signalling cascade may promote this effect (Liu et al. 2004). Long-lasting-potentiation of synaptic transmission between cultured hippocampal neurons is accompanied by an increase in the number of presynaptic α-synuclein clusters, while turning down the expression of α-synuclein expression (e.g., by antisense nucleotides or knockout techniques) blocks potentiation and the increase in the presynaptic number of functional boutons normally associated with it. Moreover, presynaptic injection of α-synuclein causes rapid and long-lasting enhancement of synaptic transmission and rescues potentiation in cultures from mice carrying a α-synuclein null mutation (Liu et al. 2004). A link between NO signalling and α-synuclein has been suggested based on findings that exogenous NO can also increase the number of α-synuclein clusters and that NOS inhibitors can block glutamate-induced increase in their density. In line with these data, α-synuclein may function as a downstream effector linking NO/cGMP/cGK signalling to increased transmitter release from presynaptic terminals. However, without doubt more experiments are needed to substantiate this view. The discussion above underlines that mechanisms of synaptic potentiation involve presynaptic changes. Retrograde

NO-dependent signalling mechanisms promote coordinate changes in the distribution of proteins in the presynaptic terminal which, ultimately, increase presynaptic transmitter release, e.g., by altering vesicle function. In addition, these mechanisms may support the alignment of presynaptic release sites with postsynaptic receptors to increase the efficacy of synaptic transmission.

G protein-mediated signalling pathways are also critically involved in the regulation of transmitter release, e.g., by a negative feedback via presynaptic autoreceptors (cf. Starke 1981). Besides the signal-receiving G protein-coupled receptor (GPCR), a linked heterotrimeric G protein and its downstream effectors, this signal transduction machinery includes modulatory RGS proteins. The more than 30 currently known RGS proteins, which have been classified into seven subfamilies (Hollinger and Hepler 2002), promote the termination of GPCR-mediated signalling by interacting with Gα–GTP and accelerating GTP hydrolysis. Studies in astrocytes and vascular myocytes indicate that members of subfamilies 2, 3, and 4 are cGK substrates (Pedram et al. 2000; Tang et al. 2003). Moreover, cGKI-dependent RGS2 phosphorylation might play a functional role for NO-dependent effects in the vasculature (Tang et al. 2003; Sun et al. 2005). Morphological and electrophysiological analyses support the idea that RGS2 is an important regulator of synaptic plasticity in hippocampal CA1 neurons (Ingi et al. 1998; Oliveira-Dos-Santos et al. 2000; Han et al. 2006). Given these findings, it is tempting to speculate that the NO/cGMP/cGK signalling cascade can modulate presynaptic transmitter release by phosphorylating the RGS2 protein.

Autoinhibition of transmitter release has been largely attributed to GPCR-dependent suppression of Ca^{2+} influx via presynaptic voltage-dependent Ca^{2+} channels (for review see Reid et al. 2003). The intracellular Ca^{2+} concentration and, hence, transmitter release are also controlled by Ca^{2+} discharged from intracellular stores (Fossier et al. 1999; Collin et al. 2005). Ryanodine-sensitive stores in presynaptic terminals have been reported to mobilize Ca^{2+} needed to induce LTD at Schaffer collateral/CA1 synapses (Reyes and Stanton 1996; Reyes-Harde et al. 1999a,b). Ca^{2+} release causing LTD can be induced by an endogenous activator of ryanodine receptors, cyclic ADP ribose (cADPR). This messenger is synthesized by ADP-ribosyl cyclase, which is expressed in various types of neurons (for review see Higashida et al. 2001). Importantly, the activity of the enzyme is regulated through the NO/cGMP/cGK signalling cascade (Galione et al. 1993; Galione 1994; Reyes-Harde et al. 1999a,b; Higashida et al. 2001). A rise in cGMP leads to an increase of the cADPR concentration in hippocampal slices. It is assumed that ADP-ribosyl cyclase is a presynaptic target of cGK. Its phosphorylation boosts cADPR synthesis, which leads to enhanced Ca^{2+} release from ryanodine-sensitive stores. This store-dependent presynaptic Ca^{2+} pool, ultimately, causes a decrease in transmitter release through unknown mechanisms. Collectively, the observations discussed above indicate that presynaptic target molecules with opposite functional impact on transmitter release, e.g., VASP (increase in release) and ADP ribosyl cyclase (decrease in release), may become activated through the NO/cGMP/cGK signalling cascade. Differential expression of these signal transduction elements may provide a mechanism enabling discrete recruitment of effectors that either facilitate or inhibit transmitter release.

cGK-Independent Presynaptic cGMP Signalling

In most of the aforementioned studies the involvement of cGKs as mediators of NO/cGMP effects has been analyzed by using pharmacological "cGK inhibitors." However, it is emerging that the presently available "cGK inhibitors" might be less specific than previously thought and, therefore, produce effects not related to cGK inhibition (Burkhardt et al. 2000; Gambaryan et al. 2004; Marshall et al. 2004; Feil R et al. 2005a). In the future, the analysis of cGK-deficient mouse models should help to dissect presynaptic NO/cGMP signalling without the use of uncertain pharmacological inhibitors. It is likely that not all effects of NO/cGMP are mediated by cGKs. Cytosolic cGMP can activate members of two ion channel families, CNG and HCN channels (Hofmann et al. 2005) leading to membrane depolarization and excitation. Moreover, both CNG and HCN channels have been reported to permit Ca^{2+} influx that may contribute to modulation of neuronal excitability and synaptic function (Koutalos and Yau 1996; Broillet and Firestein 1997; Parent et al. 1998; Yu et al. 2004). This section shortly summarizes evidence supporting the view that cGMP can indeed modulate transmitter release through these ion channels clustered in presynaptic terminals.

Recordings of membrane currents and capacitance changes from cones in the salamander retina have demonstrated that exocytosis can be triggered by a Ca^{2+} influx mediated through a cGMP-gated channel in the inner segment and in synaptic processes of cones (Rieke and Schwartz 1994). These CNG channels are clustered at high-density in cone terminals and can be activated by endogenous NO through stimulation of sGC-dependent cGMP synthesis (Savchenko et al. 1997). Furthermore, the sGC inhibitors, ODQ and LY-83583, suppress NO-induced glutamate release from cones. CNG channels are expressed in a variety of brain regions, including the hippocampus and, accordingly, have been suggested to serve as universal presynaptic transducers linking activity-dependent generation of NO and cGMP to enhanced transmitter release (Kingston et al. 1996; Bradley et al. 1997).

Garthwaite and co-workers (2006) have recently shown that rat optic nerve axons express various members of the HCN family and that HCN channels can serve as presynaptic transducers for the NO/cGMP signalling cascade. Actually, NO can cause a massive rise in cGMP levels sufficient to saturate the cyclic nucleotide binding site of HCN channels (Garthwaite 2005). The HCN channel blocker ZD7288 diminishes depolarization induced by the NO donor PAP/NO or by 8-Br-cGMP in optic nerve axons. NO-induced effects in this preparation are associated with an increase in cytosolic cGMP but not cAMP. There are a few reports suggesting that NO/cGMP can activate HCN channels also in other neurons (Pape and Mager 1992; Ingram and Williams 1996; Abudara et al. 2002; Pose et al. 2003; Kim et al. 2005). Within the HCN family, the HCN2 and HCN4 isoforms exhibit the most prominent regulation by cyclic nucleotides. The HCN2 subtype is expressed at considerable levels throughout the brain, while substantial expression of HCN4 channels is limited to a few areas, including the thalamus (Moosmang et al. 1999; Bender et al. 2001; Notomi and Shigemoto 2004). This hints at the HCN2 isoform as a possible general effector of NO signalling in the brain. As for CNG channels,

NO/cGMP-dependent activation of HCN channels leads to depolarization, increased excitability, and increased ability to conduct action potential bursts (for review see Robinson and Siegelbaum 2003).

3.2.2 NO as an Anterograde Messenger in Cerebellar LTD

LTD of the parallel fiber (PF)-Purkinje cell (PC) synapse in the cerebellum is a cellular model system, which has been suggested to underlie certain forms of motor learning. Induction of cerebellar LTD requires a postsynaptic kinase limb and a complementary phosphatase limb. The latter involves NMDA receptor-dependent generation of NO and postsynaptic cGMP synthesis followed by inhibition of protein phosphatases. Activation of both limbs promotes AMPA receptor phosphorylation and internalization resulting in LTD of synaptic transmission (Ito 2002). A postsynaptic source of NO is highly unlikely, since neither NMDA receptors nor nNOS were detected in postsynaptic PCs. Instead, and in contrast to the hippocampus, it is thought that NO acts in the cerebellum as an anterograde messenger that is produced in PF terminals or interneurons (Shin and Linden 2005) and then diffuses to the postsynaptic PC to induce LTD via a cGMP/cGKI-dependent mechanism. These postsynaptic actions will be discussed only briefly, because the focus of this review is presynaptic NO/cGMP signalling. A comprehensive review of the role of the NO/cGMP/cGKI cascade in cerebellar LTD and learning can be found elsewhere (Feil R et al. 2005b).

NO or cGMP present coincidently with a postsynaptic Ca^{2+} signal are sufficient to induce LTD (Lev-Ram et al. 1995, 1997a). LTD is blocked by inhibitors of NO synthase or guanylyl cyclase, can be restored by exogenous NO and cGMP, and is abolished in nNOS mutant mice (Hartell 1994; Boxall and Garthwaite 1996; Lev-Ram et al. 1997a,b). Among the multiple receptors for cGMP, the cGKI might serve a key function in cerebellar LTD. It is highly expressed in PCs (Hofmann and Sold 1972; Lohmann et al. 1981; Feil S et al. 2005), while cGKII has not been detected (El-Husseini et al. 1995; Geiselhoringer et al. 2004). A role for cGKI was shown by inhibitor studies (Hartell 1994; Lev-Ram et al. 1997a) and, more recently, by the analysis of PC-specific cGKI knockout mice (Feil R et al. 2003). These conditional mouse mutants are fully viable and show no generalized structural or physiological abnormalities in the cerebellum. However, they exhibit a near complete loss of cerebellar LTD. Interestingly, this LTD defect does not affect general motor performance of the mutants, but results in a rather specific defect in the adaptation of the vestibulo-ocular reflex (VOR), a simple form of motor learning (Feil R et al. 2003). Similar phenotypes, defective cerebellar LTD but relatively mild and specific defects in motor learning, have also been reported for other transgenic mouse models, including nNOS-deficient mice (Lev-Ram et al. 1997b) and mice overexpressing a protein kinase C inhibitor peptide selectively in PCs (De Zeeuw et al. 1998). On the other hand, it appears that the impaired general motor performance observed in many conventional knockout mouse models is usually associated with structural alterations of the cerebellum caused by the gene knockout (Ito 2001). Together, these

results unequivocally identify cGKI as a critical component in cerebellar LTD and, moreover, suggest that this type of synaptic plasticity is involved in specific forms of motor learning, such as VOR adaptation, rather than in general motor performance. This concept has been recently supported by the observation that pharmacological prevention of cerebellar LTD in rodents does not affect motor learning in several standard tests (Welsh et al. 2005). Indeed, there is little doubt that synaptic plasticity in other brain regions, such as the striatum, plays also an important role in motor learning (Dang et al. 2006).

How could activation of cGKI in PCs contribute to LTD and cerebellum-dependent learning? As described above, the induction of LTD is thought to depend on the balance between protein kinases and phosphatases that regulates the level of postsynaptic AMPA receptor phosphorylation and internalization. The NO/cGMP/cGKI cascade has been linked to the phosphatase limb (Ito 2002). LTD can be facilitated by inhibiting the protein phosphatase 1/2A (Ajima and Ito 1995). Intriguingly, the phosphorylated form of G-substrate, a well-characterized cGK substrate expressed in PCs, reduces the activity of protein phosphatase 1/2A (Hall et al. 1999). Decreased phosphatase activity should result in increased levels of AMPA receptor phosphorylation, which is assumed to facilitate its clathrin-mediated endocytotic removal from the postsynaptic membrane (Wang and Linden 2000; Chung et al. 2003). Thus, a likely signalling pathway is as follows: NO/cGMP-dependent activation of cGKI in PCs results in phosphorylation of G-substrate followed by inhibition of protein phosphatases 1/2A and increased phosphorylation and endocytosis of AMPA receptors. The removal of AMPA receptors from the postsynaptic site induces LTD associated with specific forms of motor learning.

4 Conclusion

Presynaptic NO/cGMP signalling modulates transmitter release and thereby supports synaptic plasticity important for learning and memory. Dysfunction of this signal transduction cascade may also contribute to various neurological disorders that involve an altered release of transmitters. Thus, components of the presynaptic NO/cGMP signalling pathway represent interesting targets for the development of new drugs improving cognition and drugs effective in neurodegenerative and psychiatric diseases, e.g., Morbus Alzheimer and schizophrenia. Indeed, initial promising findings in this direction have been reported for PDE inhibitors as well as for sGC stimulators (Chien et al. 2005; Feil R and Kemp-Harper 2006; Hebb and Robertson 2006; Menniti et al. 2006). Certainly, future therapeutic efforts will include further elements of NO/cGMP signalling involved in the modulation of transmitter release.

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