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Pharmacological manipulation of cGMP and NO/cGMP in CNS drug discovery

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Abstract

The development of small molecule modulators of NO/cGMP signaling for use in the CNS has lagged far behind the use of such clinical agents in the periphery, despite the central role played by NO/cGMP in learning and memory, and the substantial evidence that this signaling pathway is perturbed in neurodegenerative disorders, including Alzheimer's disease. The NO-chimeras, NMZ and Nitrosynapsin, have yielded beneficial and disease-modifying responses in multiple preclinical animal models, acting on GABAA and NMDA receptors, respectively, providing additional mechanisms of action relevant to synaptic and neuronal dysfunction. Several inhibitors of cGMP-specific phosphodiesterases (PDE) have replicated some of the actions of these NO-chimeras in the CNS. There is no evidence that nitrate tolerance is a phenomenon relevant to the CNS actions of NO-chimeras, and studies on nitroglycerin in the periphery continue to challenge the dogma of nitrate tolerance mechanisms. Hybrid nitrates have shown much promise in the periphery and CNS, but to date only one treatment has received FDA approval, for glaucoma. The potential for allosteric modulation of soluble guanylate cyclase (sGC) in brain disorders has not yet been fully explored nor exploited; whereas multiple applications of PDE inhibitors have been explored and many have stalled in clinical trials.

Keywords

Neurodegeneration; cGMP; Nitric oxide; NMDA receptor; GABA receptor; Migraine; Alzheimer's disease

1. Introduction

During the past three decades, nitric oxide (NO) has been recognized as one of the most versatile players in maintaining cellular homeostasis. In the CNS, NO is known to activate important physiological cascades involved in regulation of neuronal differentiation and synaptic plasticity [1]. In both neuronal and glial cells, cGMP-dependent protein kinase (PKG) is considered the primary NO effector by which NO mediates its downstream effects,

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Appendix A. Supplementary data

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and NO-sensitive soluble guanylyl cyclase (NO-GC or sGC) is the major physiological NO receptor in neurons [2]. The activation of this enzyme is achieved by conformational change upon the binding of NO to the prosthetic heme of sGC, forming a pentacoordinate ferrous-nitrosyl complex. The activated sGC rapidly converts GTP into the second messenger 3‘,5‘-cyclic GMP (cGMP), which, in turn, activates PKG. Through the activation of PKG, NO/cGMP signaling is involved in mediating CREB activation by phosphorylation of Ser133 via the MAPK-ERK cascade [3,4] and possibly in part by the CAMK pathway [3]. In addition, NO is involved in hippocampal and cortical LTP [5-8] via PKG mediated NMDA receptor activation [9,10]. Several lines of evidence also suggest that NO may act as a retrograde messenger in LTP or other forms of synaptic plasticity [11-14], modulating transmitter release under different conditions. Thus, although the major translational interest in NO/cGMP signaling has been in the periphery, there is substantial therapeutic opportunity to modulate NO/cGMP signaling in the CNS and brain. (see Table 1)

Activation of CREB by phosphorylation is necessary for memory formation and synaptic strengthening [15-17] and ultimately mediates LTP by acting upon downstream genes involved in synaptic formation and maintenance, and in neuronal plasticity and neurogenesis [18,19]. Mechanistically, it is now recognized that, in coordination with cAMP/PKA signaling, the activation of the cGMP/PKG pathway is a crucial event that contributes to synaptic plasticity and memory acquisition and consolidation through CREB-mediated changes in gene expression [20-24]. In the CSF of patients with Alzheimer's disease (AD), depressed cGMP, but not cAMP levels were observed [25]. Therefore, NO/cGMP has the potential to restore CREB signaling and thereby play a direct role in memory-related synaptic processes relevant to human disease [26-28]. Based on this knowledge, targeting synaptic dysfunction by reactivating the NO/cGMP/CREB pathway may be beneficial in multiple neurodegenerative disorders.

Since NO signaling has important functions in the brain, the etiology and progression of neurodegenerative and cognitive disorders may be associated with: dysfunction in NO production and impaired cGMP signaling [25,29]; and increased phosphodiesterase (PDE) expression levels [30] (reviewed in Ref. [31]). Specifically, aberrant CREB signaling has been linked to Alzheimer's disease (AD) pathology, reflected in mouse models of familial AD (FAD) [32,33]. Dysfunction in CREB signaling has also been implicated in other cognitive disorders such as Huntington's disease (HD) [34,35], suggesting a general role in cognitive dysfunction. Accordingly, targeting NO/cGMP/CREB signaling is now considered as a viable strategy for synaptic repair and neurogenesis, and potentially for disease modification in neurodegenerative disorders.

NO is produced by both neuronal (nNOS) and endothelial NO synthase (eNOS), the interplay between the two isoforms providing an exquisite, temporal, and spatial control of neuronal function [36,37]. In pathological conditions, the inducible isoform (iNOS) provides an important contribution to NO synthesis particularly following pro-inflammatory stimulation [38,39]. Evidence for altered expression of NOS isoforms has been reported in AD, and NO is recognized for its neuroprotective properties [40,41]. Interestingly, the deletion of the inducible *NOS2* gene in familial AD transgenic mice exacerbated AD-like pathology, neuronal loss, and behavioral impairments [42-44]. Additionally, chronic loss of

endothelial NO in late middle-aged (14–15 month old) eNOS$^{-/-}$ mice increased the amyloidogenic processing, microglial activation, and impaired performance in spatial memory tasks [45]. Therefore, through several mechanisms, chronic loss of endothelial NO, concomitant with downregulation of constitutive NOS and downstream NO/cGMP signaling, is implicated in cognitive decline during aging [45,46] and disease pathogenesis [47-50].

Importantly, activation of the NO/sGC/cGMP/CREB pathway through the application of either a NO donor, sGC potentiator, or cGMP analogue leads to re-establishment of normal levels of LTP and CREB phosphorylation [51]. Different classes of molecules targeting and enhancing components of NO/cGMP/CREB signaling to regulate synaptic plasticity represent promising disease-modifying approaches to treat cognitive dysfunction in neurodegenerative diseases. Although we will discuss nitrates, NO-donors, and alternative pharmacological agents later in this review (Fig. 1 and see Scheme 1 for structures), we begin by comparing two of the most exciting NO mimetic approaches to treatment of brain disorders including AD.

2. Nomethiazoles and nitromemantines: disease-modifying CNS therapeutics

Excitotoxicity and disrupted Ca^{2+} homeostasis have long been implicated in neurodegenerative disorders from ischemic stroke to AD [52]. The concept of pharmacologically restoring the balance between excitatory and inhibitory neurotransmission has been central to therapeutic strategies targeted at epilepsy and stroke [53-55]. In this paradigm, excitatory glutamate neurotransmission, primarily mediated at the NMDA receptor; and inhibitory neurotransmission, primarily mediated at the $GABA_A$ receptor, are primary targets. Small molecule inhibition of glutamate receptor-mediated currents and potentiation of GABAA receptor-mediated currents have led to a large number of anticonvulsant agents, some of which are used in epilepsy pharmacotherapy, and many of which have been explored as neuroprotective agents, for example, in stroke and AD [56-58].

Memantine has activity at a variety of neuroreceptors; however, it is best understood in its pharmacological use as an uncompetitive NMDA receptor antagonist, blocking NMDA receptor currents with IC_{50} ~2 μM, by binding in the open ion channel proximal to the Mg^{2+} binding site [59]. The inhibition of extrasynaptic NMDA receptor currents is thought to underlie the efficacy of memantine (Namenda) in moderate-to-severe AD [60]. In contrast to other NMDA receptor channel blockers, memantine does not cause psychotropic effects; however, in common with these channel blockers, memantine is an anticonvulsant [61]. Nitromemantines are nitrate derivatives of memantine [62], one being recently coined Nitrosynapsin (Fig. 2) [63].

Chlomethiazole (CMZ) is a non-benzodiazepine GABAA receptor potentiator and anticonvulsant. CMZ has been used clinically for treatment of seizures, epilepsy, alcoholic dementia and withdrawal, and is prescribed for anxiety and agitation in the elderly [64-70]. Under the brand name Zendra, CMZ was studied in Phase 3 clinical trials as a neuroprotective drug for use in ischemic stroke and spinal cord injury [71-78], and continues

to be recommended as a potential component of combination therapies for stroke [79]. CMZ potentiates the function of the inhibitory neurotransmitter GABA in the brain [80-82] and therefore attenuates the glutamate-induced excitotoxic cascade that leads to mitochondrial damage and neuronal loss [83-85]. CMZ is neuroprotective in animal models, attenuating levels of pro-inflammatory cytokines, including TNFα [80,82]. TNFα inhibition is itself a therapeutic goal for treatment of AD [86-88]. Selective pharmacological activation of $GABA_A$ receptors has been shown to provide neuroprotection against amyloid-β (Aβ) mediated toxicity [89-92], and a positive allosteric $GABA_A$ modulator is predicted to be of clinical utility in AD [92-94]. Nomethiazoles are nitrate analogues of CMZ [95], the most well described being GT-1061(NMZ) (Fig. 2).

Nitrosynapsin and NMZ would appear to be highly complementary in terms of mechanism of action, both adding NO mimetic activity to the complementary activity of the parent drug at NMDA receptors (NMDAR) and GABAA receptors (GABAAR), respectively (Fig. 2). Both NMZ and Nitrosynapsin have generated positive preclinical results that warrant further exploration in clinical trials. NMZ retains the activity of CMZ, both in $GABA_A$ receptor potentiation, anticonvulsant, and antiinflammatory properties; and has sedative actions, though less potent than CMZ [96-98]. NMZ and Nitrosynapsin are NO-chimeras, or hybrid nitrates, acting as NO mimetic small molecules; and we have shown that this approach adds procognitive and neuroprotective activity to diverse pharmacophore scaffolds: selective serotonin reuptake inhibitor, SSRI [99]; gamma-secretase modulator, GSM [100-102]; selective estrogen receptor modulator, SERM [103].

NMZ and related nitrates were able to rescue the AD-related impairment of LTP and restore CREB-related synaptic plasticity [96-98]; effects that were blocked by application of the sGC inhibitor 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (ODQ) [97], indicating a mechanism via NO/cGMP/CREB signaling. NMZ is neuroprotective *in vitro* in response to various insults including oxygen glucose deprivation (OGD), oligomeric Aβ, and glutamate toxicity; and restores synaptic function in hippocampal slices, in contrast to the parent molecule, CMZ [97,98]. Furthermore, NMZ reversed cholinergic cognitive deficits in rats, and demonstrated improvement of synaptic strengthening and cognition in 4 different mouse models of AD [97,104]. Remarkably, in the three FAD-Tg models (APP/PS1, 3xTg, and 5xFAD/h*APOE4*), NMZ treatment attenuated hallmark pathology, and the toxic forms of Aβ and tau [97].

Nitrosynapsin and nitromemantines also retain the properties of the parent drug, memantine, and are proposed to provide dual allosteric modulation of extrasynaptic NMDA receptors [105]. Nitromemantines showed superior neuroprotection and efficacy at lower doses than memantine. In addition, nitromemantines were able to reduce Aβ-induced Ca^{2+} excitotoxicity, synaptic depression, and tau phosphorylation, and in one FAD-Tg mouse model significantly restored synaptic markers of hippocampal function [106]. In the MEF2C haploinsufficiency mouse model of human autism, Nitrosynapsin displayed promising results when administered b.i.d., improving excitatory and inhibitory neuronal imbalance, synaptic markers, LTP, and autistic-like behavior [63].

Reports on Nitrosynapsin and NMZ show obvious commonalities; however, the NO-mimetic mechanism of action has been interpreted quite differently. The blockade of effects in hippocampal slices by ODQ in studies on NMZ and other nitrates and NO-donors has led to a focus on sGC activation by NMZ; whereas, ODQ has not been used in studies on Nitrosynapsin and nitromemantines, which have been interpreted to function exclusively via *S*-nitrosylation of specific cysteines located specifically in the extrasynaptic NMDA receptor. Pharmacokinetic data on nitromemantines have not been reported, nevertheless the dose used (2.5 mg/kg/day i.p.) [106] is comparably low to that of NMZ (1 mg/kg/day i.p. + 20 mg/kg/day p.o). An oral dose of NMZ (20 mg/kg delivered continuously over 24 h), representing a procognitive dose, resulted in brain concentrations of NMZ and its metabolite, HMZ, of 0.73 nM and 3.41 nM, respectively (not significantly different from plasma concentrations) in male C57BL/6 mice. These measurements indicate that the brain concentration of NMZ required for memory consolidation after amnestic insult is low or sub-nanomolar. Using the concentration of the HMZ metabolite as a surrogate for the concentration of NO released from NMZ, yields [NO] ≤ 3.4 nM. These data emphasize the high potency and promise of the NO mimetics, NMZ and Nitrosynapsin, and since both deliver disease-modifying effects in animal models after 3 months treatment, the phenomenon of nitrate tolerance is clearly not relevant to the action of these organic nitrates in the CNS.

3. Nitroglycerin and NO/cGMP: migraines are not headaches

The observation that nitroglycerin (glyceryl trinitrate, GTN) exposure and ensuing nitrate tolerance causes headaches was made over a century ago in dynamite factories [107-112]. More recently, GTN was shown to induce specific headaches and other symptoms of migraine attacks in migraineurs, which cannot be directly linked to the very rapid vasodilatory effects induced by GTN [113-118]. Debilitating migraine without aura affects 8% of Americans, with prevalence being strongly linked to age and biased threefold towards females. Migraine symptoms include: allodynia, a central pain sensitization following normally non-painful, often repetitive, stimulation; and hyperalgesia, an increased sensitivity to stimuli normally associated with mild pain. Mechanical allodynia/hyperalgesia caused by cutaneous application of Von Frey filaments to periorbital or plantar surfaces of mice mimics the symptoms of migraine without aura: the pain threshold (mechanical response) decreases as hyperalgesia increases [119,120]. In response to GTN administration, the threshold is lowered: more remarkably, when a single dose of GTN is administered every 2 days for 9 days, there is a chronification of hyperalgesia, which does not rebound to the normal, pretreatment threshold until approximately day 15, many days after GTN has been cleared from the system [121].

GTN must undergo reductive bioactivation in the body to yield NO, and it is commonly believed that depletion of the bioactivation apparatus and concomitant induction of oxidative stress cause the phenomenon of clinical nitrate tolerance, possibly through peroxynitrite formation [122-124]. Recognizing that: (i) both oxidative stress and peroxynitrite have been associated with the development of migraines [125,126]; (ii) doses of GTN used in migraine induction are relatively high; and (iii) there is debate over the relative importance of NO signaling via cGMP versus *S*-nitrosylation, we chose to define the mechanism of action

using pharmacological interventions. We demonstrated that direct activation of sGC by the novel sGC stimulator, VL- 102, replicated the pattern and chronification of migraine-associated hyperalgesia in mice, which was rescued by both acute and preventive clinical migraine treatments [120]. VL-102 treatment also increased the expression of migraine markers such as neuropeptide CGRP in trigeminal ganglia [120]. We also demonstrated that the sGC inhibitor, ODQ, completely blocked GTN induced acute and chronic hyperalgesia, establishing the role of the sGC-cGMP pathway in migraine. ODQ also effectively inhibited the established chronic migraine-associated pain in the absence of GTN or VL-102 [120]. Therefore, blocking this maladaptation by targeting of the sGC-cGMP pathway could represent an attractive approach to treat chronic migraine (Fig. 3). NOS inhibitors have been explored in the last two decades as potential treatment of migraine and headache. Specifically, the non-selective NOS inhibitor, NG-monomethyl-L-arginine hydrochloride (L-NMMA), provided pain relief compared to placebo in chronic tension-type headache in a clinical study [127]. However, selective inhibition of iNOS using GW274150 was ineffective in treating migraine in both prevention [128] and treatment [129] paradigms, suggesting that an upregulation of iNOS in experimental animal models, is unlikely to be a key mediator in migraine pathophysiology [130,131]. The potential of NOS inhibitors in migraine has recently been reviewed [132]. It should also be noted that sildenafil, a PDE5 inhibitor (*vide infra*), was shown to induce both acute and chronic hyperalgesia in mice [133].

Simplistically, the potentiation of allodynia/hyperalgesia by NO/cGMP (Fig. 3) is reminiscent of the potentiation of LTP by NO/cGMP in memory consolidation. However, the patient population susceptible to migraine and the population suffering age-related dementia are quite different, since migraine prevalence decreases after age 50 [134].

4. Hybrid nitrates: enhanced activity?

Nitromemantines and nomethiazoles are examples of hybrid nitrates, in which a parent drug has been modified to incorporate an organic nitrate moiety to deliver NO mimetic activity together with the actions of the parent drug or pharmacophore, thus enhancing activity [135,136]. Considerable research has been conducted over the past 20 years by the biotech industry, notably NitroMed, and NicOx, to develop hybrid nitrates; however, to date only latanoprostene bunod (NCX-116; Scheme 1) has received FDA approval, in this case for topical treatment of glaucoma. NCX-116 undergoes ester hydrolysis to yield hydroxybutyl nitrate, with the evidence for NO release being the measurement of increased ocular cGMP [137].

NCX-116 is typical hybrid nitrates prodrug that links an alkyl or benzyl nitrate to a parent drug by a labile ester linkage. In the majority of literature examples the parent drug is a nonsteroidal anti-inflammatory drug (NSAIDs) such as aspirin (acetylsalicylic acid, ASA) [138-140]. Hybrid NO-donating NSAIDs (NO-NSAIDs) were originally conceived to overcome NSAID gastrotoxicity by releasing NO and overcoming the effects of COX-1 inhibition [136,141-143]. Many preclinical studies focused on the promising spectrum of cancer chemo-preventive and chemotherapeutic activity reported in cell cultures and in animal models. Frequently, the “NO enhanced activity” of NO-NSAIDs was growth inhibition of cancer cell lines, multifold more potent than the parent NSAID [144,145].

However, in many cases, the enhanced activity could be replicated by analogues of NO-NSAIDS lacking any nitrate or NO-donating group [143,146-149]. Rigas, who pioneered the exploration of hybrid nitrates and NO-NSAIDs in cancer chemoprevention, has himself moved away from NO-NSAID nitrate esters to NSAID phosphate esters that have similarly enhanced activity relative to the parent NSAID, which is obviously unrelated to release of NO by the prodrug [150].

With respect to pharmacological use in the brain, two flurbiprofen containing NO-NSAIDs, HCT-1026 and NCX-2216, were compared and shown to have efficacy in a rat AD model [151]; one having been previously shown to attenuate neuroinflammation *in vivo* [152]. As in the case of NO-NSAIDs in cancer chemoprevention, these studies were stimulated by the epidemiology of NSAIDs associated with AD chemoprevention, and using the NO-NSAID modification to circumvent GI toxicity [153]. Several epidemiological studies have reported that long-term use of NSAIDs reduces AD risk [154], and many neuroin-flammatory contributors to AD pathology exist [155-157], and are considered therapeutic targets for AD [153,158,159]. HCT-1026 was shown to reverse scopolamine induced cognitive deficits in behavioral assays [102], and reduce Aβ load and microglial activation in an APP/PS1 transgenic mouse model [160].

Flurbiprofen is one of a subset of NSAIDs reported to reduce the levels of neurotoxic $A\beta_{42}$ in cell culture and FAD-Tg mice. Hence, these NSAIDs were referred to as selective amyloid lowering agents (SALAs) [161-164]. The $A\beta_{42}$ lowering activity of these SALAs required a mechanism of action associated with $A\beta_{42}$ production or clearance, which was ascribed to γ-secretase modulator (GSM) activity [165,166]. However, the potency of these SALA NSAIDs was an order of magnitude lower than contemporary designer GSMs; and, many alternative mechanisms relevant to $A\beta_{42}$ lowering have been identified for NSAIDs, including: activation of PPAR-γ and decreased BACE1 gene transcription [167]; inhibition of Rho-kinase activity [168]; and the direct interaction with APP [169].

To explore the SALA activity of NO-flurbiprofens, the mechanism of $A\beta_{1\text{-}42}$ lowering was explored in neuronal cells expressing human Aβ, showing problematic involvement of γ-secretase [101]. A library of flurbiprofen and NSAID analogues was tested for SALA activity and several flurbiprofen analogues were modified and studied as hybrid nitrates [170]. The hybrid nitrates possessed enhanced anti-inflammatory activity and reduced toxicity relative to the parent NSAIDs, and the SALA activity was attributed to the intact hybrid nitrate. A hybrid nitrate based upon CHF-5074/CSP-1103 was an efficacious SALA, which is of interest, because CHF-5074 was reported to reverse contextual memory deficits in an FAD-Tg mouse model, and in clinical trials, to reduce biomarkers of neuroinflammation in patients with mild cognitive impairment (MCI) [171] [172,173]. CHF-5074 continues to be studied in clinical trials [174,175]. That R-flurbiprofen failed to provide any benefit in either cognition or function in a large Phase 3 clinical trial [176,177], and the failure of trials on the related COX-2 inhibitors [178-181], are likely to dampen enthusiasm for pursuit of NSAIDs and NO-NSAIDs in clinical trials for AD.

NSAID NO-donating hybrids have also been reported that incorporate a diazeniumdiolate (NONOate) [182] or a furoxan [183]. Conversely, hybrid nitrates have been designed for

brain disorders, which incorporate a parent drug, other than an NSAID. These include hybrids of tacrine, a cholinesterase inhibitor not currently used clinically in AD [184-186], including one containing a ferulic acid linker [186], *in simile* with the linker incorporated in NCX-2216. Tacrine hybrid nitrates are potent inhibitors of acetylcholinesterase and butyrylcholinesterase, and observed to be effective in scopolamine-induced amnesia.

5. Organic nitrates

Glyceryl trinitrate (GTN; nitroglycerin) (Fig. 4) has been used in treatment of angina pectoris for almost 150 years. Nitrates are believed to elicit biological effects via reductive bioactivation to yield NO, stimulating the production of cGMP by sGC [187]. A small number of enzymes have been shown to mediate NO formation from GTN; while a larger number are capable of converting GTN (and organic nitrates in general) to inorganic nitrite (NO_2^-) (Fig. 4A). This reductive denitration is also mediated by proteins such as deoxyhemoglobin, transition metals, and certain, reactive small molecule thiols [188,189]. In the past decade, the biological activity of NO_2^- has been recognized to have physiological and potential therapeutic relevance, via reduction to NO in hypoxic tissues [190]. The venodilator activity of organic nitrates is characterized by bioactivation to NO in hypoxic tissues, suggesting that NO_2^- might be an intermediate in GTN bioactivation. The exact mechanism of nitrate bioactivation is not fully understood and likely involves more than one mechanism [191,192]. Since NO_2^- is often measured as a metabolite of NO and is used as a surrogate for NO, many studies have mistaken the direct production of NO^{2-} from nitrates as a measure of NO production. Organic nitrates are capable of direct oxidation of thiols such as cysteine, in a reaction that may yield NO or NO_2^-, and convert the thiol to a disulfide, sulfonate, or sulfinate. This published chemistry is largely overlooked in the biomedical literature, but has been shown to mediate the cGMP-independent activation of PKG1α (Fig. 4A), leading to the intriguing hypothesis that GTN nitrovasodilator activity, and indeed nitrate tolerance, are both mediated by oxidation of PKG1α by GTN, and not by bioactivation of GTN to NO [193].

The concept of using intramolecular reactions to provide models for enzyme-mediated reactions is well proven [194,195] and has been applied to modeling sulfhydryl-dependent nitrate reduction. Cysteine, at physiological pH, has low reactivity towards nitrates [196], therefore incorporating a thiol group adjacent to the nitrate group has been used to facilitate an intramolecular reaction, modeling nitrate bioactivation by cysteine-dependent enzymes/proteins. 1,2-Dinitrooxy-3-mercapto-propane (GT-150), at neutral pH, is a spontaneous NO donor, generating fluxes of NO comparable to NO-donor NONOates. GT-150 acts as an NO donor in activating sGC and inhibiting lipid peroxidation [197,198]. The disulfanyl nitrate (GT-715) is a prodrug of GT-150, and with related aryl disulfanyl dinitrates liberates NO after prodrug activation to give GT-150 [197]. The mechanistic data on GTN, GT-150, GT-715, and model compounds such as $tBuSNO_2$ [199], suggests possible sulfhydryl-dependent mechanisms for NO release (Fig. 4B), and several transition metal facilitated mechanisms. However, the major nitrogen product of organic nitrate metabolism is NO_2^-.

While the role of GTN and organic nitrates in the cardiovascular system has been extensively investigated, activity in the CNS has been more rarely pursued. Lipton's seminal

work on NMDA receptor function showed some nitrovasodilators to be neuroprotective in models of NMDA receptor-mediated excitotoxic neuronal injury [200]. GTN (25 mg/kg), administered i.v. during the 2 h ischemic period, reduced total infarct volume by 20% in a standard rat model of ischemic stroke [201]. Isosorbide dinitrate (ISDN) also provided neuroprotection in the rat stroke model, but required pretreatment before ischemia to demonstrate a neuroprotective effect [202]. The use of potent vasodilators such as GTN is contraindicated in stroke, because of risk of exacerbated hemorrhage, hence the GTN experiment required continuous co-administration of the pressor agent, phenylephrine. GT715, being a weaker vasodilator, with minimal effects on mean arterial pressure in the whole animal compared to GTN, was more potent and more effective as an activator of sGC in the brain, and more effective in elevating cGMP levels in hippocampal brain slices, compared to GTN [203]. GT-715 was shown to reduce infarct volume in the rat MCAO model of ischemic stroke, when administered 4 h after the ischemic event, to be a potent neuroprotective agent in several animal models, and to reverse cognitive deficits induced in animal behavioral models [40,203,204].

6. Furoxans (1,2,5-oxadiazole-N-Oxides)

Compounds containing furoxan (1,2,5-oxadiazole-N-oxide) or benzofuroxan heterocycles are thiol-bioactivated NO-mimetics that demonstrate bioactivation and release of NO [205]. The reactivity of furoxan rings can be manipulated via the incorporation of substituents adjacent to the furoxan ring system, potentially avoiding the cytotoxic effects of high NO concentrations. Lower concentrations of NO in the CNS have been shown to be neuroprotective [205], making furoxans an attractive candidate for CNS drug development, however, in the literature there are few attempts to utilize furoxans in the CNS. The furoxan **9a** (Scheme 1) was shown to restore LTP following an Aβ-induced synaptic deficit in mouse hippocampal slices [205], demonstrating the potential of furoxans to restore synaptic function. This neuroprotection could be blocked by the addition of ODQ, indicating that the NO/sGC/cGMP pathway was involved in the restoration of synaptic function [205].

7. Diazeniumdiolates

Diazeniumdiolates (NONOates) decompose spontaneously under physiological conditions to generate NO [206]. Modifications to the structure of NONOates influence the rate of NO generation [206-209]. DEA/NO [51,210,211] and DETA.NO [120,212,213] are amongst the most commonly used NO donating molecules. While NONOates have been studied as potential drug candidates for cardiovascular and oncologic applications, their use in the CNS has been largely associated with neurotoxic effects [211,214,215]. Garthwaite et al., concluded that prolonged exposure to NO was potentially toxic to both axons and glial cells in central white matter, whereas higher NO concentrations, imposed for shorter periods, exclusively damaged axons [216]. Paradoxically, other studies have demonstrated the neuroprotective effects of NONOate derived NO [217]. Lu et al. showed that the NONOate DETA/NO significantly improved the neurological functional outcomes of rats with traumatic brain injury (TBI) [213]. Fernández-Tomé et al. demonstrated DETA/NO-induced stimulation of sGC led to elevation of cGMP, which conferred protection against neuronal cell death induced by H_2O_2 [212]. SPER/NO was used to demonstrate that elevated

extracellular NO levels induced reversible axonal conduction deficits in guinea pig spinal cord neurons [218]. These effects were reversed on washout, at low concentration of SPER/NO (0.5 mM), but were only partially reversed at higher concentrations. PROLI/NO was used to demonstrate the effect of extracellular NO concentration on the permeability of the blood brain barrier (BBB) [219]: PROLI/NO selectively increased intratumoral uptake of radiotracers without significant changes in cerebral and tumor blood flow or arterial blood pressure, an effect blocked by the sGC inhibitor LY83583.

The greatest contribution to pharmacological manipulation of NO has been that of Keefer in his extensive development of diazeniumdiolates, designed to release NO at different rates and in some cases, with specific bioactivation by, for example, glutathione-S-transferase [220], or cytochrome P450 (CYP) [221]. A recent paper from a Merck research team presented a diazeniumdiolate designed to be bioactivated by CYP3A4 and to circumvent the development of tolerance associated with nitrates [222]. The observations in this paper on blood pressure lowering are important, because tolerance developed over 28 days, indicating that NO itself is associated with tolerance in the vascular system.

8. Sydnonimines

The sydnonimine, SIN-1, is often referred to as a source of peroxynitrite [223]. SIN-1 is believed to react with heme proteins and other electron acceptors in biological systems to produce NO. *In vivo*, SIN-1 will predominantly release NO rather than the superoxide. Molsidomine is a prodrug of SIN-1, metabolized in the liver to SIN-1 to induce slow release of NO [224,225]. Molsidomine crosses the BBB [226], and it has been demonstrated to increase its permeability [227]. Molsidomine (2–4 mg/kg) was found to be effective in restoring memory deficits in several animal models [228-233].

9. Nitroxyl-donors

Nitroxyl (HNO/NO^-) is the reduced form of nitric oxide [234]. HNO is more reactive towards thiol groups, leading to the formation of sulfonamides or disulfide bonds [234-236]. HNO donors have been explored in cardiovascular diseases, with one example in Phase 2 clinical trials [237,238]. In contrast to the most commonly used HNO donor, Angeli's salt (AS) [211,235], which spontaneously releases HNO at physiological pH and temperature, the clinical HNO donors are prodrugs with more controlled bioactivation characteristics. AS has been studied both *in vitro* [239-242] and *in vivo* [239,242] with regard to neuronal and brain physiology, once again showing neuroprotection [241]. *In simile* with the proposed mechanism of nitromemantines, a mechanism via HNO reaction with critical thiol groups of the NMDA receptor was proposed to block excessive Ca2+ influx and excitotoxicity [242].

10. S-nitrosothiols

S-Nitrosothiols are stable compounds at 37 °C and pH 7.4, however, in the presence of trace transition metal ions, or photolysis, release of NO can occur [243,244]. *S*-Nitrosocysteine (Cys-NO) is commonly used as a surrogate for NO *in vitro*, although since it readily undergoes transnitrosation reactions with protein-thiols, it behaves more as a nitrosonium

(NO^+) donor than an NO donor [245]. The glutathione *S*-nitroso adduct, GSNO, is a biologically relevant mediator of NO signaling, and has been proposed as a therapeutic approach to stroke, via stabilization of the HIF-1alpha/VEGF pathway [246]. Transnitrosation of cysteine residues in the NMDAR receptor inhibits the receptor activity [247], which is argued to be central to the mechanism of action of nitromemantines, although direct transnitrosation is not a chemically feasible reaction for nitrates. GSNO reductase (GSNOR), otherwise known as formaldehyde dehydrogenase, is a class III alcohol dehydrogenase that is argued to regulate cellular GSNO levels be degradation of GSNO. Interestingly, GSNOR was reported to be upregulated in the hippocampus of aging humans and mice; and 8–10 week old transgenic mice overexpressing neuronal GSNOR showed a significant deficit in contextual fear and Y-maze tasks [248]. In these studies, focused on protein *S*-nitrosylation, the involvement of NO/sGC/cGMP signaling was not explored.

11. sGC activators and stimulators

YC-1 was the first reported positive allosteric modulator of sGC, causing a 10–20-fold increase in activity of sGC over basal activity, and a left-shift of the response to NO [196,249,250]. YC-1 and subsequent small molecules derived from this benzylindazole scaffold have been referred to as NO-independent sGC stimulators; differentiated from sGC activators, by the dependence of activators on a reduced heme moiety [249,251]. Stimulators allosterically inhibit dissociation of NO from the heme group of sGC and although described as NO-independent, these agents potentiate activation of sGC by NO, which is the likely mechanism of action [252,253]. In brain slices, YC-1 activates cGMP/PKG signaling to enhance LTP [254]. In mice, YC-1 enhanced both learning and memory in Morris water maze and avoidance tasks in the presence or absence of scopolamine, effects antagonized by NOS and PKG inhibitors [255,256]. YC-1 was also reported to attenuate glutamate-induced excitotocity in a cGMP-dependent manner [257]. However, some activity of YC-1 could be attributed to off-target effects as a PDE inhibitor [258].

Optimization and scaffold-hopping from the benzylindazole lead structure has led to highly potent and selective sGC stimulators [259,260]. BAY 41–2272 and BAY 41–8543 potentiate the effects of NO up to 200-fold [261], and Riociguat (BAY 63–2521) has had success in multiple clinic trials in cardiopulmonary indications [262]. The effect of BAY 63–2521 was studied in atherosclerotic lesions in $APOE^{-/-}$ mice [263], and although ApoE is highly relevant to AD, no CNS studies have been reported. Further structural modifications have led to a new family of selective sGC stimulators with a 5-(isoxazol-3-yl)-1H-pyrazole scaffold, exemplified by IWP-051 [264]. A 5-(isoxazol-3-yl)-1H-pyrazole photoaffinity probe was used to identify the binding site of IWP-051 and displacement of the probe by BAY 41–2272 was used to confirm this as the allosteric site for sGC stimulator binding: a conserved cleft between two subdomains in the sGC heme domain [265]. As yet, no peer-reviewed publications have appeared on the activity of contemporary sGC stimulators/activators in the CNS. As described above, YC-1 and its analogue VL-102 replicate the actions of NO-donors in learning and memory, and in hyperalgesia associated with migraines [120].

12. sGC inhibitors

In contrast to research on sGC stimulators, few sGC inhibitors have been reported [266-268]. The mechanism of action of ODQ and NS2028 in inhibition of sGC requires binding to the ferrous-heme (FeII) in the β-subunit of the enzyme, yielding ferric-heme that cannot bind NO to achieve an activated state [268]. Furthermore, ODQ oxidation of sGC ferrous-heme may lead to conformational change and loss of ferric-heme from the β-subunit [269]. Unsurprisingly, there are examples of sGC inhibition by metal chelators and oxidants. The mechanism of action of ODQ predicts off-target actions at other ferrous-heme proteins and interactions with heme-proteins and enzymes such as hemoglobin and CYPs; however, these have not been extensively nor quantitatively explored. Feelisch et al. implicated CYPs as ODQ targets using the indirect evidence that ODQ inhibited nitrovasodilator bioactivation [270]. Similarly, 300 μM myoglobin attenuated the actions of both NO-donors and ODQ (50 μM) in cardiomyocytes, which might be explained by the ability of myoglobin to trap NO and ODQ [271]. Although the chemistry of NS2028 suggests a mechanism of action identical to ODQ [272], examples of divergent phenotypes exist [273]. Finally, ODQ does not completely replicate the effects of knockout of sGC *in vivo*, or *ex vivo* [274].

The universal use of ODQ to define the involvement of sGC in physiology and pathophysiology is demonstrated by over 2000 publications in PubMed. However, ODQ itself has potential therapeutic activity. ODQ has been reported to reverse basal ganglia dysfunction and akinesia in animal models of Parkinson's disease (PD), reversing the increased striatal cGMP levels and neuronal activity in the subthalamic nucleus in the 6-OHDA rat model of PD [266]. ODQ was also effective in improving deficits in forelimb akinesia induced by both 6-OHDA and MPTP [266].

13. cGMP-phosphodiesterase inhibitors

cGMP and cAMP are regulated by phosphodiesterase (PDE) enzymes: cAMP-specific PDE4, PDE7 and PDE8; cGMP-specific PDE5 and PDE9; and dual-substrate PDE1, PDE2 and PDE10 [275,276]. Inhibitors of at least seven PDEs families have been implicated in behavioral changes related to cognition, depression, and anxiety, namely those for PDE 1, 2, 4, 5, 9, 10, and 11 [277] (see Scheme 1). It has been reported that an increase in PDE expression and activity and a decrease in cGMP concentration occurs in the aging brain [278]; therefore brain bioavailable PDE inhibitors activating cGMP signaling are therapeutic targets for AD [23,46].

Research has targeted PDE5 inhibitors to elevate cGMP in the brain [279] [280]. Although the presence of PDE5 in neurons has been a matter of debate [281], aberrant expression of PDE5 in the temporal cortex of AD patients has been reported [25]. The clinical PDE5 inhibitors sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis) have been widely studied. Sildenafil has been shown to rescue cognitive impairment in FAD-Tg mouse models. In an APP/PS1 mouse model, sildenafil activated cGMP/CREB signaling to improve synaptic function and memory, and attenuate Aβ hallmark pathology [282]. In an aging mouse model, sildenafil was neuroprotective and reduced neurotoxic $A\beta_{1\text{-}42}$ [283]. Tadalafil has been proposed as a superior candidate for AD treatment, because of observed

brain bioavailability in primates; and both sildenafil and tadalafil were observed to restore behavior in the J20 mouse model (an FAD model with APP KM670/671NL, and APP V717F mutations). In this model, neither altered brain Aβ levels, nor improvement in tau pathology was reported [284-286]. The brain impenetrable PDE5 inhibitor, UK-343,664, improved memory in an object recognition task in rats with cognitive deficits induced by muscarinic or NMDA receptor blockade [287], implying a mechanism via peripheral actions of cGMP, in accord with high expression level of PDE5 in the smooth muscle of the meningeal arteries and blood vessels [284]. Research has been ongoing to optimize PDE5 inhibitors for use in the CNS. The potent PDE5 inhibitor, YF012403, rescued LTP and deficits in contextual memory in the APP/PS1 FAD mouse model [288], and further improvements to this PDE5 inhibitor have been reported and validated in a FAD-Tg (APP/PS1) mouse model [289].

Positive data on the combination of the pan-HDAC (histone deacetylase) inhibitor vorinostat with tadalafil, led to the design and testing of hybrid or chimeric PDE5 inhibitors that incorporate the metal chelating hydroxamate warhead standard to HDAC inhibitors [290]. CM- 414 is a relatively weak inhibitor of Class-I HDACs and of PDE5. In APP/PS1 and Tg2576 FAD-Tg mice treatment led to increased pCREB, rescued synaptic and neuronal function, and amelioration of Aβ and tau hallmark pathology [291]. Hydroxamate HDAC inhibitors have recently been shown to have off-target effects activating cell stress response pathways via the nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) and Hypoxia-inducible factor 1 (HIF-1), which can also contribute to efficacy in FAD models [292].

PDE9 has high affinity for cGMP [293], and is insensitive to pan- PDE inhibitors: 3-isobutyl-1-methyl-xanthine (IBMX), vinpocetine, EHNA, enoximone, rolipram, and dipyridamole [294]. Selective PDE9 inhibitors, BAY73–6691 and PF-04447943, improved memory and synaptic plasticity in older rats [295-297]. In the Tg2576 FAD mouse model, PF-04447943 improved memory, LTP, and hippocampal spine density; however, in phase 2 clinical trials, PF-04447943 did not improve cognition over placebo [298]. A novel PDE9 inhibitor, BI-409306, recently completed Phase 1 clinical trials, and entered Phase 2 trials in patients with prodromal or mild to moderate AD [299].

While there are no specific PDE1 inhibitors reported in the literature, many PDE5 inhibitors offer some inhibition of PDE1 [300]. The PDE5/1 dual inhibitor SCH 51866, was unsuccessful in attenuating progression in a R6/2 mouse model of HD [301].

The PDE2 inhibitor, BAY 60–7550, in age impaired rats, produced enhanced learning, memory acquisition, and memory consolidation [23,302-304]. PF-05180999, a pyrazolopyrimidine based PDE2 inhibitor [305], underwent phase I clinical trials for the treatment of schizophrenia and migraine, however, was terminated prematurely due to safety concerns [306]. In 2005 a patent for benzo-1,4-diazepin-2-one based PDE2 inhibitors (i.e. ND7001), for the treatment of various diseases of the central or peripheral nervous system was published [307].

The opium alkaloid, papaverine, a PDE10A inhibitor has been shown to inhibit conditioned avoidance responding in rats and mice and to inhibit PCP–and amphetamine-stimulated

locomotor activity in rats [308]. However, chronic administration of papaverine led to motor perturbations, mild cognitive disturbance and anxiety-like behavior [309]. PF-02545920 (Amaryllis) showed promise in HD, but a Phase II clinical trial failed [310]. The PDE10A inhibitor TAK-063 [311], produced dose-dependent antipsychotic-like effects in METH-induced hyperactivity and prepulse inhibition in rodents, in contrast to PF-02545920 [312]. The highly potent PDE10A inhibitor from Pfizer, “compound 96“, reversed MK-801 induced hyperactivity and conditioned avoidance response in rats [313].

14. Conclusions

As outlined in this review, there has been substantial activity over the past decade using selective PDE inhibitors to regulate cGMP in the CNS; however, this has not been matched by efforts to explore alternative therapeutic approaches to regulation of cGMP in the CNS. Striking progress has been made exploring sGC activators and stimulators in the periphery; and, as discussed in this review, extensive studies on hybrid nitrates have led to a single clinical drug for glaucoma.

NO/cGMP signal transduction is important for modulating synaptic transmission, plasticity, and memory in the brain, and this signaling pathway has been shown to be perturbed in many neurodegenerative disorders, making targeting of this pathway an attractive therapeutic strategy. The evidence strongly suggests that NO-donors and sGC modulators effectively regulate NO/cGMP signaling to elicit beneficial effects in many preclinical models of CNS disorders, in particular neurodegenerative diseases. The impressive preclinical data on NMZ and Nitrosynapsin, in particular, should support progress to clinical trials. sGC stimulators in the relatively few studies focused on the CNS have shown promise, often replicating the activity of NO-donors.

Despite significant advances in our understanding of NO and cGMP-dependent signaling mechanisms, important questions remain unsolved. Most importantly, gaps in our knowledge exist with the NO receptor, sGC, notably: the precise mechanism of sGC activation; the role of post-translational modification; modulation by allosteric ligands, such as ATP, GTP and endogenous sGC stimulators; and interactions with protein partners. Therefore, continued progress towards elucidating the structure and mechanism of sGC activation is needed to enable the development of novel drugs that target sGC to treat CNS disorders. For example, targeting the PDZ domain of the sGC α2 subunit using protein-protein interaction inhibitors would yield interesting chemical probes; however, this isoform of sGC, enriched in the brain, is poorly studied.

Modern drug discovery is dominated by development of small organic molecules that bind an individual protein target, with selectivity defined against specific off-target proteins. Increased affinity and potency is best achieved with multiple co-crystal structures of the protein target. The molecule preferably should be stable with a very small number of defined and measurable metabolites. Generally, a single mechanism of action is preferred to polypharmacy. The characteristics of NO-donors and sGC modulators are not compatible with some, or all, of these drug-like characteristics desired in modern drug discovery. NO-donors are by design and definition metabolically labile. In addition, the extensive literature

on protein modification caused directly or indirectly by NO, increases the potential targets of any NO-donor. Tolerance to GTN, or “nitrate tolerance” is also perceived to increase risk of development of NO-donors, although no evidence for such a phenomenon in the CNS has been revealed with agents such as NMZ and Nitrosynapsin. Furthermore, based upon doses of NMZ and Nitrosynapsin administered chronically in preclinical animal models, the effective potency of these agents is very high.

In neurodegenerative disorders, but especially in AD, the high rate of Phase 3 clinical trial failures of drugs singularly targeting one protein and one aspect of disease neuropathology has been unprecedented. Targeting NO-sGC signaling in the CNS will inherently modulate more than one aspect of the disease, and multiple preclinical studies with PDE inhibitors and NO-chimeras have demonstrated this approach to be disease-modifying with respect to hallmark neuropathology. The pursuit of these strategies in clinical trials is eagerly awaited.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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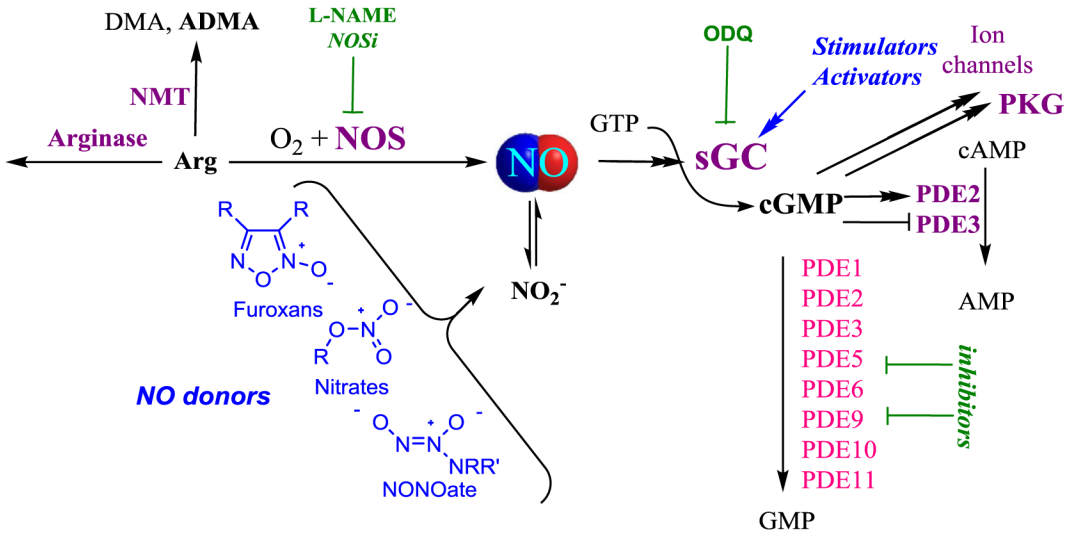


Fig. 1. Opportunities for pharmacological intervention in canonical NO/cGMP signaling.

Under physiological conditions, NO, endogenously synthesized by nitric oxide synthase (NOS), stimulates soluble guanylate cyclase (sGC), increasing cGMP production above basal levels. cGMP binds to and activates cGMP-dependent protein kinases (PKG) and certain ion channels (not shown). cGMP hydrolyzing phosphodiesterases (PDEs) temporally and spatially regulate cGMP levels. Exogenous NO donors spontaneously release NO, or require bioactivation to give NO and nitrite ion (NO_2^-); nitrite may provide an alternative source of NO after further reductive bioactivation. NOS inhibitors (NOSi), such as L-NAME, have been extensively explored and are not discussed in this review. sGC stimulators directly activate or potentiate the effects NO, enhancing cGMP production by the ferrous-heme enzyme at low levels of bioavailable NO. sGC activators activate the NO-unresponsive, heme-oxidized or heme-free enzyme. 1H-[1,2,4]oxadiazolo [4,3-a]quinoxalin-1-one (ODQ) is a heme-dependent sGC inhibitor. ADMA, asymetric dimethyl arginine; ATP, adenosine 5′-triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DMA, dimethyl arginine; GTP, guanosine 5′-triphosphate; NMT, *N*-methyl transferase.

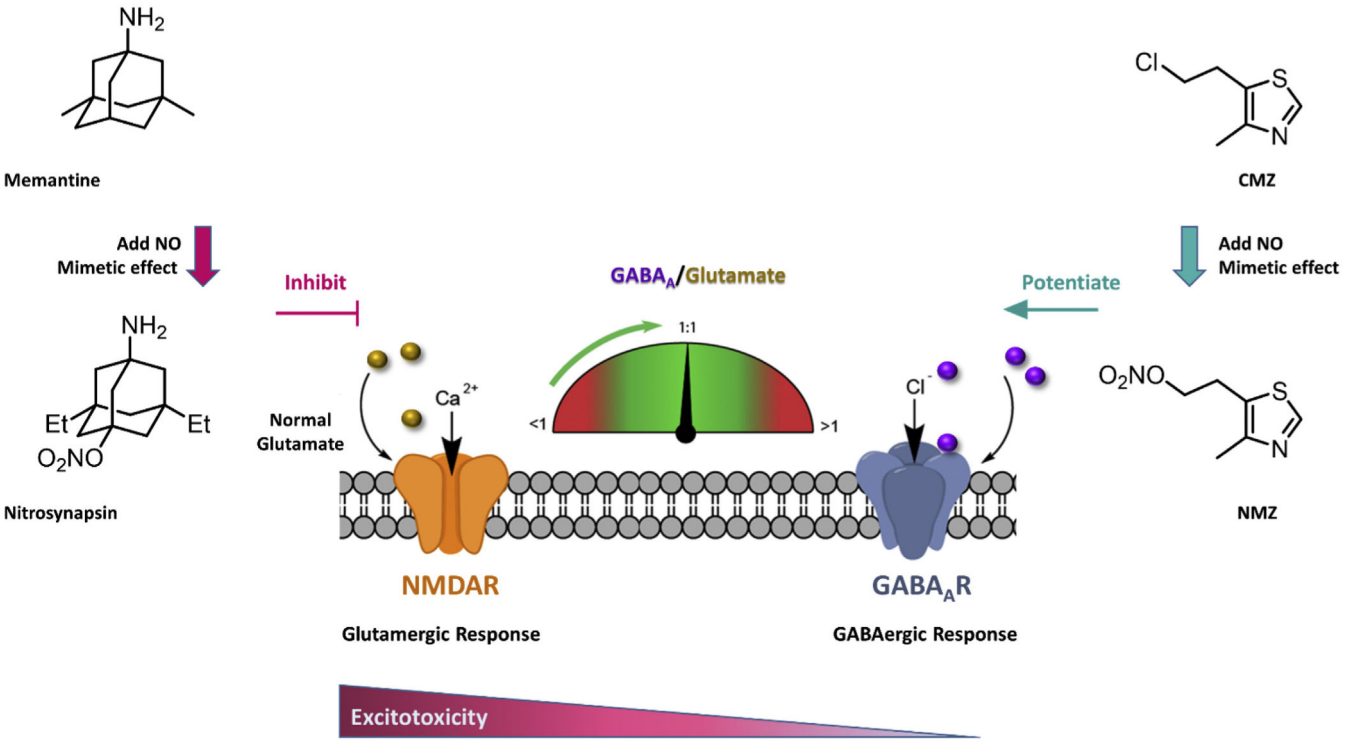


Fig. 2.

The complementary mechanisms of NMZ and Nitrosynapsin help restore the balance between excitatory and inhibitory neurotransmission.

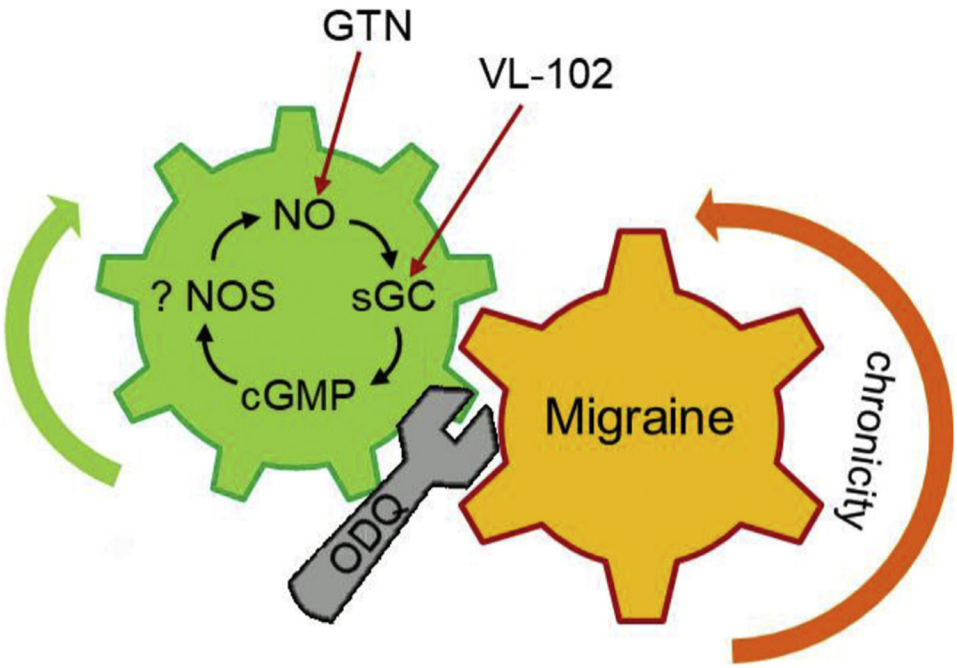


Fig. 3.

Pharmacological activation of NO/cGMP every other day for 9 days causes chronic hyperalgesia that does not revert to baseline until days 13–15[120]. Thus, days after clearance of exogenous NO/cGMP activators, endogenous NO/cGMP signaling is upregulated and potentiates chronicity. Blocking sGC using ODQ restores baseline response on day 10. This migraine model is responsive to various anti-migraine drugs in human use.

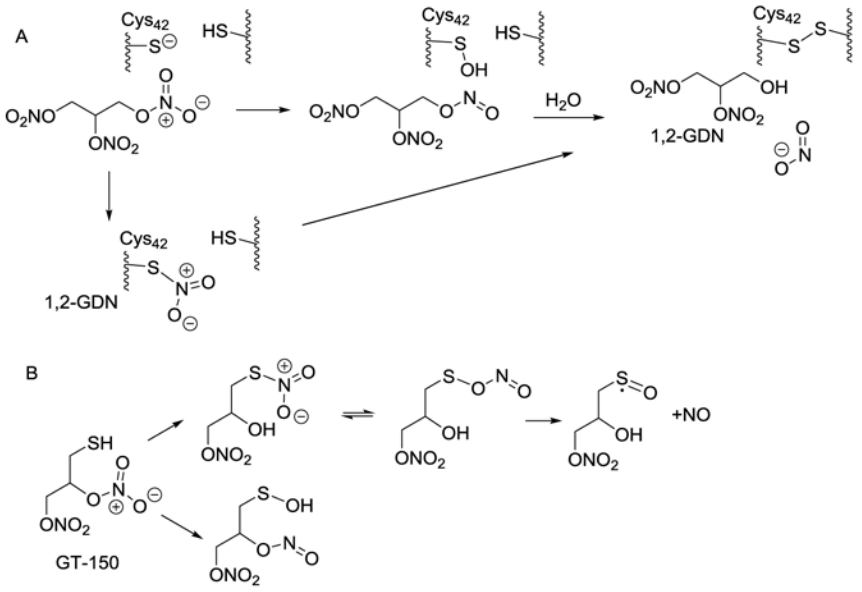
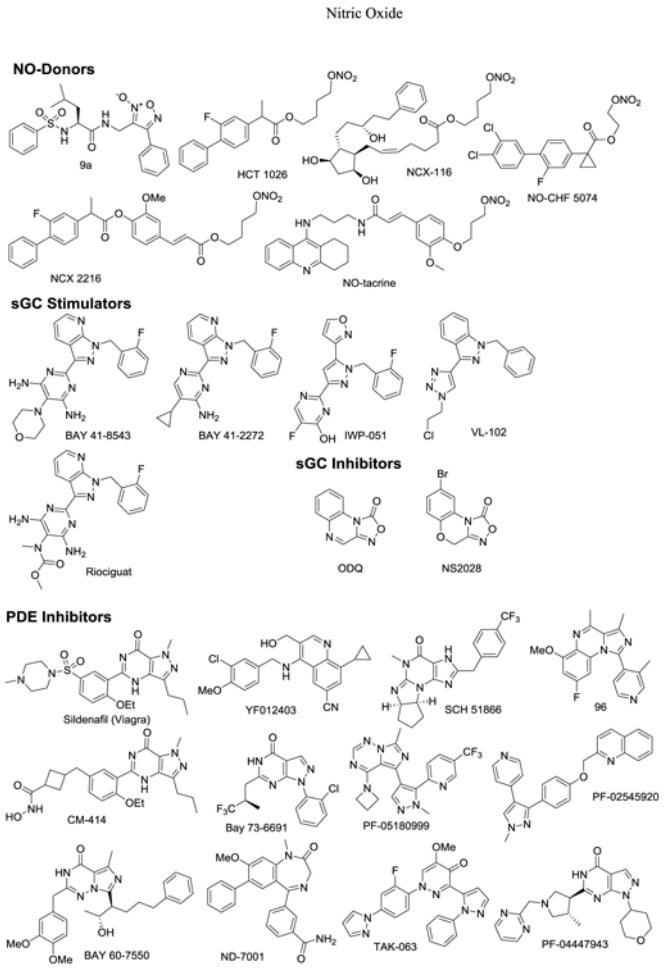


Fig. 4.

Oxidation of thiols by nitrates: **A)** Potential mechanism of PKG1α Cys42 oxidation (and NO-independent activation of PKG1α) by GTN. **B)** Mechanism of spontaneous release of NO from organic nitrate, GT-150.



Scheme 1.
Structures of pharmacological modulators of NO/cGMP signaling.

Nitric Oxide. Author manuscript; available in PMC 2020 November 06.

Table 1

Pharmacological modulators of NO/cGMP signaling in the literature that have been utilized in disorders of the CNS, including their pharmacological targets and mode of action.

	Drug	Pharmaceutical Mechanism	Disease Target	Observed Effect	Model	Clinical	Refs
NO-Donors	Nitrosynapsin	NO-Hybrid drug	AD/Autism	↓Aβ-induced synapse damage	3xTg	-	[106]
	NMZ (GT-1061)	NO-Hybrid drug	AD	LTP restoration in hippocampal slices, ↑ memory, and ↓ Aβ	APP/PS1, 3xTg, and 5xFAD/hAPOE4		[97,104]
	NCX-116	NO-NSAID	AD	-		FDA approved for Glaucoma	
	HCT-1026	NO-NSAID	AD	Reversed cognitive deficits induced by scopolamine, ↓ the $A\beta^{1-42}$-induced glia reaction, iNOS ↑ and p38MAPK activation	APP/PS1	-	[102,151] [152]
	NCX-2216	NO-NSAID	AD	↓$A\beta_{1-42}$-induced glia reaction, ↑ iNOS and ↑ p38MAPK		-	[151]
	CHF-5074	NO-NSAID	AD	Reversal of contextual memory deficit	Tg2576	Phase II [176,177]	[171] [172,173]
	9a	Furoxan	AD	↑ LTP in hippocampal slices treated with oligomeric Aβ		-	[205]
	Sin-1	NO-Donor	AD	↓ 7-nitroindazole induced learning deficit, scopolamine-induced amnesia and hypermotility in rats		-	[228-233]
HNO-Donors	Angeli's salt	HNO-Donor	AD	↑ cerebral ischemia-reperfusion injury	Experimental stroke model - C57BL6/J	-	[239,242]
sGC Stimulators	YC-1	sGC Stimulator	AD	LTP restoration in hippocampal slices, attenuated scopolamine-induced amnesia	adult Wistar rats	-	[255,256]
	VL-102	sGC Stimulator	Migraine	Acute and chronic mechanical cephalic and hind-paw allodynia	C57BL/6	-	[120]
sGC Inhibitors	ODQ	sGC Inhibitor	PD, Migraine	Improved deficits in forelimb akinesia induced by 6-OHDA and MPTP. ↓ acute and chronic hyperalgesia induced by nitroglycerin	6-OHDA and MPTP treated rats	-	[266]
NOS Inhibitors	L-NMMA	NOS inhibitor	Migraine	-		Phase II [127]	
PDE Inhibitors	Sildenafil	PDE5 Inhibitor	AD	↑ synaptic function, CREB phosphorylation, and memory. Reversed cognitive impairment of Tg2576 mice	APP/PS1, aging mouse model, J20, Tg2576	-	[282] [283]
	Tadalafil	PDE5 Inhibitor	AD	↑ performance of J20 mice in the Morris water maze test	J20	-	[284-286]
	UK-343664	PDE5 Inhibitor	AD	Ineffective at preventing MK-801-induced memory disruption, however, ↓ the memory impairment of scopolamine	MK-801	-	[287]
	YF012403	PDE5 Inhibitor	AD	Rescued the defects in LTP, synaptic, plasticity and memory	APP/PS1	-	[288] [289]

	Drug	Pharmaceutical Mechanism	Disease Target	Observed Effect	Model	Clinical	Refs
	CM-414	PDE5 Inhibitor	AD	LTP restoration in hippocampal slices, ↓ brain Aβ and tau phosphorylation, reversed a decrease in dendritic spine density on hippocampal neurons, and reversed cognitive deficits	APP/PS1, Tg2576		[291]
	BAY 73-6691	PDE9 Inhibitor	AD	↑ acquisition, consolidation, and retention of long-term memory (LTM) in a social recognition task ↓ a scoplamine-induced retention deficit in a passive avoidance task, and MK-801-induced short-term memory deficits.	FBNF1 rats		[295]
	PF-04447943	PDE9 Inhibitor	AD	LTP restoration in hippocampal slices, ↑ indicators of hippocampal synaptic plasticity and improved cognitive function	Tg2576	Phase II [298]	[296,297]
	BI-409306	PDE9 Inhibitor	AD, Schizophrenia	-		Phase II [299]	
	SCH-51866	PDE1/5 Inhibitor	HD	No effect in the R6/2 mouse model of HD	R6/2 HD		[301]
	BAY 60-7550	PDE2 Inhibitor	AD	↑ performance of rats in social and object recognition memory tasks, and reversed MK801-induced deficits	MK-801	-	[23,302-304]
	PF-05180999	PDE2 Inhibitor	Schizophrenia, Migraine	-		Phase I [306]	[305]
	ND7001	PDE2 Inhibitor	Various CNS	-			[307]
	Papaverine	PDE10A Inhibitor	Psychosis	↓ conditioned avoidance responding in rats and mice and ↓PCP induced hyperlocomotion	Male CD rats	-	[308] [309]
	PF-02545920	PDE10A Inhibitor	HD	-		Phase II [310]	
	TAK-063	PDE10A Inhibitor	Schizophrenia	↓ PCP induced hyperlocomotion	C57BL/6	-	[311]
	“compound 96”	PDE10A Inhibitor	Psychosis	Reversal of MK-801 induced hyperactivity and conditioned avoidance responding	MK-801	-	[313]

Nitric Oxide. Author manuscript; available in PMC 2020 November 06.