Chapter

Targeting the NO/cGMP/CREB Phosphorylation Signaling Pathway in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of senile dementia. Recently, scientists have put significant effort into exploring the molecular mechanisms involved in the pathological processes leading to the disease. A vast number of studies have focused on understanding the nitric oxide (NO) signaling pathway, which culminates with the phosphorylation of the transcription factor cAMP-responsive element-binding protein (CREB) through the increase of the second messenger cyclic guanosine monophosphate (cGMP) and activation of cGMP-dependent protein kinase. This book chapter provides an overview of the progress being made in modulating the hippocampal synaptic transmissions, which are critical for learning and memory, by targeting the different components of the NO/cGMP/CREB phosphorylation signaling pathway. Furthermore, a description of recent research on this pathway through the use of phosphodiesterase inhibitors is emphasized.

Keywords: Alzheimer's disease, nitric oxide, cyclic guanosine monophosphate, cGMP-dependent protein kinase, phosphodiesterases, phosphodiesterase inhibitors, cAMP-regulatory element-binding protein

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that involves cognitive impairment, such as loss of memory and reasoning and decline in mental ability. The AD brain is characterized by cell death and intra- and extracellular accumulation of amyloid-beta (Aβ) and tau proteins that form senile plaques and neurofibrillary tangles, respectively. Nowadays, medical treatments available on the market comprise two classes of drugs, acetylcholinesterase (AchE) inhibitors (i.e. donepezil, galantamine, and rivastigmine) and *N*-methyl-D-aspartate (NMDA) receptor antagonist (i.e. memantine). Based on the AD cholinergic hypothesis, acetylcholine-containing neurons project diffusely to the cortex and modulate cognitive processing. Damage of these projections has been associated with learning and memory impairment. Thus, AchE inhibitors block the acetylcholine-degrading enzyme consequently raising the levels of the acetylcholine neurotransmitter in the brain [1]. Differently from AchE inhibitors, memantine antagonizes the NMDA receptors, modulating dysfunctions in the glutamatergic

neurotransmission associated to AD. Even if glutamate NMDA receptors are essential for synaptic transmission [2], excessive stimulation of glutamatergic signaling results in excitotoxicity, a condition in which nerve cells are damaged leading to gradual loss of synaptic function and ultimate neuronal cell death. Thus, memantine reduces glutamate excitotoxicity effects [3]. Although these medications have been used for decades, they help with cognitive and behavioral symptoms but fail stopping or reversing the progression of the disease. Because of this limitation, the discovery of new therapeutic strategies for the treatment of AD has become a critical and shared goal to academia and industry.

Much progress has been made since Aβ and tau proteins were recognized as the major hallmarks of AD. With the aim of finding novel and more effective therapeutic targets, scientists have put enormous effort in understanding the molecular mechanisms causing the development and progression of the disease. Long-term potentiation (LTP) is the primary experimental model for investigating synaptic transmission and strength in the hippocampus [2]. Changes in synaptic strength, resulting from specific patterns of synaptic activity, define the biological process called synaptic plasticity, which is thought to contribute to learning and memory [4]. It is widely recognized that LTP at the CA3-CA1 synapse is triggered by postsynaptic NMDA receptors in response to high-frequency synaptic transmission. During the induction of LTP, the depolarization of the postsynaptic membrane, induced by tetanic stimulation, removes the Mg^{2+} block from the NMDA receptor channel that would otherwise occupy the lumen of the channel at resting membrane potential levels. At the same time, the neurotransmitter L-glutamate is released to activate NMDA receptors, upon which Ca^{2+} as well as Na^{+} ions enter the dendritic spine. Consequently, the elevation of intracellular Ca^{2+} triggers LTP. The implication of the NMDA receptors in the process of LTP has been proven by a variety of NMDA antagonists, such as MK-801 and 2-amino-5-phosphopentanoate that are able to prevent the induction of LTP [2, 5]. Likewise, Ca^{2+} chelators injected intracellularly block the induction of LTP as demonstrated by Lynch and coworkers [6]. Ca^{2+} triggers activation of second messenger cascades relevant to memory formation such as the NO cascade [7] on which we have focused in this chapter. LTP has been used as an electrophysiological model to investigate the correlation between memory impairment and synaptic strengthening in hippocampal slices of mice and to evaluate the effect of various compounds on synaptic transmission. Interestingly, $A\beta_{1-42}$ has been found to block LTP through the disruption of different molecular pathways, such as the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase (MAPK) as well as the metabotropic glutamate receptor type 5 [8], the extracellular signal-regulated kinase (ERK)-MAPK cascade [9], the cyclic adenosine monophosphate (cAMP)/cAMP-dependent-protein kinase/cAMP-regulatory element-binding protein (CREB) pathway [10], and the NO/cyclic guanosine monophosphate (cGMP)/CREB pathway [11].

This chapter provides an overview of the NO/cGMP/CREB phosphorylation signaling pathway and its role in learning and memory mechanisms during aging and neurodegenerative diseases. Several studies have demonstrated the association between NO, cGMP and CREB phosphorylation and synaptic plasticity [11–13]. The overall pathway includes the gaseous molecule NO, which is synthesized by the enzyme nitric oxide synthase (NOS) from arginine and induces an increase in the levels of second messenger cGMP by activating the enzyme soluble guanylyl cyclase (sGC). cGMP, consequently, activates the cGMP-dependent protein kinases (PKGs), a family of enzymes that is involved as transduction mediators in a number of cellular signaling systems. Lastly, PKGs phosphorylate the transcription factor CREB at its serine 166 (Ser-166), leading to the transcription of genes relevant

to learning and memory during LTP. Additionally, phosphodiesterase enzymes (PDEs) act on the pathway by hydrolyzing cGMP and therefore lowering the intracellular levels of the second messenger (**Figure 1**). CREB phosphorylation has been recognized as a crucial event during synaptic plasticity. Indeed, not only does the increase of phosphorylated CREB (pCREB) levels regulate the transcription of important neuronal genes, such as the gene for brain-derived neurotrophic factor (BDNF) [14] but also leads to the generation of new dendritic spines that represent morphological changes crucial in LTP in central neurons [15]. The fundamental role of the NO/cGMP/CREB signaling pathway in strengthening the synaptic transmissions has been explored by observing the effect of inhibiting the single components of the pathway on CREB phosphorylation [16–18]. On the contrary, the stimulation of this pathway has shown to restore the levels of pCREB and improve age-related learning and memory in *in vivo* tests [19, 20]. Importantly, in the presence of Aβ protein the NO/cGMP/CREB pathway is inhibited. In fact, findings have shown that the increase of pCREB during synaptic plasticity is blocked by Aβ in cultured cortical and hippocampal neurons [10, 21] as well as in mouse hippocampal slices [11]. Furthermore, to correlate the molecular mechanisms involved during LTP to the cognitive functions of learning and memory *in vivo*, the NO/cGMP/CREB cascade has been explored in a variety of animal models using different memory-related tasks [11, 19].

Due to the high relevance of the NO/cGMP/CREB pathway in aging and neurodegenerative disorders, a growing number of studies have focused on developing therapeutic strategies aimed at regulating this signaling pathway. The following sections summarize the single components of the pathway and their implication in neurodegenerative disorders, with particular emphasis on AD, as well as the therapeutic approaches advanced for targeting each of these pathway

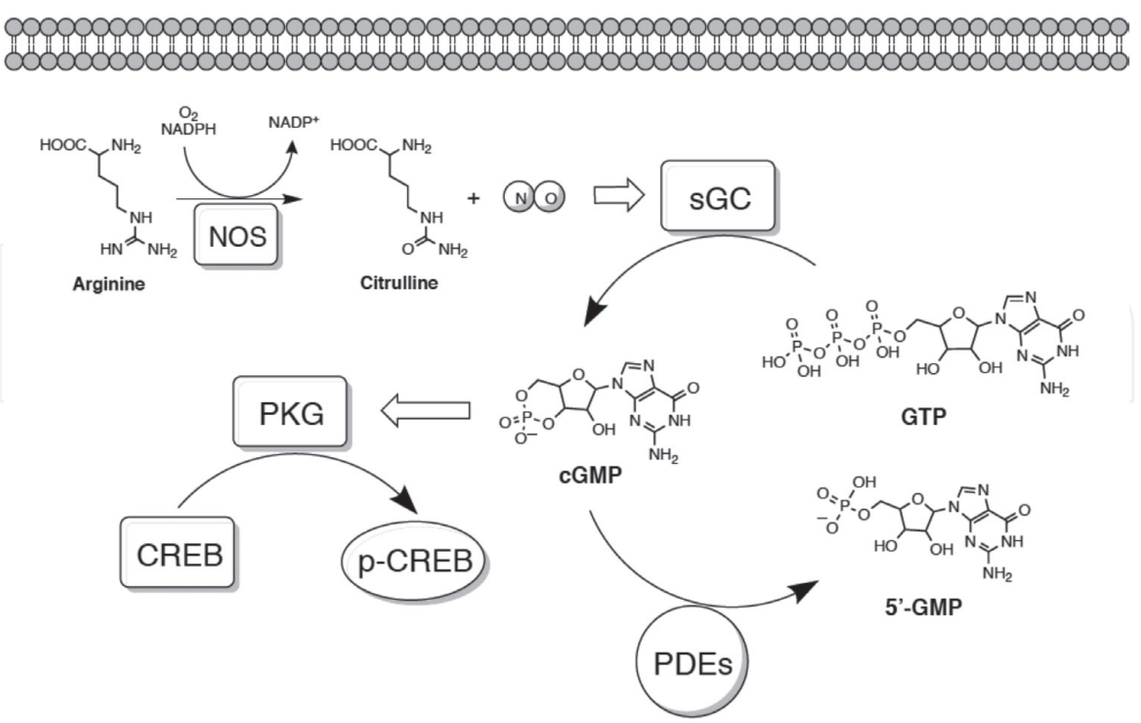

Figure 1.
NO/cGMP/CREB phosphorylation signaling pathway. Nitric oxide (NO) is produced during the conversion of arginine into citrulline by the enzyme nitric oxide synthase (NOS). NO activates soluble guanylyl cyclase (sGC), which stimulates cyclic guanosine monophosphate (cGMP) production from guanosine triphosphate (GTP). cGMP is degraded into 5'-GMP by the phosphodiesterases (PDEs). The increase of cGMP levels activates cGMP-dependent protein kinase (PKG), which induces phosphorylation of cAMP-responsive element binding (CREB).

effectors. Among them, inhibitors of PDEs have been the most studied and developed agents modulating the NO/cGMP/CREB pathway.

2. NO and NO donors

Nitric oxide, •N═O (abbreviated as NO) is a diatomic molecule with an unpaired electron in its outer orbit. NO is a highly diffusible gaseous molecule, which easily crosses cell membranes due to its high lipophilicity [22]. NO is involved in different metabolic pathways. NO can react with molecular oxygen (O_2) or superoxide anion ($O_2^{\bullet-}$) to produce nitrogen reactive species, including peroxynitrite [22–24]. At a cellular level, NO is a signaling molecule that regulates important processes such as cell differentiation and death, immune response, vascular tone and function, platelet aggregation, angiogenesis, and neurotransmission [25–27]. NO is predominantly produced along the biosynthetic process that converts the amino acid arginine into citrulline, in the presence of oxygen and cofactors (**Figure 1**). This metabolic pathway is catalyzed by nitric oxide synthases (NOS) [28, 29]. NOS occur in three isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). nNOS and eNOS are constitutively expressed and their activities are regulated by calcium-bound calmodulin. Both constitutive NOS isoforms respond immediately to increased levels of calcium and produce low levels of NO rapidly. The endothelial isoform is a key regulator of NO production in vascular endothelial cells and has a major role in the regulation of vascular tone and platelet aggregation. In the brain, the basal concentration of NO is mainly regulated by nNOS and, in a smaller extent, by eNOS [28]. iNOS is tightly bound to calmodulin and acts independently of calcium levels; its activity is induced by a number of cytokines, such as interferon-gamma and tumor necrosis factor. While several studies have associated iNOS with the development of disease such as atherosclerosis, others have proposed that the activity of iNOS in pathological conditions has a protective mechanism [28, 30]. The main receptor for NO is sGC. The binding of NO to the heme Fe center present in the catalytic site increases the enzymatic basal activity for conversion of guanosine 5′-triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) [31–33]. Most recently, Martin and coworkers have reported the mechanism of binding of NO to sGC [34]. By using isotopic ^{14}NO and ^{15}NO in rapid-freeze quench experiments, different intermediates of the complex NO–sGC were trapped and analyzed by electron paramagnetic resonance (EPR) spectroscopy. This study confirmed that NO binds to the distal side of heme Fe and then a second molecule of NO binds to the proximal side, leading to the release of NO from the distal side of the transient bis-NO-sGC complex. Also, a concerted mechanism in which the dissociation of the His-105 proximal ligand occurs simultaneously with the binding of the second NO has been unveiled [34].

In the central nervous system, NO plays crucial physiological functions as a neurotransmitter as well as regulator of the cGMP levels [35]. Specifically in the hippocampus, NO is involved in the processes of LTP, the persistent increase in synaptic strength upon high-frequency stimulation of a neuronal synapse [7, 36]. In the early 1990s, two studies demonstrated the link between NO and LTP concluding that the messenger NO was required in LTP [37, 38]. In electrophysiology experiments using hippocampal slices, NOS inhibitors such as *N*-nitro-L-arginine and N^G-methyl-L-arginine were found to block LTP when applied either extracellularly or intracellularly to the postsynaptic cell. At the same time, these findings have suggested that NOS is localized in the postsynaptic cell and that NO is a retrograde messenger that diffuses to the presynaptic terminal, leading to enhanced transmitter release [37, 38]. Extensive research has been done to unveil the effect of NO

on learning and memory in a range of behavioral tasks [39–44]. The use of NOS inhibitors has provided a means for exploring the link between NO and memory formation. Several studies have found that NOS inhibitors significantly decrease rodent performance in a number of memory and behavioral paradigms, such as the radial arm water maze and novel object recognition tests.

2.1 Nitric oxide and neurodegeneration/neuroprotection

Unbalance in the concentration of NO plays an important role in the development of neurodegenerative damage in AD [45]. For one thing, neural cell damage in the amygdala and hippocampus of AD brain has been associated with NO reactive species, which leads to the generation of oxidative stress [46]. Immunohistochemistry of hippocampal slices from AD human brains has specifically detected nitrotyrosine, a product of nitration of tyrosine residue by NO-reactive species peroxynitrite [47, 48]. In addition, neurotoxicity caused by excess of the excitatory neurotransmitter glutamate (defined as glutamatergic excitotoxicity) leads to the overexpression of NO through an increase in Ca^{2+} intraneuronal levels and activation of NOS. Yamauchi and colleagues measured the concentration of NO and survival of rat cultured cortical neurons upon treatments with NOS inhibitor (L-NMMA), NO donors (S-nitroso-N-acetyl-D,L-penicillamine-SNAP) and NMDA receptor agonist (glutamate) and antagonists (MK-801, ketamine) [49]. Application of glutamate to the cultured medium increased NO concentration, while both pretreatment with NMDA antagonists prevented glutamate-induced NO increase and neuronal death. L-NMMA prevented glutamate-induced NO production and neuronal death. The nitric oxide donor also caused neuronal death, and MK-801, ketamine and L-NMMA did not prevent SNAP-induced toxicity. This study demonstrated the link between changes of NO concentration and neuronal death [49].

Differently from above, other studies have reported the neuroprotective effects of NO. In cultures of differentiated cerebellar granule cells (CGCs), the inhibition of NO production for 3–4 days, obtained by using the NOS inhibitor L-NAME, resulted in progressive apoptotic death of CGCs. Cell death was rescued by adding to the culture medium slow-releasing NO donors, DETA-NONOate and Glyco-SNAP2 [50]. In addition, to confirm the essential role of cGMP in NO-mediated action, inhibition of sGC through the specific inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), replicated the pro-apoptotic effect of NOS inhibition [50]. The NO neuroprotection effect was evaluated in the NMDA-mediated neurotoxicity model, in which prolonged stimulation of NMDA receptors causes excitotoxic cell death [51, 52]. These studies indicate that NO protects against such excitotoxicity by S-nitrosylating the NMDA receptor subunits, thus reducing the intracellular Ca^{2+} influx that is responsible for neuronal death. S-nitrosylation is a post-translational modification that regulates the activity of important signaling effectors [53]. Prolonged nNOS stimulation during excitotoxicity generates superoxide radicals that react with NO to form peroxynitrite and S-nitrosylate the NMDA subunits, leading to a reduction of either the formation of peroxynitrite or Ca^{2+} influx and promoting neuronal survival [51, 52].

2.2 NO donors

Since the handling of NO is particularly challenging, drugs that release NO have been developed as a useful means of systemic nitric oxide delivery. Although several types of NO donors (e.g., nitrates, nitrites, metal-NO complexes, and furoxans) have been reported over the years, sodium nitroprusside and organic nitrates such

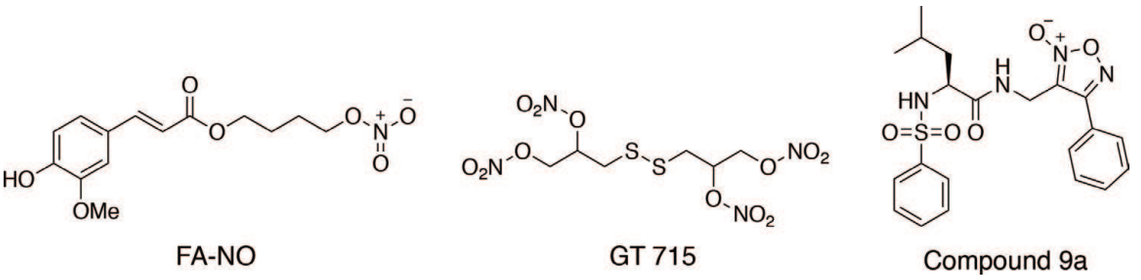

Figure 2.
Structures of NO-donors.

as glyceryl trinitrate, isosorbide mononitrate, and pentaerythritol tetranitrate have been used for many years as effective therapies for cardiovascular diseases [22].

New NO donors have been investigated for their neuroprotective activity, together with anti-inflammatory and antioxidant effects. A NO-releasing derivative of ferulic acid (NO-FA, also named NCX 2057) has been studied on lipopolysaccharide LPS-infused rats, an animal model of chronic neuroinflammation (**Figure 2**) [54]. Treatment with NO-FA for 14 days after LPS infusion produced a dose-dependent reduction in the level of microglial activation in the hippocampus and entorhinal cortex, demonstrating beneficial effects at a lower dose than that of the antioxidant ferulic acid. NO-FA or drug combining anti-inflammatory and antioxidant properties have been suggested as treatments that might significantly attenuate the processes driving the pathology associated with AD [54].

The importance of NO signaling in modulating synaptic plasticity and its correlation to enhanced learning and memory, as well as its neuroprotective effects, has also supported the development of NO donor for the treatment of neurodegeneration and AD. The nitrate ester GT 715 (**Figure 2**) is a NO mimetic drug that has shown to improve task acquisition in scopolamine-treated animals in a time and dose-dependent manner, activate hippocampal sGC and increase cGMP accumulation in hippocampal brain slices *in vitro* [55]. Most recently, Schiefer et al. have proposed another class of compounds, furoxans (1,2,5-oxadiazole-N-oxides) as neuroprotective and pro-cognitive agents [56]. Furoxan 9a (**Figure 2**) has exhibited neuroprotective effects in primary rat neuronal cell cultures subjected to oxygen glucose deprivation. Interestingly, neuroprotection was abolished by coincubation with the sGC inhibitor, ODQ, implicating the involvement of the NO/sGC cascade.

3. cGMP and PKG

3.1 cGMP and cGMP analogs

cGMP, as well as cAMP, is a cyclic nucleotide that functions as an intracellular second messenger in a variety of signal transduction cascades. cGMP is a hydrophilic molecule and therefore transmits signals within the cytosol, activating mainly protein kinases and ion channels. Synthesis of cGMP is regulated by sGC, which converts GTP into cGMP (**Figure 1**). However, the most important regulation of this cyclic nucleotide is seemingly not achieved by its synthesis but its breakdown in an inactive form, 5'-GMP. The enzymes responsible for this process are PDEs. Initially, the increase in cGMP has been associated with relaxation of tracheal, intestinal, and vascular smooth muscle [57, 58]. These studies led to the first proposed role of cGMP in the regulation of smooth muscle relaxation. In the hippocampus, cyclic nucleotides play an important role in the regulation of CREB phosphorylation through the

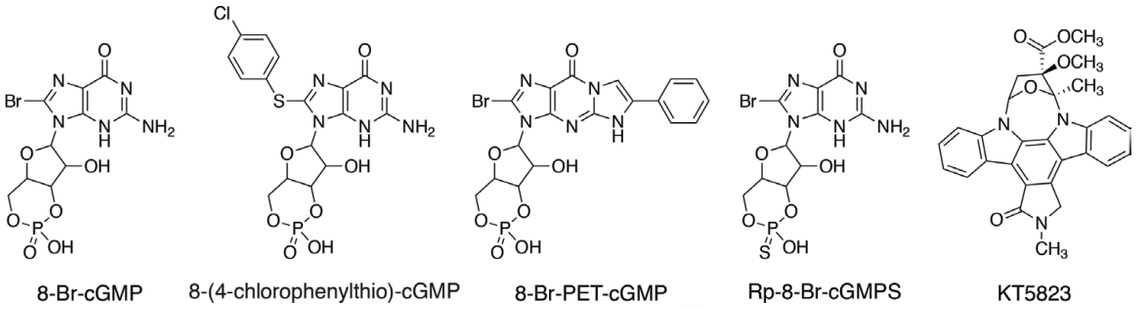

Figure 3.
Structures of cGMP analogs (8-Br-cGMP, 8-(4-chlorophenylthio)-cGMP, and 8-Br-PET-cGMP) and PKG inhibitors (Rp-8-Br-cGMPS and KT5823).

activation of cyclic nucleotide-dependent protein kinases. While some studies provide evidence that cAMP is critically involved in the processes of LTP and memory formation and consolidation [10, 59], others recognize cGMP as a key player as well [11, 59–61]. In an effort to identify biomarkers for AD, it was recently found that the levels of cGMP in the cerebrospinal fluid (CSF) of AD patients are reduced, supporting the relevance of cGMP in dementia and depression [62]. Molecules with molecular structures similar to cGMP (cGMP analogs) have been employed to provide insights into the mechanisms and functional role of the cGMP-dependent component of LTP [11, 60]. cGMP analogs mimic the endogenous cGMP, thus activating PKG. Examples of such molecules are 8-Br-cGMP, 8-(4-chlorophenylthio)-cGMP, and 8-Br-PET-cGMP (**Figure 3**) [11, 63, 64].

3.2 PKG and PKG inhibitors

Along the NO/cGMP/CREB cascade, cGMP activates the cGMP-dependent, serine/threonine protein kinase PKG that, in turn, phosphorylates CREB [16, 65]. Two families of PKG are known, PKG-I and PKG-II. PKG-I is found in various regions of nervous system, including the hippocampus, and its isoforms (PKG-Iα and β) are more commonly involved when NO mediates the cGMP signaling [65, 66]. Both PKGs exist as homodimers and each monomer contains a regulatory domain that is located in the more N-terminal portion of the protein and a catalytic domain that is located in the C-terminal portion. Two molecules of cGMP bind to the regulatory domain at an allosteric site. In the catalytic domain, there are two major subdomains: (1) a subdomain that binds Mg^{2+}/ATP and (2) a substrate-binding subdomain [66]. Arancio et al. studied the role of pre- and post-synaptic PKG in LTP [67]. To this end, inhibition of PKG by injecting a highly specific peptide (Gly-Arg-Thr-Gly-Arg-Arg-Asn-(D-Ala)lle-NH_2) into the presynaptic but not the postsynaptic neuron has been found to block LTP in rat hippocampal neurons. This work supported the hypothesis that PKG functions as a target of NO during the induction of LTP in the hippocampus [61] and possesses a predominant pre-synaptic role. Other inhibitors of PKG, such as Rp-8-Br-cGMPS and KT5823 (**Figure 3**), have revealed the importance of the cGMP/PKG pathway in learning and memory in either electrophysiological experiments or animal models [68, 69].

4. PDEs and PDE inhibitors

An important part of the signal transduction process is the rapid degradation of cGMP or cAMP by cyclic nucleotide PDEs. Specifically, PDEs catalyze the hydrolysis of the cyclic phosphate bond in cAMP and cGMP to generate the products

5'-AMP and 5'-GMP, respectively [70]. PDEs include 11 families of enzymes, namely PDE1–11, that show specificity for one only or both cyclic nucleotides. PDE1–3, 10, and 11 hydrolyze both cAMP and cGMP; PDE4, 7, and 8 are highly specific for cAMP while PDE5, 6, and 9 are cGMP-hydrolyzing enzymes. Each family of PDE comprises multiple isoforms, generated from 21 PDE genes by alternative splicing or transcription from distinct promoters [71]. PDEs exhibit tissue-specific differences in expression and functional characteristics. Some PDEs are expressed in a variety of tissues (PDE1, 2, 3, and 4) whereas others are more restricted, such as the PDE6 family that is mainly localized in retinal photoreceptors and regulates light perception [70, 72]. Importantly, splice variants of PDE1, 2, 4, 5, 7, 9, and 10 have been identified in different regions of human brain [72–79]. It is worth to mention that studies aimed at measuring the levels of PDEs in various tissues have provided inconsistent results. This could be due to differences in age, tissue species and specific technique involved for the measurement of either the mRNA or the protein level.

PDEs are homodimers with the exception of PDE1 and PDE6, which are typically heterotetramers under physiological conditions. The representative structure for most PDE monomers includes an NH_2-terminal regulatory domain (R domain) and a COOH-terminal catalytic domain (C domain). With exception of PDE4, which contains regulatory features also in the C domain, the R domain provides regulatory control through different types of domains, such as calcium-calmodulin binding (PDE1), GAF-A and -B (PDE2, 5, 6, 10, and 11), PAS (PDE8), and upstream conserved regulatory domain (PDE4) [70]. With regard to the C-terminal catalytic domain, approximately 270 amino acids are conserved, with a sequence identity of 35–50% among different PDE families. The catalytic site contains two major regions: (1) a region that interacts with the purine-like base in the nucleotides, and (2) a distinctive histidine-rich region that forms a binuclear metal-ion binding site where a catalytic hydroxide ion is generated and catalysis occurs. The first region is formed of hydrophobic, aromatic residues that engage with the purine-like ring through π-π stacking interactions. The presence of a conserved tyrosine residue (Tyr-612) in this pocket contributes to its hydrophobicity. The histidine-rich region contains two metal ions that play a critical role in the hydrolysis of the cyclic phosphate bond. Several studies have confirmed the zinc ion as the metal occupying the M-1 site in all the PDEs, while the second ion in the M-2 site is magnesium [80]. The whole catalytic machinery is made of two histidines, two aspartic acid residues, and water molecules coordinating the two metal ions. The nucleophile responsible for the attack to the phosphorous atom and breakage of the cyclic phosphate bond has been identified as a bridging hydroxide ion [80].

By hydrolyzing the second messenger cGMP and/or cAMP, PDEs are related to specific intracellular transduction signals, ranging from cell proliferation and apoptosis to smooth muscle contraction to neuronal functions [81]. In the brain, an important target of both cyclic nucleotides in neuronal signaling is the CREB protein. CREB is a transcription factor that regulates the gene expression of neurotransmitters, growth factors, and other signaling molecules [82]. Therefore, changes in PDEs expression and subsequently cyclic nucleotides alter the level of neuroprotection via CREB [83, 84]. For instance, an increase in PDE4 expression has been observed in primary cultures of cortical neurons of rats, while significant increase in PDE5 expression, together with a decrease in cGMP in the CSF, has been detected in the temporal cortex of AD patients [84, 85]. In animal studies, however, PDE4 activity was found to be reduced in the striatum and frontal cortex of aged monkey [86] and aged rat brains [87].

4.1 Phosphodiesterase inhibitors

The important role of cGMP (and cAMP) levels and CREB phosphorylation in learning and memory has led to a growing interest in exploring PDE inhibitors for the treatment of neurodegenerative disorders, especially AD. The inhibition of PDEs has been proposed as a novel therapeutic approach based on a number of evidence showing that several PDE inhibitors have exhibited remarkable effects in animal models related to AD when tested in different behavioral tests, including the Morris water maze, passive avoidance, and object recognition test (ORT). Recent studies have demonstrated that certain PDE inhibitors ameliorate memory impairment or enhance cognitive functions in rodent models. Examples include inhibitors of PDE2 (BAY60-7550, [88–90]), PDE3 (cilostazol, [91–93]), and PDE5 (sildenafil, [19, 94]). Herein, a list of well-studied cGMP-degrading PDE inhibitors that modulate the NO/cGMP/CREB signaling pathway and their effects on learning and memory is presented.

4.1.1 Phosphodiesterase 1 inhibitors

PDE1 is a Ca^{2+}/calmodulin-dependent PDE family comprising three isoforms, PDE1A, 1B, and 1C). PDE1 hydrolyzes both cGMP and cAMP and is highly distributed in the brain. PDE1 has been considered as a pharmacological target for the improvement of cognitive impairment in neurodegenerative disorders, such as AD, Parkinson's disease (PD), and schizophrenia.

A handful of selective PDE1 inhibitors have been discovered thus far [95] (**Figure 4**). Vinpocetine is a nutraceutical derivative of the alkaloid vincamine with moderate potency (PDE1, IC_{50} = 30 μM). In streptozotocin-induced rat model, chronic treatment with vinpocetine significantly improved learning and memory abilities in the Morris water maze and passive avoidance tests [95]. Intra-cellular therapies has identified a potent PDE1-inhibiting pyrazolopyrimidinone, namely ITI-214 with much higher potency than vinpocetine against PDE1B specifically (IC_{50} = 0.058 nM) [96]. ITI-214 has shown to improve memory performance of rats in the novel object recognition test at a dose of 3 mg/kg, i.p. [97]. Most recently, a thienotriazolopyrimidinone PDE1 inhibitor, DNS-0056 (PDE1B, IC_{50} = 0.026 μM) has been reported. In a rat model of recognition memory, DNS-0056 (0.3 mg/kg, p.o.) notably increased long-term memory, without altering exploratory behavior [98]. However, at odds with these findings, administration of the ICOS PDE1 inhibitor IC354 (IC_{50} against PDE1 of 80 nM; ratio of IC_{50} value for the next most sensitive PDE to IC_{50} value for PDE1 equal to 127) failed to rescue the defect in LTP in a mouse model of amyloid elevation [19].

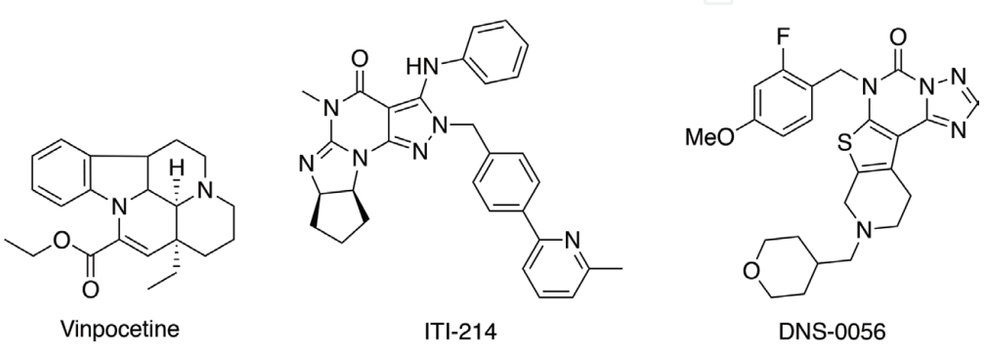

Figure 4.
Structures of PDE1 inhibitors.

4.1.2 Phosphodiesterase 2 inhibitors

PDE2 is found in the brain, where it hydrolyzes both cAMP and cGMP [72, 99]. PDE2A is the only isoform recognized in several brain regions [72]. In most peripheral tissues, except the spleen, PDE2 levels are relatively low. Due to this tissue distribution, PDE2 inhibitors exhibit less cardiovascular side effects than other PDE inhibitors. Thus, PDE2 inhibitors have been considered attractive therapeutic agents against cognitive disorders.

BAY-60-7550 is a highly selective PDE2A inhibitor developed by Bayer. It shows a high potency (IC_{50} = 4.7 nM) and selectivity versus the other PDEs. BAY-60-7550 has been used in numerous behavioral tasks and animal models for testing learning and memory [88–90]. A study by Boess and coworkers has explored the effect of BAY-60-7550 on the synaptic plasticity as well as memory in rats. BAY-60-7550 at a concentration of 100 nM was able to increase hippocampal LTP. In the ORT, BAY-60-7550 improved the recognition performance of adult rats at a dose of 1–3 mg/kg. Interestingly, similar doses of the PDE2 inhibitor reversed the memory impairment caused by an NMDA antagonist (MK-801) in a T-maze spontaneous alteration task [88]. Additionally, BAY-60-7550 has been tested in scopolamine-induced and MK-801-induced memory deficit mouse models. A dose of 1–3 mg/kg given by oral gavage rescued the memory defects in the ORT [90]. Recently, young mice have shown a dose-dependent memory enhancement upon treatment with BAY-60-7550 (0–6 mg/kg, i.c.v.) in the ORT. In this recent study, researchers have proven that the enhancement of memory in the ORT following PDE2 inhibition during early consolidation is mediated via NOS/cGMP/PKG pathway by using a NOS inhibitor and an sCG inhibitor. In support of these results, an increase in CREB phosphorylation was observed as well [89].

Two PDE2 inhibitors sharing the same chemical scaffold were developed by Pfizer, PF-05085727 and PF-05180999 (also called PF-999) [100, 101]. PF-05085727 showed an IC_{50} of 2.0 nM and selectivity of up to 4000-fold over other PDEs was identified by Pfizer as well [101]. PF-05085727 increased the level of cGMP in rodent brain regions expressing the highest levels of the PDE2A enzyme. PF-05085727 (0.032–1 mg/kg, s.c.) significantly attenuated memory impairments induced by ketamine in rats subjected to the radial arm maze task. Additional behavioral experiments using the MK-801-induced memory deficit mouse model revealed that the PDE2 inhibitor is able to reverse the MK-801-induced local field potential disruption. This study represents another evidence of the potential use of selective PDE2A inhibitors in treating neurological and neuropsychiatric disorders [101].

Likewise, PF-05180999 showed remarkable inhibitory activity (IC_{50} = 2.3 nM) and selectivity over other PDEs. PF-05180999 was found to increase the level of cGMP in the CSF of rats, attenuate ketamine-induced memory deficits, and reverse spatial learning and memory in scopolamine-induced models [100]. In 2015, a study that explored the primarily presynaptic mechanism of PDE2A inhibition was also performed by using PF-05180999 [102]. These results showed that the inhibition of PDE2 might be involved in short-term synaptic plasticity by modulating the hydrolysis of cAMP to accommodate changes in cGMP levels associated with presynaptic short-term plasticity.

In 2017 Takeda disclosed the discovery of compound 20 as a novel PDE2 inhibitor [103]. Compound 20 increased cGMP levels in the frontal cortex, hippocampus and striatum of rats in a dose-dependent manner (1–10 mg/kg), while no increase of cAMP was observed in the same rat brain regions. Also, compound 20 was effective on MK-801-induced episodic memory deficits in a passive avoidance task in rat. The ability of compound 20 to reverse deficits in episodic memory produced by

MK-801, suggests its potential for the treatment of cognitive deficits seen in a range of psychiatric disorders with impaired glutamatergic neurotransmission [103].

Finally, through structure-based drug design approaches and molecular modeling, DNS-8254 has been proposed as a potent and selective PDE2 inhibitor with good brain-penetrant properties. DNS-8254 was evaluated in a test of rat NOR, and improved visual recognition memory was observed 24 h after training [104].

4.1.3 Phosphodiesterase 3 inhibitors

Similar to PDE1 and PDE2, PDE3 is another subfamily responsible for hydrolyzing both cAMP and cGMP and has two isoforms: PDE3A and PDE3B. In the brain, the expression of PDE3A and PDE3B is relatively low and is mainly in the cerebellum [72]. Cilostazol is a PDE3 inhibitor clinically used as an antiplatelet drug (**Figure 5**) [105]. As cilostazol increases the cerebral blood flow [106], this drug has been explored for its effectiveness in treating the type of dementia associated with a decrease and stoppage of the cerebral blood flow in brain blood vessels. A study conducted by Hiramatsu and coworkers has revealed that cilostazol prevents $A\beta_{25–35}$-induced memory impairment and oxidative stress in mice [93]. The effect of cilostazol was examined on mice with memory impairment induced by treatment with $A\beta_{25–35}$. Two behavioral testes were performed: the Y-maze and the step-down type passive avoidance tests. Repeated administration of cilostazol (30 and 100 mg/kg, p.o.) significantly and dose dependently attenuated the impairment of spontaneous alternation the shortened step-down latency induced by $A\beta_{25–35}$. Cilostazol prevented the accumulation of lipid peroxide (malondialdehyde—MDA levels) in the frontal cortex and hippocampus in the early period after $A\beta_{25–35}$ treatment, as MDA levels in both regions returned to control levels by 7 days after $A\beta_{25–35}$ injection [93]. Interestingly, an *in vitro* study using N2a cells stably expressing human amyloid precursor protein Swedish mutation (N2aSwe) showed that cilostazol decreased Aβ and tau phosphorylation levels in the conditioned medium and cell lysates [92]. Cilostazol (10–20 mg/kg) also reduced Aβ accumulation and tau phosphorylation levels in $A\beta_{25–35}$-injected mice when given orally 2 weeks before and daily for 4 weeks after $A\beta_{25–35}$ injection. The brain level of apolipoprotein E (ApoE), a protein associated with Alzheimer's neurofibrillary tangles and Aβ aggregation, was decreased. These results were consistent with the reduction of Aβ aggregation observed in N2aSwe cells and improvement of spatial learning and memory detected in $A\beta_{25–35}$-injected mice [92]. While the aforementioned studies assessed cilostazol for its cognitive enhancing properties, Yanai et al. were interested in understanding the effect of this PDE3 inhibitor on memory function [91]. To this end, the effect of cilostazol on wild-type C57BL/6J mice as they perform various behavioral tasks was examined. Importantly, cilostazol improved long-term memory, which was correlated with an increase in phosphorylated CREB-positive cells in the dentate gyrus.

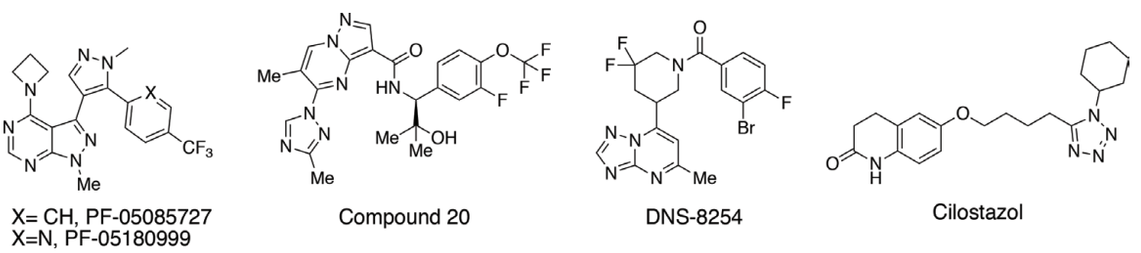

Figure 5.
Structures of PDE2 (PF-05085727, PF-05180999, and compound 20, and DNS-8254) and PDE3 (cilostazol) inhibitors.

4.1.4 Phosphodiesterase 5 inhibitors

PDE5 specifically hydrolyzes cGMP and has one isoform, PDE5A. While according to Lakics and colleagues the expression of PDE5A in the brain is relatively low [72], others have proved that PDE5 protein is significantly present in human brain as well as neurons and the low expression previously detected was due to methodological inaccuracies [79]. PDE5 inhibitors have been proposed as novel therapeutics for the treatment of AD and other neurological disorders (**Figure 6**). Sildenafil, vardenafil, and tadalafil are PDE5 inhibitors approved by the FDA for the treatment of erectile dysfunction and pulmonary arterial hypertension. Both sildenafil and tadalafil have been explored for their effects in neurodegenerative disorders. Sildenafil has shown an IC_{50} of 2.2 nM against PDE5A and selectivity across other PDEs, except for PDE1 and PDE6. The ability of sildenafil to cross the blood-brain barrier (BBB) together with its lower toxicity, indicate that this drug is a suitable candidate in treating neurodegenerative processes related to low levels of cGMP and down-regulation of the NO/cGMP/CREB signaling pathway. Sildenafil produced an immediate and long-lasting improvement of synaptic function, CREB phosphorylation, and memory in the APP/PS1 mouse model of AD [19]. Furthermore, sildenafil has been shown to regulate the level of Aβ, possibly by modifying its production, metabolism, or clearance, as well as presenting an anti-inflammatory effect [107].

Tadalafil (PDE5 IC_{50} = 5.0 nM) shows a better selectivity against PDE6 and a longer half-life compared to sildenafil [108, 109]. At a dose of 1 mg/kg and administered intraperitoneally, tadalafil failed to improve either contextual fear conditioning or spatial working memory in APP/PS1 mice, most likely due to the poor brain permeability of the drug [19]. A derivative of tadalafil, 3c•Cit, with improved water solubility and BBB permeability has been developed and tested on a scopolamine-induced cognitive impairment mouse model. In the passageway water maze test, mice treated with 3c•Cit (10 and 30 mg/kg, orally) showed reduced escape latency and number or errors [110].

Lately, two novel PDE5 inhibitors have been generated at Columbia University, a quinoline-based compound, 7a, and a naphthyridine-based molecule, 6c [111, 112]. Both compounds have exhibited a high inhibitory activity (IC_{50} = 0.27 and 0.056 nM, respectively) and better selectivity than sildenafil, vardenafil and tadalafil. Levels of cGMP in the hippocampus of mice were increased upon *in vivo* treatment with these two compounds and pharmacokinetic studies showed that 7a and 6c crossed the BBB readily. Compound 7a restored LTP and memory damage caused two different mouse model of AD, the APP/PS1 and Aβ-induced cognitive impairment model. Similarly, synaptic plasticity and spatial and associative memory were improved by compound 6c (3 mg/kg, i.p.), which showed a better aqueous solubility compared to 7a.

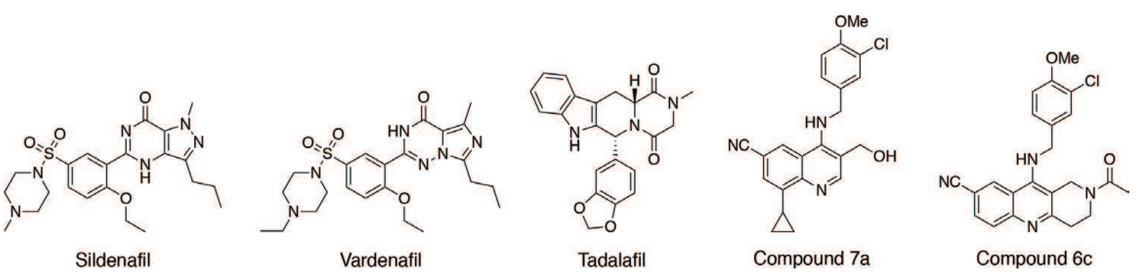

Figure 6.
Structures of PDE5 inhibitors.

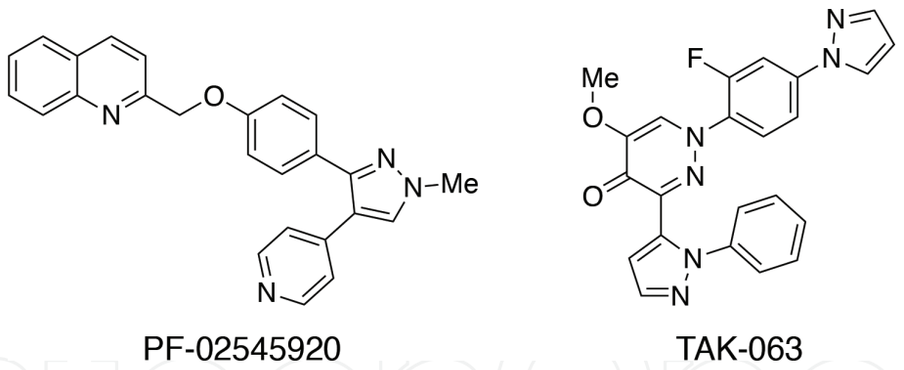

Figure 7.
Structures of PDE10 inhibitors.

4.1.5 Phosphodiesterase 10 inhibitors

PDE10A is a dual-specificity subfamily that hydrolyzes cAMP and cGMP, with a higher affinity for cAMP. The highest expression of PDE10A in the brain is in the caudate nucleus, and it is also the most prevalent PDE species in this tissue, together with PDE1B. The level of PDE10A is relatively high in the nucleus accumbens. In other parts of the brain and the peripheral tissues examined, the level of PDE10 mRNA was very low. Currently, PDE10 is considered a promising target for CNS diseases, especially schizophrenia and Huntington's disease (HD). Although numerous studies have reported that PDE10A expression in the striatum and different other brain regions of post-mortem HD patients [113–115] and HD animal models [113, 116] is reduced, inhibition of PDE10A has shown rescue of behavioral, neurodegenerative, and electrophysiological deficits in HD animal models.

PF-02545920 (also named MP-10) was developed by Pfizer [117] and tested for schizophrenia [118] and HD [119] in preclinical and clinical studies (**Figure 7**).

Developed by Takeda by using structure-based drug design techniques, TAK-063 has a potency of 0.30 nM against PDE10 and high selectivity over other PDEs (**Figure 7**). The potential antipsychotic-like effects of the compound were evaluated in mice showing phencyclidine (PCP)-induced hyperlocomotion. At a minimum dose of 0.3 mg/kg, p.o., TAK-063 reversed the induced deficits, while had no effects on the hyperactivity produced by PCP in PDE10A-knockout mice [120]. Additional studies reported the dose-dependent antipsychotic-life effects of TAK-063 in methamphetamine-induced hyperactivity in rodents [121] as well as attenuation of PCP-induced and MK-801-induced working memory deficits in a Y-maze behavioral test in mice and eight-arm radial maze task in rats, respectively [122].

5. Conclusion

In summary, activation of the NO/cGMP/CREB pathway has been greatly evaluated as a critical molecular mechanism responsible for learning and memory. The impact of this signaling pathway on synaptic strengthening and memory formation has been explored pharmacologically through the use of activators and/or inhibitors of the single components. NO donors, well-known drugs in use for the treatment of cardiovascular diseases, have been considered as therapeutics in AD due to their ability to activate sGC. A number of analogs of the second messenger cGMP are commercially available and have been used to target the pathway by stimulating PKG. Moreover, inhibitors of PKG have proven that CREB phosphorylation leading to improved learning and memory is correlated to the increase in cGMP levels.

Most importantly, PDE inhibitors have received much attention by pharmaceutical industries and research institutions. Overall, the safe clinical profile of the PDE inhibitors—some are FDA approved for other diseases—and the pharmacological effects observed both in electrophysiological and animal experiments has contributed to the development of new therapeutic strategies for the treatment of AD.

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Conflict of interest

OA is a co-founder of Neurokine Therapeutics LLC. DWL and OA have received research funding from Appia Pharmaceuticals LLC. Columbia University owns equity in Appia Pharmaceuticals LLC.

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