Chapter 9
Role of PDE9 in Cognition

C. Dorner-Ciossek, K.S. Kroker, and H. Rosenbrock

Abstract Inhibition of phosphodiesterases (PDEs) has been demonstrated to enhance performance of animals in various cognition tasks and accordingly PDE inhibitors have been proposed as new approach for treatment of cognitive dysfunction (Reneerkens et al. Psychopharmacology 202:419–443, 2009; Schmidt Curr Top Med Chem 10(2):222–230, 2010). One of the eleven PDE isoforms, showing expression in cognition relevant brain regions across species, is PDE9, which hydrolyzes cGMP only. Furthermore, it is well established that the nitric oxide (NO)/cGMP pathway and NMDA receptor signaling has a crucial function in synaptic plasticity and cognitive function. In this chapter, we will provide an overview on PDE9, its expression and function in the brain, and hence, its relevance for synaptic plasticity and cognitive performance. Moreover, the recent advances of PDE9 inhibition as potential therapeutic approach for treatment of cognitive dysfunction in CNS disorders will be discussed.

Keywords PDE9 • Inhibitor • Cognition • Behavior • LTP • Enzyme

9.1 Introduction

Inhibition of phosphodiesterases (PDEs) has been demonstrated to enhance performance of animals in various cognition tasks and accordingly PDE inhibitors have been proposed as new approach for treatment of cognitive dysfunction (Reneerkens et al. 2009; Schmidt 2010). One of the eleven PDE isoforms, showing expression in cognition relevant brain regions across species, is PDE9, which hydrolyzes cGMP only. Furthermore, it is well established that the nitric oxide (NO)/cGMP pathway and NMDA receptor signaling has a crucial function in synaptic plasticity and cognitive function.

In this chapter, we will provide an overview on PDE9, its expression and function in the brain, and hence, its relevance for synaptic plasticity and cognitive performance. Moreover, the recent advances of PDE9 inhibition as potential therapeutic approach for treatment of cognitive dysfunction in CNS disorders will be discussed.

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9.2 Gene Organization, Splice Variants and Expression

PDE9A cDNA was discovered in 1998 and classified as the ninth family of PDEs with no additional members of this family identified (Fisher et al. 1998; Soderling et al. 1998). Accordingly, we refer to PDE9A in this review as PDE9. PDE9 is cGMP specific and, unlike other PDEs, in the N-terminal region it contains no protein domain of known function; the PDE domain is localized at the C-terminus of the protein. PDE9 is encoded by a single gene, which is localized on chromosome 21q22.3 in human and is split into 25 exons that extend over 122 kb. In total, 28 splice variants have been identified, 13 of which are protein coding (Ensembl Database a). Up to now, only little research has been dedicated to the investigation of PDE9 splice variants, and we need to wait on single-cell NGS data to collect the full picture of PDE9 splice variants across different tissues and particular cell types. The longest transcript variant 1 (PDE9A1) translates into a protein containing 593 amino acids. Other splice variants investigated in a cellular context are PDE9A2, PDE9A3, and PDE9A17 which mRNA differ in the use of specific combinations of exons located at the 5′-end of the gene while the 3′ half coding for the catalytic PDE domain is always the same combination of exons (Rentero and Puigdomènech 2006). The corresponding proteins differ in their subcellular localization as shown in transient overexpression in HeLa and Cos-1 cells: While PDE9A1 was found via immunofluorescence staining to be localized in membrane ruffles at the cell projections, in the perinuclear region, the ER and Golgi apparatus, PDE9A2 (533 amino acids) was found in membrane ruffles, the perinuclear region as well as other membrane regions. In contrast, the proteins PDE9A3 (466 aa) and PDE9A17 (567 aa) seem to have lost the targeting towards membranes but instead show a cytosolic localization with only weak co-localization at the endoplasmic reticulum (Rentero and Puigdomènech 2006). In a different study upon transient overexpression in HEK293 cells, PDE9A1 was co-localized with the nucleus whereas PDE9A6 (published as PDE9A5; 492 aa) was found exclusively in the cytoplasm using immunofluorescence and cellular fractionation/western blot (Wang et al. 2003). However, due to the lack of splice variant specific antibodies, no data are available describing the localization of splice variants endogenously expressed in primary cells.

PDE9 expression is conserved across species and PDE9 orthologues have been identified in primates, rodents, laurasiatheria, sauropsidia, fish, but also invertebrates (enseml database b). Homology within vertebrates is high, for example, sequence identity between human PDE9A2 and the corresponding mouse homologue is 93% and 83% at the amino acid and nucleotide level, respectively (Guipponi et al. 1998).

The PDE9 mRNA has been detected more or less ubiquitously over all organs; however, signals are highest in hematopoietic cells, brain, prostate, colon, small intestine, spleen, kidney, and thymus (Almeida et al. 2008; Fisher et al. 1998; Guipponi et al. 1998; Rentero and Puigdomènech 2006).

9.3 Enzymology

9.3.1 PDE9 Enzymatic Profile and Crystal Structure

PDE9 forms a separate family amongst the PDEs with very low sequence similarity to any other PDE outside the catalytic domain. Closest neighbor based on sequence similarity in the catalytic domain is PDE8A sharing 34.4% amino acid identity (Fisher et al. 1998; Figs. 9.1 and 9.2). Recombinant full-length human PDE9A1 shows ~1400 fold selectivity for cGMP, with a K_M value of 170 nM for cGMP compared to a K_M value of 230 μM for cAMP (Fisher et al. 1998), the V_{max} for cGMP is 4.9 nmol/min/μg. The K_M for cGMP makes PDE9 one of the highest affinity PDEs known and clearly distinguishes PDE9 from the two other cGMP selective PDEs, namely 5 and PDE6, as well as from the dual specific PDEs PDE1, PDE2 and PDE10 being also expressed in the brain. The kinetic properties reported for the catalytic domain (amino acid residues 181–506) are very much comparable to the full-length enzyme: the K_M value for cGMP is 139 nM, V_{max} is 1.53 nmol/min/μg (Huai et al. 2004). As expected from the sequence similarity, the K_M value of the mouse PDE9A2 for cGMP is - with 70 nM—comparable to the human enzyme (Soderling et al. 1998). These data are suggesting that indeed the N-terminal part of PDE9 does not have a significant impact on the enzymatic properties of PDE9.

As for all PDEs, the catalytic activity is dependent on the presence of divalent cations which are bound at the bottom of the active site. The first metal ion binding

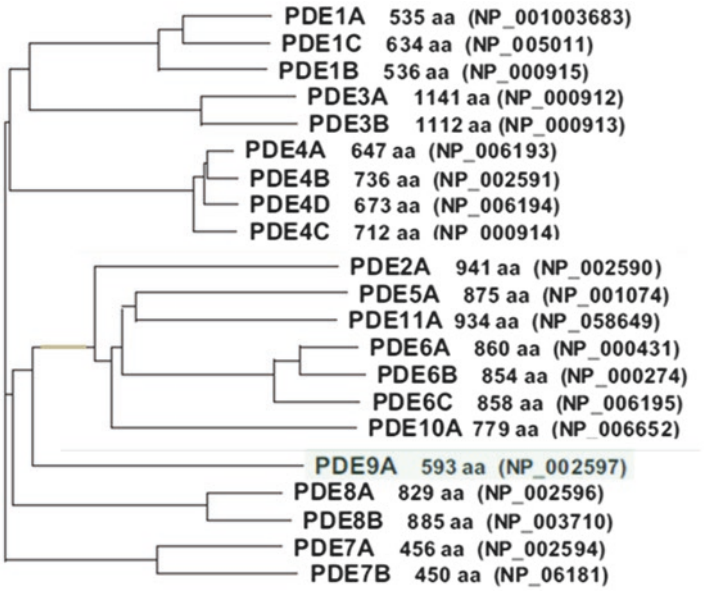


Fig. 9.1 Phylogenetic tree of human PDE families, adapted from Omori and Kotera 2007

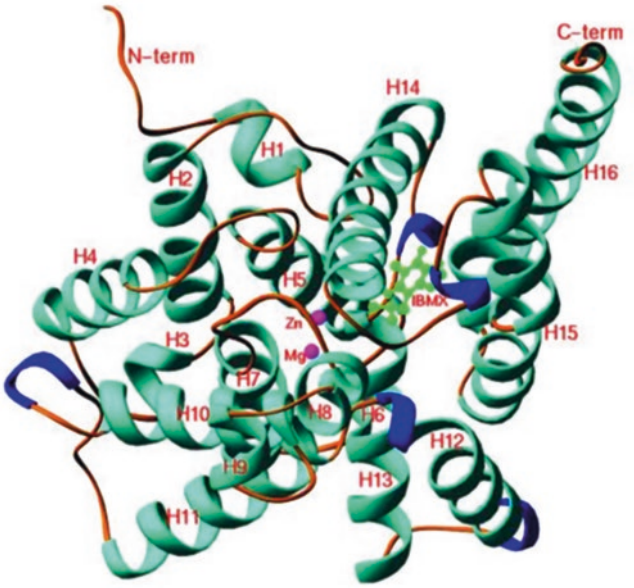


Fig. 9.2 X-ray structure of PDE9, adapted from Huai et al. 2004

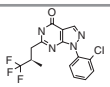
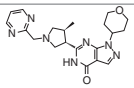
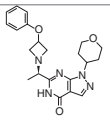
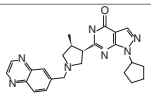
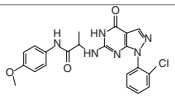
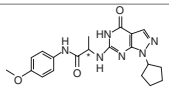
presumably is zinc as in other PDEs, the nature of the second ion however is unknown. Biochemical studies have shown that *in vitro* manganese (Mn^{2+}) activated PDE9 twice as much as magnesium or calcium (Fisher et al. 1998). Maximal activity of PDE9 is achieved at Mn^{2+} concentration of 1–10 mM.

The catalytic domain of PDE9A2 (amino acid residues 181–506) has been crystallized as dimer and consists of 16 alpha-helices (Huai et al. 2004; Hou et al. 2011). Structure comparison of the catalytic domains of PDE9 and the cGMP specific PDE5 revealed significant difference in the conformation of the catalytic domains while in contrast similarity to the catalytic domain of the cAMP specific PDE4D2 is highest. By applying freeze-trapping technique, Liu et al. (2008) reported the capture of ligand/enzyme complexes of PDE9 spanning the entire reaction path during the hydrolysis of the phosphor-ester bond of a cyclic nucleotide. The unique polarization of glutamin Gln-453 of PDE9 makes the hydrogen bond pattern with Gln-453 a more pronounced feature than in other PDEs, accounting for the high specificity of PDE9 toward cGMP over cAMP. Up to now, various groups have applied structure guided design of inhibitors towards PDE9 (Hassaan et al. 2015; Shao et al. 2014; Meng et al. 2012; Deninno et al. 2009).

9.3.2 Selective PDE9 Inhibitors

In 2005, the first potent and selective inhibitor of PDE9, BAY 73-6691 (1-(2-chlorophenyl)-6-((2R)-3,3,3-trifluoro-2-methylpropyl)-1,5-dihydro-4H-pyrazolo(3,4-d)pyrimidine-4-one), was published by a research group from Bayer

Table 9.1 Structure, potency and selectivity of reported PDE9 inhibitors

PDE9 inhibitor	Structure	PDE9 IC_{50} (K_i)	Minimum selectivity factor	Reference
BAY 73-6691		55 nM	25-fold against PDE1C	Wunder et al. (2005)
PF-04447943		12 nM (2.8 nM)	78-fold against PDE1C (>1000-fold against PDE6)	Kleiman et al. (2012), Hutson et al. (2011)
Pf-04449613		24 nM	33-fold against PDE1C	Kleiman et al. (2012)
Pf-4181366		1.8 nM	30-fold against PDE1C	Verhoest et al. (2009)
Compound 28		21 nM	157-fold against PDE5	Meng et al. (2012)
Compound 3r		0.6 nM	150-fold against PDE5	Shao et al. (2014)

(Wunder et al. 2005). This compound shows a potency of 55 nM against recombinant human PDE9 and is minimum 25-fold selective against other PDE enzymes (see Table 9.1). Its *in vitro* and *in vivo* pharmacological profile was described by Wunder et al. (2005) and van der Staay et al. (2008), respectively. In 2011, a group of Merck, and shortly thereafter a group of Pfizer, described the pre-clinical pharmacology profile of another, more potent and selective PDE9 inhibitor, PF-04447943 (6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H pyrazolo[3,4-d]pyrimidin-4-one; Hutson et al. 2011; Kleiman et al. 2012); this compound was investigated in a clinical phase II trial in Alzheimer's Disease patients (Schwam et al. 2014; Sect. 5.1). Further potent and selective PDE9 inhibitors were described in literature by Pfizer and other groups, but with only limited data published on their pharmacological profiles (see Table 9.1; Verhoest et al. 2009; Kleiman et al. 2012; Meng et al. 2012; Shao et al. 2014). In addition, there are several patents on PDE9 inhibitors disclosed by various pharmaceutical companies; overviews on patents related to cognitive disorders have been published previously (Bales et al. 2010; Blokland et al. 2012).

9.4 Protein Expression and Function

9.4.1 Brain Expression

Based on *in situ* hybridization studies in rodents, PDE9 is widely expressed in the brain (Andreeva et al. 2001; van Staveren et al. 2003), and—at least in the hippocampus - it was found to be restricted to neurons as demonstrated by van Staveren et al. (2004). As described in the latter study, the expression pattern closely resembles that of soluble guanylyl cyclase (sGC) and neuronal nitric oxide synthase (NOS) which indicates an involvement of PDE9 in the sGC-NO pathway by regulation of neuronal cGMP levels. The strongest expression of PDE9 was found in cognition relevant regions such as cortex and hippocampus as well as in basal forebrain, basal ganglia, pons and olfactory bulb (Andreeva et al. 2001; van Staveren et al. 2003). The rodent brain expression pattern of PDE9 mRNA was confirmed in principle for the human brain by *in situ* hybridization (Reyes-Irisarri et al. 2007) and quantitative RT-PCR analysis (Lakics et al. 2010). Available data on cerebral PDE9 expression on protein level are sparse - probably due to a lack of suitable anti-PDE9 antibodies. As to our knowledge, there is only one study published using immunohistochemistry in human brain tissue demonstrating PDE9 expression consistent with the regions and cell types as shown by *in situ* hybridization, e.g. cortical and hippocampal areas (Kleiman et al. 2012). At the cellular level, PDE9 protein was found primarily in neuronal cell bodies and primary dendrites presumably indicating a post-synaptic localization of PDE9. Indeed, results of a study in rat hippocampal slices on synaptic transmission and neurotransmitter release by paired pulse facilitation are in line with the suggested localization of PDE9 at the post-synapse and not the pre-synapse (Fernández-Fernández et al. 2015).

9.4.2 Cognition

9.4.2.1 The Second Messenger cGMP

Inhibition of specific PDEs has come into the focus of interest for treating memory dysfunction (Menniti et al. 2006; Reneerkens et al. 2009; Schmidt 2010) as PDEs play an essential role in signal transduction by regulating the intracellular levels of cAMP and cGMP. The cyclic nucleotide cGMP is a key intracellular mediator of signal transduction and plasticity. PDE9 contributes to the intracellular NO-sGC-cGMP signaling cascade by its cGMP hydrolytic activity (Arancio et al. 2001; Son et al. 1998; Zhuo et al. 1994). Under physiological conditions, cGMP is formed by NO-sensitive soluble guanylyl cyclases (NO-GCs; Garthwaite 2008). These NO-GCs are activated by NO, which is generated by calcium/calmodulin-dependent neuronal NO synthases (Christopherson et al. 1999). NO is believed to act as a retrograde messenger and has been implicated as a neuromodulator in synaptic transmission (Boehme et al. 1991; Boehning and Snyder 2003; Garthwaite 2008;

O'Dell et al. 1991; Schuman and Madison 1991). Indeed, pre-synaptic cGMP facilitates glutamate release (Neitz et al. 2011). Furthermore, cGMP acts post-synaptically as part of the sGC-cGMP-PKG pathway, known to indirectly activate the transcription factor CREB (Ko and Kelly 1999; Lu et al. 1999; Sect. 4.2.2). Thus, overall elevation of cGMP results in increased glutamate release pre-synaptically and increased phosphorylation of CREB post-synaptically, both being important mechanisms for learning and memory (Blokland et al. 2006; Prickaerts et al. 2004; Rutten et al. 2007; Silva et al. 1998; Son et al. 1998). Indeed, it was shown that PDE9 inhibitors can increase cGMP levels in cells - as demonstrated in reporter cell line studies (Wunder et al. 2005) - and in the animal brain (Verhoest et al. 2009; Kroker et al. 2014). Based on the described cellular expression profile of PDE9 (Sect. 4.1), it is hypothesized that PDE9 inhibition post-synaptically increases cGMP levels. PDE9 inhibition leads to increased cGMP levels in CSF of animals and humans demonstrating PDE9 inhibition in the brain as functional target engagement biomarker (Hutson et al. 2011; Nicholas et al. 2009; Kleiman et al. 2012; Rosenbrock et al. 2015). This also demonstrates the translatability of this biomarker from rodent to human.

9.4.2.2 PDE9 Inhibition and Synaptic Plasticity

The core component of the brain is the neuron. A neuron is an electrically excitable cell that processes and transmits information by electro-chemical signaling. Each neuron may be connected to up to 10,000 other neurons, passing signals to each other via as many as 1000 trillion synaptic connections. Information storage in the brain involves changes in the strength of these synaptic connections. Synaptic plasticity is the ability of synapses to strengthen or weaken over time, in response to increases or decreases in their activity. PDE9 inhibition was shown to strengthen synaptic plasticity on the functional level of long-term potentiation (LTP) and on the structural level of neurites and spines, which will be described in detail in the next paragraphs.

LTP was first published in Bliss and Lomo 1973 by Bliss and Lomo. They reported that trains of high frequency stimulation to the rabbit perforant path caused a sustained increase in efficiency of synaptic transmission in the granule cells of the dentate gyrus. This report and others, which followed during the 1970s, confirmed the Hebbian nature of this form of synaptic plasticity, namely cooperativity, associativity and input specificity. Cooperativity (Lee 1983; McNaughton 2003), associativity (Barrionuevo and Brown 1983; Levy and Steward 1979) and input specificity (Dunwiddie and Lynch 1978; Nishiyama et al. 2000), being the characteristics of LTP, as well as the durability of LTP (Abraham et al. 1995; Reymann et al. 1985) support the hypothesis that LTP may be a biological mechanism for at least some forms of memory. Hippocampal LTP *in vitro* is widely regarded as a measure of synaptic strengthening and plasticity and used as a model for learning and memory. A distinction is made between two different types of hippocampal LTP (Fig. 9.3). A weak stimulation pattern can induce early LTP, which lasts one to three hours and

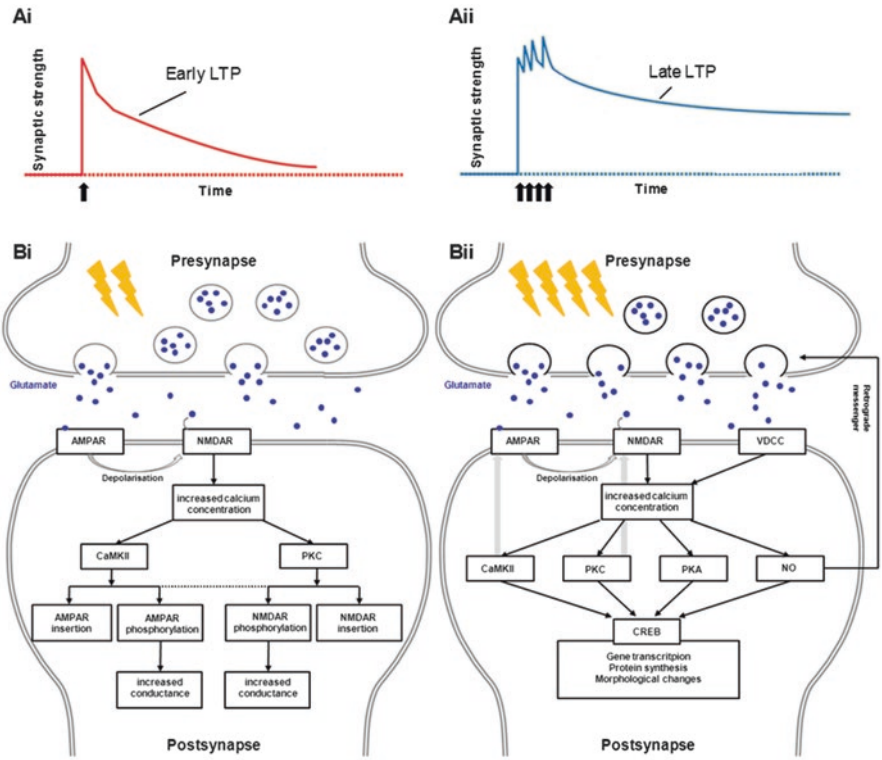


Fig. 9.3 Mechanisms underlying early and late LTP in the CA1 region. (**a**) The durations of early (i) and late (ii) LTP are shown schematically. *Arrow/s* indicate/s the different stimulation protocols. Adopted from Huang 1998. (**b**) Schematic drawing of the mechanisms of early (i) and late (ii) LTP

is protein-synthesis independent (Lynch 2004). The use of a stronger stimulation pattern causes the induction of a more persistent phase of LTP, namely late LTP. Late LTP is the natural extension of early LTP being defined as lasting longer than three hours and being protein-synthesis dependent (Frey et al. 1988; Frey et al. 1996; Lu et al. 1999). The stimulation pattern to induce early LTP causes a simultaneous pre-snaptic release of glutamate and post-synaptic depolarization leading to the release of the magnesium blockage of the NMDA receptor. Thus, the NMDA receptor is dually regulated by ligand and voltage and thereby acts as a coincidence detector (Coan and Collingridge 1987; Cotman et al. 1988). After release of the magnesium ion, calcium can enter through the NMDA channel into the post-synaptic cell (Collingridge et al. 1983; Harris et al. 1984; Jahr and Stevens 1987). Due to stronger stimulation to induce late LTP and thus stronger depolarization, besides NMDA receptors, also voltage-dependent calcium channels are activated. Thus, even more calcium, which triggers a whole series of events (Fig. 9.3), enters the cell. A post-synaptic calmodulin-dependent protein kinase II (CaMKII) pathway (Sweatt 1999) and pre-synaptic cGMP/PKG pathway (Arancio et al. 1995) have been implicated

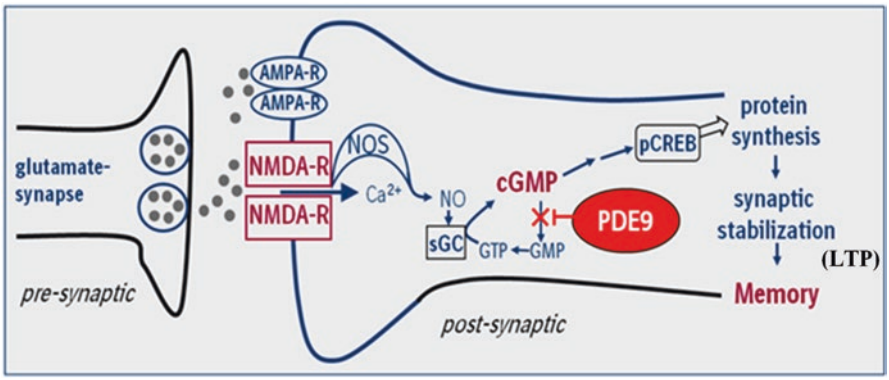


Fig. 9.4 Putative mechanism of action of PDE9 inhibition for strengthening synaptic plasticity

in early LTP. Recently it has been suggested that a post-synaptic cGMP pathway is also involved in early LTP (Taqatqeh et al. 2009). A post-synaptic cAMP/PKA/CREB pathway (Impey et al. 1996) and a cGMP/PKG/CREB pathway (Lu et al. 1999) are involved in late LTP. It has been assumed that early-LTP is related to short term memory and late LTP to long-term memory (Izquierdo et al. 2002).

Since PDE9 inhibitors influence the level of the second messenger cGMP, it seems likely that the procognitive effects of PDE9 inhibitors are related to the facilitation of LTP (Fig. 9.4). The PDE9 inhibitor BAY 73-6691 was found to increase LTP induced by weak stimulation protocols in CA1 hippocampal area of young, and, even more prominent, in aged rats (31–35 months old; van der Staay et al. 2008). A different PDE9 inhibitor PF-04447943 enhanced hippocampal LTP in mice induced by a weak tetanus, but failed to affect the magnitude of LTP induced by a strong theta burst protocol. This effect on tetanus induced LTP was reported to have an inverted U-shaped concentration response curve (Hutson et al. 2011). In the CA1 region of hippocampal rat slices, it was demonstrated with BAY 73-6691 that PDE9 inhibition is able to convert early LTP, induced by weak high frequency stimulation, to protein synthesis dependent late LTP (Kroker et al. 2012). Again this effect followed an inverted U-shaped concentration response curve. Furthermore, it was shown that this transformation into late LTP was dependent on the NO-cGMP-PKG pathway (Kroker et al. 2012).

The reciprocal process of LTP is LTD, which is defined as an activity-dependent reduction in the efficacy of neuronal synapses (Collingridge et al. 2010). A deficit in LTD can result in memory impairment (Griffiths et al. 2008), which is in line with the theoretical neural network models that depend on bidirectional synaptic plasticity (LTP and LTD) to mediate learning and memory (Malenka 1994). However, the exact underlying mechanisms of LTD remain elusive and the role of PDE9 inhibition in these processes requires further investigation.

Beneath induction of functional changes, PDE9 inhibition was shown to modulate plasticity on the structural level of neurites and spines, specialized dendritic

protrusions where the majority of excitatory synapses are located typically formed by a single synapse at the spine head. It was reported that after 24 h treatment, PF-04447943 significantly increased neurite outgrowth and the number of synapses in cultured hippocampal rat neurons (Hutson et al. 2011). This was indicated by increased synapsin 1 expression with maximal effects at 30–100 nM. Furthermore, in a conference abstract and the corresponding poster (Kleiman et al. 2010), it has been reported that chronic dosing of PF-04447943 demonstrated synaptoprotective effects in Tg2576 transgenic mice. The PDE9 inhibitor attenuated the reduction of hippocampal spine density in these mice. Interestingly, PF-04447943 did not increase spine density in control animals at the same dose.

9.4.2.3 Effects of PDE9 Inhibition on Cognition

By means of neurophysiological models monitoring auditory evoked potentials in the hippocampus, it was shown that the PDE9 inhibitor PF-04447943 reversed the amphetamine-induced deficit in auditory gating in anesthetized rats (Kleiman et al. 2012) and in a transgenic rat model of Huntington's disease (Nagy et al. 2015). Although these studies were performed in anesthetized or transgenic animals, the data suggest that PDE9 inhibition might improve auditory information processing, which is impaired in CNS disorders such as schizophrenia and presumably Huntington's disease. Regarding sensorimotor gating, PF-04447943 had no effect on pre-pulse inhibition (PPI) in the poor-gating C57BL/6 J mice per se, but administered together with a sub- threshold dose of the antipsychotic drug risperidone it significantly increased PPI. However, PF-04447943 did not show any improvement of PPI disrupted by the NMDA receptor antagonist MK-801 (Kleiman et al. 2012). These effects on PPI might suggest an interaction of cGMP levels modulated by PDE9 and the dopamine/serotonin system in the striatum. But, on the other hand, PF-04447943 had no effect on psychostimulant-induced hyperlocomotion, neither via a dopaminergic (amphetamine) or glutamatergic (MK-801) stimulation, which indicates that enhanced basal ganglia GMP signaling alone is not sufficient to produce effects on these behaviors.

Regarding cognition, as of our knowledge, only two PDE9 inhibitors have been published so far, for which pharmacology on cognitive performance on various memory domains in animals have been described extensively. BAY 73-6691 demonstrated enhanced memory performance in a variety of rodent cognition tasks in naïve or pharmacologically impaired animals (van der Staay et al. 2008). It showed efficacy in the object and social recognition, passive avoidance and T- maze continuous alternation tasks, and regarding the process of memory formation, it was efficacious on memory acquisition, consolidation and retrieval in the social recognition test. Additionally, BAY 73-6691 showed efficacy on object place memory in a transgenic mouse model related to Aβ-pathophysiology of Alzheimer's disease (Kroker et al. 2014). For the other well-characterized PDE9 inhibitor, PF-04447943, pro-cognitive efficacy was demonstrated in the object and social recognition,

Y-maze, Morris water maze and 8-arm radial arm maze tasks (Hutson et al. 2011; Kleiman et al. 2012). Furthermore, PF-04447943 was shown to reverse a scopolamine-induced deficit in the conditioned avoidance attention task in rats demonstrating enhanced attentional performance by PDE9 inhibition (Vardigan et al. 2011). In summary, PDE9 inhibitors have been demonstrated to cause pro-cognitive efficacy in a variety of animal tasks assessing episodic and working memory as well as attentional performance, which are impaired in several CNS disorders such as schizophrenia and Alzheimer's disease. It is noteworthy, that in several of these cognition tasks the efficacy of PDE9 inhibition followed an inverted U-shaped dose-response curve. This suggests that an optimum of central cGMP increase has to be reached by PDE9 inhibition for pro-cognitive efficacy, like for strengthening of synaptic plasticity as determined by hippocampal LTP enhancement serving as molecular/cellular model for memory formation (Sect. 4.2.2). More detailed information about the pro-cognitive pharmacology of PDE9 inhibitors in animals is summarized in Table 9.2.

9.4.3 Role of PDE9 in Other Physiological and Pathophysiological Conditions

9.4.3.1 Heart Failure

Heart failure is a disease in which the heart cannot pump sufficient blood to meet the needs of the body. It is a leading cause of death and disability worldwide (Sharma and Kass 2014). An important risk factor for heart failure is a persistent high blood pressure as it increases the heart's workload, which in turn induces cardiac hypertrophy, i.e. it increases the size and strength of the cardiomyocytes. After some time, this can cause permanent molecular and structural changes in cardiomyocytes, impairing cardiac contraction and relaxation. To counteract this risk, numerous studies have shown that the body produces cGMP (Kuhn 2003; Lugnier 2011), which is believed to have protective function in the heart, accelerating relaxation, decreasing the stiffness of cardiomyocytes and moderating adverse cardiac remodeling (Frantz et al. 2013; Holtwick et al. 2003; Takimoto 2012; Kuhn 2015).

Lee et al. (2015) showed that PDE9 was expressed in myocardial tissue from mice as well as humans, and was increased in the myocardium of patients with various forms of heart failure, especially in patients with heart failure with preserved ejection fraction. Pharmacologic inhibition or genetic silencing of PDE9 by PF-04447943 or siRNA was protective in pressure-overload mediated cardiac hypertrophy and in a mouse model of cardiac pressure overload. This study suggests PDE9 inhibition as a new therapeutic strategy to increase cGMP in the failing heart.

Table 9.2 Overview of PDE9 inhibitors on cognition in animals

Task	Memory domain	Species (Model)	Compound	Results	Reference
Conditioned avoidance attention	Attention	Rat (impaired by scopolamine)	Pf-04447943	1 mg/kg i.p. Reversed scopolamine-induced deficits, higher dose not	Vardigan et al. (2011)
Object recognition	Recognition, episodic memory	Rat (unimpaired, 24 h ITI, memory acqusition)	Bay 73-6691	0.1–0.3 mg/kg p.o. improved memory performance, higher doses not	van der Staay et al. (2008)
		Rat (impaired by scopolamine, 1 h ITI)	Pf-04447943	3 mg/kg p.o. reversed scopolamine-induced deficits, higher doses not	Hutson et al. (2011)
		Rat (impaired by scopolamine, 2 h ITI)	Pf-04447943	1–3.2 mg/kg s.c. Reversed scopolamine-induced deficits	Kleiman et al. (2012)
Object location	Spatial, episodic memory	Tg2576 APP-tg-mouse (impaired by Aβ over-expression, 4 min ITI)	Bay 73-6691	0.2–5 mg/kg p.o. reversed Aβ-related deficits	Kroker et al. (2014)
Y-maze	Spatial, episodic memory	Mouse (unimpaired, 24 h ITI, memory acqusition)	Pf-04447943	1–3 mg/kg p.o. improved memory performance	Hutson et al. (2011)
Morris water maze	Spatial, episodic memory	Rat (impaired by scopolamine, 24 h ITI)	Pf-04447943	3.2–10 mg/kg p.o. attenuated scopolamine-induced deficits	Kleiman et al. (2012)
Social recognition	Recognition, episodic memory	Rat (unimpaired, 24 h ITI, memory acqusition)	Bay 73-6691	0.3–3 mg/kg p.o. improved memory performance	van der Staay et al. (2008)
		Rat (unimpaired, 24 h ITI, memory conslidation)		0.03–3 mg/kg p.o. improved memory performance	
		Rat (unimpaired, 24 h ITI, memory retrieval)		0.03–3 mg/kg p.o. improved memory performance	
		Mouse (unimpaired, 24 h ITI, memory acqusition)		0.3–3 mg/kg p.o. improved memory performance	

(continued)

Table 9.2 (continued)

Task	Memory domain	Species (Model)	Compound	Results	Reference
		Mouse (unimpaired, 24 h ITI, memory acquisition	Pf-04447943	1 mg/kg p.o. improved memory performance, higher doses not	Hutson et al. (2011)
Passive avoidance	Aversive learning	Rat (impaired by scopolamine, 24 h ITI, memory acqusizion)	Bay 73-6691	1–3 mg/kg p.o. attenuated scopolamine-induced deficits	van der Staay et al. (2008)
T-maze continouns alternation	Working memory	Mouse (impaired by MK-801)	Bay 73-6691	10 mg/kg p.o. attenuated MK-801 induced deficits	van der Staay et al. (2008)
8-arm radial maze	Working memory	Rat (impaired by ketamine)	Pf-04447943	1 mg/kg s.c. Reversed ketamine-induced deficits, higher doses not	Kleiman et al. (2012)

ITI inter-trial-intervall between trial 1 and trial 2, *p.o.* per oral, *s.c.* subcutan, *i.p.* intraperitoneal

9.4.3.2 Sickle Cell Disease

Sickle cell disease (SCD) is a recessive hereditary disorder caused by a single amino acid substitution in the β-globin gene that leads to a polymerization of the hemoglobin and finally red blood cell sickling under hypoxic conditions. The complex pathophysiology of SCD is characterized by hemolysis, chronic inflammation, elevated cell adhesion, leukocytosis, and endothelial dysfunction, culminating in the episodic vaso-occlusive processes responsible for much of the morbidity, observed in patients (Platt et al. 1994; Stuart and Nagel 2004). Vaso-occlusion comprises multistep and multicellular processes that appear to be initiated by the adhesion of red cells and leukocytes to the activated endothelium; mechanisms considered involved in this process are inflammation, hypoxia, oxidative stress, and reduced nitric oxide (Hebbel et al. 1980; Frenette and Atweh 2007; Turhan et al. 2002; Belcher et al. 2003; Aslan and Freeman 2007). PDE9 mRNA as well as protein expression is elevated in SCD neutrophils compared to healthy controls (Almeida et al. 2008). In an *in vitro* and an *in vivo* model of TNFalpha-induced acute vaso-occlusion in SCD mice, inhibition of PDE9 by BAY73-6691 reduced leukocyte adhesion to the microvascular endothelium and extravasation in a cGMP-PKG pathway dependent manner and enhanced the beneficial effects of the NO donor hydroxyurea (Almeida et al. 2012; Miguel et al. 2011). Accordingly, Almeida and colleagues suggest inhibition of PDE9 in combination with hydroxyurea as a promising treatment approach.

9.4.3.3 Erectile Dysfunction

Penile erection is caused by the relaxation of corpus cavernosum smooth muscles, which is initiated by the release of NO from nitrergic nerves and endothelial cells, activating soluble guanylate cyclase in the cavernosal smooth muscle, thus increasing intracellular cGMP and activating cGMP dependent protein kinase PKG. Next to PDE5, also PDE9 is expressed in the human (Küthe et al. 2001) and mouse corpus cavernosum. In mice, prolonged inhibition of PDE9 with BAY 73-6691 over 21 days amplifies NO-cGMP mediated corpus cavernosal responses, i.e. the relaxation of corpus cavernosum. This supports a beneficial effect of PDE9 inhibition for erectile dysfunction via increase of cGMP in the corpus cavernosum (da Silva et al. 2012).

9.4.3.4 Retina

Only few studies are available on PDE9 expression and function in the retina. Early investigations in cells of rat retina have found PDE9 mRNA in retinal pigment epithelium cell layers (Diederen et al. 2007) and in ON-bipolar cells, a specific cell type within the inner retina, (Dhingra et al. 2008) by *in situ* hybridization and RT-PCR analysis, respectively. Furthermore, an analysis of the retinal transcriptome database by Siegert et al. (2009) has elucidated that several amacrine and ganglion cell types also express PDE9, which might suggest a function of PDE9 in the NO/cGMP pathway in the retina and hence on retina physiology. Indeed, recently it could be shown that retinal processing by recording the electroretinograms (ERG) is changed in PDE9$^{-/-}$ (ko) mice (Dhingra et al. 2014). In this study, the greatest effect was found on the recovery of the b-wave, a deflection of the ERG derived predominantly from Muller and ON-bipolar cells. Additionally, the falling phase and the b-wave duration were significantly longer in the PDE9$^{-/-}$ compared to wild-type mice for all photopic stimuli. In summary, the authors concluded that PDE9 might control cGMP levels in specific retinal cells, thereby modulate inhibitory processes in the retina and restrict the duration of the inhibitory processes and thus sharpening and accelerating retinal signaling.

9.5 PDE9 and Its Relevance in Cognitive Disorders

9.5.1 Link to Alzheimer's Disease

Synaptic dysfunction accompanied by impaired structural plasticity and progressive neuronal loss in cortical and hippocampal brain areas are in addition to neurofibrillary tangles and amyloid plaques the key pathophysiological hallmarks of Alzheimer's Disease (AD) leading to the cardinal symptoms of cognitive impairment and progressive memory loss (reviewed by Selkoe 2002; Scheff and Price 2006).

Results from several studies performing quantitative correlations of post-mortem histopathology with pre-mortem cognitive deficits demonstrated that synapse loss is correlated with the cognitive performance better than numbers of plaques or tangles, degree of neuronal perikaryal loss, or extent of cortical gliosis in the early stages of the disease (DeKosky and Scheff 1990; Terry et al. 1991; Masliah et al. 2001). The neurotransmitter systems most prominent affected in AD are the cholinergic and glutamatergic neurons. Whereas the acetylcholine deficits are targeted in clinical practise with inhibitors of the acetylcholine degrading enzyme acetylcholinesterase, the hypofunction of the glutamatergic system and its related synapse loss is so far not addressed by available medication.

Glutamate as the major excitatory neurotransmitter in the human brain is most prominently associated with functions of memory formation and learning. Glutamatergic transmission is mediated by various receptors with the post-synaptic NMDA receptor playing an essential role, which, upon activation, induces a cascade of intracellular post-synaptic signalling events triggered through elevation of second messengers such as cAMP and cGMP finally manifesting in LTP and synaptic plasticity (Sect. 4.2.2). Impaired NMDA receptor signalling and reduced guanylate cyclase activity indicating decreased cGMP levels have been shown in patients suffering from AD (Bonkale et al. 1995; Olney et al. 1997; Lee et al. 2002). Recently, Ugarte et al. (2015) showed, that cGMP, but not cAMP levels, were significantly lower in the CSF of patients diagnosed with mild AD when compared with nondemented controls and importantly the CSF levels of cGMP showed a significant association with MMSE-diagnosed clinical dementia and with CSF biomarker Aβ42 in AD patients. Data on PDE9 expression in AD patients are mixed. While Ugarte et al. (2015) showed a trend for a ~ 2-fold increase of PDE9 expression in Brodman area 20 as part of the temporal cortex in post-mortem AD brains (7 AD brains vs. 8 age-matched controls), Reyes-Irisarri et al. (2007) have not observed such difference in their cohort.

Multiple evidence as been collected that amyloid pathology, namely soluble Abeta oligomers, impair LTP and affect cognition in rodents (Ferreira and Klein 2011; Klein 2013; Puzzo et al. 2015). Recently, the effects of PDE9 inhibition in the context of Abeta have been analysed by Kroker et al. (2014). The PDE9 inhibitor BAY 73-6691 was found to restore LTP in rat hippocampal slices impaired by Abeta oligomers. It was furthermore demonstrated that the rescue of the LTP requires the activity of sGC and protein kinase PKG indicating that the effect is based on the NO/cGMP/PKG pathway. The relevance of the sGC signaling pathway in APP transgenic mice had previously been introduced by Puzzo et al. (2005). Numerous mouse models which by overexpressing pathogenic mutations causing an accumulation of the Abeta peptide show impariments in cognitive tasks. A single application of BAY 73-6691 showed efficacy on object place memory in the APP transgenic mouse model tg2576 and fully restored performance to the level of the wildtype non-transgenic control mice at doses far below the one required to detect changes in brain cGMP concentrations (Kroker et al. 2014).

Based on these clinical observations and the pre-clinical findings of PDE9 inhibition to strengthen synaptic plasticity, improving memory acquisition,

consolidation and retrieval in paradigms assessing episodic memory being strongest affected in AD as well as working memory and attention (Sect. 4.2.3), PDE9 inhibitors are explored in clinical trials in patients with AD. To date, only one clinical proof-of-concept trial with a PDE9 inhibitor in patients with AD has been completed (Schwam et al. 2014). This trial was designed to assess the efficacy, safety and pharmacokinetics of PF-04447943 compared with placebo in mild-to-moderate probable AD patients. Ninety-one subjects received 25 mg PF-04447943 for 12 weeks and were compared to a control group of 100 patients. Although generally safe and well-tolerated, no improvement of cognition, behavior, and global change assessed by Alzheimer's Disease assessment Scale-cognitive subscale ADAS-cog, The Neuropsychiatric Inventory NPI and Clinical Global Impression-Improvement scale CGI-I respectivly, compared with placebo was observed. In healthy volunteers, PF-04447943 has been shown to increase cGMP in the CSF up to threefold (Nicholas et al. 2009). However, it has to be noted that only one dose was tested in the proof of concept trial, and it could be that, based on the pre-clinical data demonstrating pro-cognitive efficacy at an optimal dose range, the optimal degree of PDE9 inhibition for achieving efficacy in the clinical population was not reached or may be lower as suggested also by Schwam et al. (2014). Thus, it is still too early to draw final conclusions on the effects of PDE9 inhibition in AD and additional trials exploring the potential of PDE9 inhibition in more detail and by using a broader dose range are needed. Recently, another clinical proof of concept phase II trial designed to compare the effects of four different doses of BI 409306 to placebo in patients with AD (clinicaltrial.gov: NCT02240693) has been initiated. This compound was shown to increase cGMP levels in the CSF of in rats and healthy volunteers after oral administration (Rosenbrock et al. 2015).

9.5.2 Link to Huntigton

Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder with characteristic motor, cognitive, and behavioral disturbances (Shannon and Fraint 2015). This disease is caused by an expanded CAG repeat in the coding region of the huntingtin gene, and its pathology involves early and prominent degeneration of striatal medium spiny neurons and eventually more widespread loss of cortical, thalamic, hippocampal, and hypothalamic neurons, with cortical thinning and generalized loss of cerebral tissues. Changes in dopaminergic, glutamatergic, and gamma-aminobutyric acid (GABA)-ergic systems are believed important in the genesis and evolution of motor and perhaps other symptoms. Although HD is mainly a movement disorder, cognitive impairment appears early, even before the onset of motor symptoms, both in patients and mouse models (Giralt et al. 2012). However, the molecular events and the relvant brain circuitries involved in cognitive decline of patients stills need to be understood. Recently, it was found that cGMP levels in CSF of patients with HD and in a genetic mouse model of HD (R6/1) were decreased (Saavedra et al. 2013). Interestingly, these transgenic mice

showed cognition deficits in the object recognition and passive avoidance tasks which could be reversed by increase of cGMP through PDE5 inhibition; however the effects of PDE9 inhibition need further investigation. In another study using a transgenic rat model of HD (BACHD rats), the PDE9 inhibitor PF-04447934 was demonstrated to reverse auditory gating deficits - as compared to wild-type rats - in the hippocampus and primary auditory cortex after sub-chronic treatment (Nagy et al. 2015). In summary, the findings in patients with HD and in transgenic rodent models of HD indicate that abnormal auditory gating deficits and cognitive impairment in HD could be ameliorated by increasing cGMP levels in the brain through inhibition of cGMP-hydrolyzing phosphodiesterases. However, so far only two PDE10 inhibitors have been progressed to clinical trials of patients with HD (Fusco and Giampà 2015).

9.5.3 Link to Schizophrenia

Beyond the neurodegenerative diseases, in particular Alzheimer's disease (Sect. 5.1), PDE inhibitors are currently considered as promising therapeutic targets for treating cognitive impairment also in psychiatric disorders like Schizophrenia. Besides showing positive symptoms (e.g. hallucinations and delusions) and negative symptoms (e.g. flat affect and anhedonis), many patients diagnosed with schizophrenia also suffer from cognitive impairment. The cognitive dysfunction includes problems with working memory as well as attentional function (Keefe and Harvey 2012) and results in significant disabilities in social, occupational and economic functioning (Barch and Ceaser 2012; Rajji et al. 2009; Dickson et al. 2012; Stip et al. 2005). Evidence from neuroimaging studies in patients with schizophrenia suggests marked structural changes within the brain which might be related to the cognitive deficits (Fusar-Poli et al. 2013; Lesh et al. 2011; Habets et al. 2008; Yao et al. 2013; Vita et al. 2012). Furthermore, Schizophrenia patients display reduced mismatch negativity (Light and Näätänen 2013), a neurophysiological information filtering process, which has a well-established relationships to cognition (Light et al. 2007; Rissling et al. 2013) and psychosocial functioning in both healthy volunteers and schizophrenia patients (Light and Braff 2005a, b). Since mismatch negativity is dependent on NMDA receptor function, it is hypothesised that the impairment of mismatch negativity and hence cognition in patients with schizophrenia is caused by NMDA receptor hypofunction. Indeed, this hypothesis has become increasingly accepted as an etiopathological model of this illness, based on clinical observations that phencyclidine induces a schizophrenia-like psychosis by blocking neurotransmission at NMDA receptors (Javitt et al. 2012). The NMDA receptor hypofunction in turn influences GABAergic circuits (Lewis and Moghaddam 2006) and thereby causes impaired functioning of glutamatergic/GABAergic pathways in (pre-) frontal cortical but also limbic areas of the brain (Lewis and Moghaddam 2006; Tamminga 2006; Stephan et al. 2009). The hypothesis regarding NMDA receptor hypofunction in schizophrenia is further supported

by a recent meta-analysis of double-blind, placebo-controlled studies in patients with schizophrenia that examined the efficacy of prototype NMDA receptor-enhancing agents like e.g. D-cycloserine (Tsai and Lin 2010). Moreover, patients with Schizophrenia show decreased levels of cGMP in the CSF compared to healthy controls (Gattaz et al. 1983; Beckman and Gattaz 2002). These observation, along with pre-clinical research implicating cGMP signaling pathways in cognitive functioning (Sect. 4.2.1), suggests that PDE9 inhibition, by improving the NMDA receptor signalling cascade via increasing cGMP levels, might represent a therapeutic strategy for the treatment of cognitive deficits associated with Schizophrenia.

In animals, PDE9 inhibitors demonstrated cognition enhancing effects in various models (Sect. 4.2.3) including working memory (T-maze continuous alternation, 8-arm radial maze) and attention tasks (conditioned avoidance attention). To mimic the supposed NMDA receptor hypofunction in schizophrenia patients in these memory tasks, the animals were cognitively impaired by using moderate doses of NMDA receptor antagonists (Kleiman et al. 2012; van der Staay et al. 2008). Furthermore, the PDE9 inhibitor PF-04447943 fully restored D-amphetamine-induced deficits in sensory gating (Sect. 4.2.3; Kleiman et al. 2012), an information filtering process known to be disturbed in schizophrenia (Bramon et al. 2004).

Taken together, based on the clinical observations related to NMDA receptor hypofunction in schizophrenia and the pre-clinical findings of PDE9 inhibition to strengthen synaptic plasticity as well as improving working memory and attentional function, PDE9 inhibitors have started to be explored in clinical trials in patients with schizophrenia. So far, only one PDE9 inhibitor, namely BI 409306, has started a clinical proof of concept phase II trial. This clinical study is designed to investigate the efficacy, safety and tolerability of four different doses of BI 409306 once daily compared to placebo given for 12 weeks in patients with schizophrenia on stable antipsychotic treatment (clinicaltrials.gov: NCT02281773).

Conflict of Interest The authors declare that they have no conflicts of interest**.**

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