From Age-Related Cognitive Decline to Alzheimer's Disease

Chapter 6
From Age-Related Cognitive Decline to Alzheimer's Disease: A Translational Overview of the Potential Role for Phosphodiesterases

Pim R.A. Heckman, Arjan Blokland, and Jos Prickaerts

Abstract Phosphodiesterase inhibitors (PDE-Is) are pharmacological compounds enhancing cAMP and/or cGMP signaling. Both these substrates affect neural communication by influencing presynaptic neurotransmitter release and postsynaptic intracellular pathways after neurotransmitter binding to its receptor. Both cAMP and cGMP play an important role in a variety of cellular functions including neuroplasticity and neuroprotection. This chapter provides a translational overview of the effects of different classes of PDE-Is on cognition enhancement in age-related cognitive decline and Alzheimer's disease (AD). The most effective PDE-Is in preclinical models of aging and AD appear to be PDE2-Is, PDE4-Is and PDE5-Is. Clinical studies are relatively sparse and so far PDE1-Is and PDE4-Is showed some promising results. In the future, the demonstration of clinical proof of concept and the generation of isoform selective PDE-Is are the hurdles to overcome in developing safe and efficacious novel PDE-Is for the treatment of age-related cognitive decline and cognitive dysfunction in AD.

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H.-T. Zhang et al. (eds.), *Phosphodiesterases: CNS Functions and Diseases*, Advances in Neurobiology 17, DOI 10.1007/978-3-319-58811-7_6

Keywords cAMP • cGMP • long-term potentiation • PDE • dementia • Alzheimer's disease

6.1 Age-Related Cognitive Decline, Alzheimer's Disease and Phosphodiesterases

In an increasingly aging society cognitive dysfunction is becoming a growing issue. Increasing age is, up to now, inevitably accompanied by cognitive decline, ranging from age-related cognitive decline up to cognitive dysfunction due to neurodegenerative disorders like Alzheimer's disease (AD). AD leads to dementia and is characterized by dysfunction and deterioration of neurons within the cerebral cortex resulting in loss of memory and progressive cognitive decline. In the earliest stages patients suffer from difficulties in storage and recall of episodic memory. During later stages other cognitive domains like executive functioning and language also become affected. On a neuropathological level, patients suffer from progressive amyloid-β (Aβ) plaque deposition, neurofibrillary tangle formation and synaptic dysfunction in brain regions involved in learning, memory and other higher cognitive functions. Up till now, loss of synapses and the death of neurons are assumed to be responsible for the majority of AD symptoms (Terry et al. 1991).

Phosphodiesterases (PDEs), a group of intracellular enzymes, are receiving increased attention as possible therapeutic targets for treatment of cognitive decline in aging and AD. This chapter will start with a description of the different mechanism of action of phosphodiesterase inhibitors (PDE-Is). Subsequently, the currently available data, both preclinical and clinical, will be discussed.

6.1.1 Phosphodiesterases and Signal Transduction

Neurotransmitter receptors can be divided into the ionotropic or ion channel receptors and metabotropic or GTP binding protein (G-protein) coupled receptors. G-protein activation engages second messenger cascades (Shah and Catt 2004). Second messengers translate an extracellular signal, such as the binding of a neurotransmitter to its receptor, into a non-structural (increased neurotransmitter release) or structural (receptor and/or synapse formation) cellular responses (Wei et al. 1998; Lu and Hawkins 2002).

Traditionally, the cAMP second messenger system, next to the phosphoinositol second messenger system, received the most attention. The second messenger cAMP is synthesized from adenosine triphosphate (ATP) by adenylate cyclase (AC), which is stimulated or inhibited by Gs or Gi, respectively, and degraded by different PDEs. Cyclic adenosine monophosphate (cAMP) activates the cAMP-dependent protein kinase (PKA), which phosphorylates cAMP response element binding protein (CREB). P-CREB is an activated transcription factor, which initiates

transcription of specific genes coding for neurotransmitter receptors such as ionotropic AMPA receptors or growth factors as brain-derived neurotrophic factor (BDNF) (Scott Bitner 2012).

Effects of cAMP activation after receptor binding are located postsynaptically. However, the enzyme AC is also present presynaptically, where it is mainly involved in synthesis, metabolism and release of neurotransmitters including glutamate and dopamine (DA) (Schoffelmeer et al. 1985; Imanishi et al. 1997; Rodriguez-Moreno and Sihra 2013), most likely via a presynaptic CaMK/cAMP/PKA cascade.

The synthesis of the other cyclic nucleotide, cGMP, starts with Ca^{2+} influx. The Ca^{2+} activates nitric oxide synthase (NOS) producing NO (Murad et al. 1978). In turn, NO stimulates the enzyme guanylate cyclase (GC) which converts guanosine triphosphate (GTP) into cGMP. cGMP activates the cGMP-dependent protein kinase (PKG), which can also induce CREB phosphorylation (Lu et al. 1999). NO is also known to act as a retrograde messenger and can thus stimulate presynaptic GC. Just like cAMP, cGMP can influence the neurotransmitters glutamate and dopamine via a cGMP/PKG cascade (Arancio et al. 1995; Sanchez et al. 2002). Figure 6.1 provides a schematic overview of the pre- and postsynaptic cellular processes related to the second messengers cAMP and cGMP involved in signal transduction.

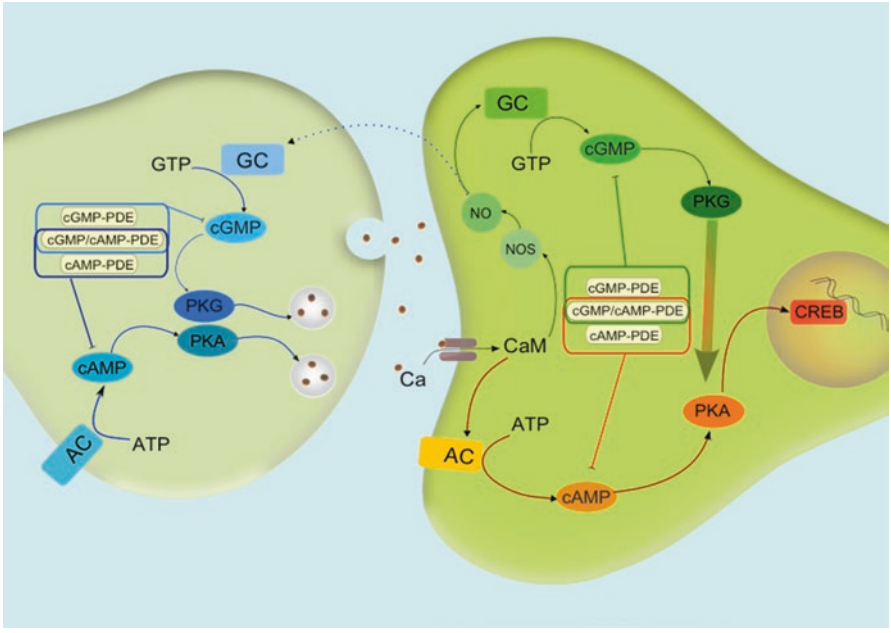

Fig. 6.1 Schematic diagram of pre- and postsynaptic cellular processes related to the second messengers cAMP and cGMP involved in LTP-related signal transduction (Heckman et al. 2015a; reprinted with permission)

Table 6.1 Overview of the different PDE families

Type	Genes	Property	Substrate
PDE1	A, B, C	Ca^{2+}-CaM-stimulated	cAMP/cGMP
PDE2	A	cGMP-stimulated	cAMP/cGMP
PDE3	A, B	cGMP-inhibited	cAMP/cGMP
PDE4	A, B, C, D	cAMP-specific	cAMP
PDE5	A	cGMP-specific	cGMP
PDE6	A, B, C	Photoreceptor	cGMP
PDE7	A, B	cAMP high affinity	cAMP
PDE8	A, B	cAMP high affinity	cAMP
PDE9	A	cGMP high affinity	cGMP
PDE10	A	cAMP-inhibited	cAMP/cGMP
PDE11	A	Dual substrate	cAMP/cGMP

PDEs degrade cAMP and/or cGMP and currently there are eleven families of PDEs of which most have more than one gene and each gene can consist out of several different splice variants and isoforms (see Table 6.1). In total, there are estimated to be over 100 specific human PDEs (Bender and Beavo 2006). One fundamental distinction between PDE families is made on the basis of the difference in the affinity for the two distinct cyclic nucleotides. Dual-substrate PDEs, which have affinity for both cyclic nucleotides, include PDE1, PDE2, PDE3, PDE10 and PDE11. PDE4, PDE7 and PDE8 are cAMP-specific enzymes, whereas PDE5, PDE6 and PDE9 are cGMP-specific enzymes (see Table 6.1) (Beavo 1995). Most of these PDEs can also be found in the brain, having a distinct localization in specific brain structures and neurons (see also Sect. 6.2). This indicates that each PDE may be related to a distinctive neurobiological function.

A PDE-I is a pharmacological compound blocking one or more of the subtypes of PDE (Bender and Beavo 2006). Thus, PDE-Is may affect neuronal communication by influencing presynaptic neurotransmitter release and postsynaptic intracellular pathways after extracellular neurotransmitter binding. This enhanced neuroplasticity is linked to improved cognition as explained in the next section.

6.1.2 Neuroplasticity

Both the cAMP/PKA/CREB and the cGMP/PKG/CREB pathways are implicated in long-term potentiation (LTP), the supposed neurophysiological correlate of memory (Lu et al. 1999; Bliss and Collingridge 1993; Frey et al. 1993). LTP can be induced and measured in vitro and in vivo, when high frequency stimulation produces a stable and lasting increase of synaptic responses (Bliss and Collingridge 1993; Reymann and Frey 2007). A distinction is made between two different types of hippocampal LTP. Early-phase LTP (E-LTP) lasts less than 3 h, while late-phase LTP (L-LTP) lasts 3 h or longer. Furthermore, it has been suggested that E-LTP is

involved in early consolidation processes, while L-LTP is involved in late consolidation processes in long-term memory (Bollen et al. 2014; Bollen et al. 2015) A presynaptic cGMP/PKG pathway (Arancio et al. 1996) as well as a postsynaptic cGMP/PKG pathway has been implicated in E-LTP (Taqatqeh et al. 2009). In contrast, cAMP/PKA signaling appears not to be involved in E-LTP (Bollen et al. 2014; Bollen et al. 2015). A postsynaptic cAMP/PKA/CREB pathway (Impey et al. 1996) as well as a cGMP/PKG/CREB pathway (Lu et al. 1999) is involved in L-LTP.

Recently, it has been demonstrated that early phase cGMP/PKG signaling actually requires late-phase cAMP/PKA-signaling in L-LTP and long-term memory (Bollen et al. 2014), suggesting that enhancement of cGMP-PKG signaling in early consolidation phases requires PKA signaling in a later stage of consolidation. Acquisition processes and short-term memory might be related to the presynaptic release of neurotransmitters regulated by cAMP/PKA as well as cGMP/PKG signaling (Akkerman et al. 2016).

One of the target genes of the cAMP/PKA/CREB and the cGMP/PKG/CREB pathways could be *bdnf* as it is transcribed by the activated transcription factor CREB (Scott Bitner 2012). The protein BDNF is involved in the generation of synapses (synaptogenesis) and the proliferation, survival and differentiation of new neurons (i.e., neurogenesis in the brain) (Minichiello 2009). First, a precursor protein (proBDNF) is produced consisting of a pro-domain and a mature domain of the BDNF protein itself. BDNF is packed into vesicles by the endoplasmatic reticulum, to be secreted either constitutively or in a regulated activity-dependent way (Lu et al. 2005). After release, BDNF binds to the tropomyosin-related kinase B (TrkB) receptor, which is the receptor with the highest affinity for BDNF. Particularly the activity-dependent release of BDNF and subsequent TrkB-mediated activation of CREB is an important mechanism of enhancing neuronal communication, specifically in active neurons of the brain. For instance, BDNF increases synaptic strength with adjacent neurons by processes like LTP, thus ameliorating their connectivity (Minichiello 2009; Lu et al. 2008). Furthermore, TrkB-mediated phosphorylation of CREB is linked to molecular mechanisms that ultimately lead to increased synaptogenesis and neurogenesis (Minichiello 2009). The latter processes have been shown to be involved in learning and memory (Gould et al. 1999). Interestingly, LTP has been linked to both synaptogenesis and neurogenesis (Bruel-Jungerman et al. 2006). Neuroplasticity is therefore a first mode by which PDE-Is exert their effects on cognition.

6.1.3 Neuroprotection

Through their downstream signaling cascades, cyclic nucleotides can reduce the release of inflammatory cytokines (e.g. TNF-α, NF-κB) (Taguchi et al. 1999; Sanchez et al. 2005). Additionally, they induce the synthesis of BDNF and the recruitment of its TrkB receptor. The result is activation of MAPK and phosphatidylinositol-3-kinase/Akt (PI3K/Akt) cascades, which beneficially

influence neuronal proliferation or survival via activation of anti-apoptotic factors (e.g. Bcl-2) and inactivation of pro-apoptotic factors (e.g. Bad) (Jin et al. 2002; Bonni et al. 1999; Brunet et al. 2001; Wang et al. 2015). In various in vitro neurotoxicity models, including hypoxia/hypoglycemia-induced and glutamate-induced neurotoxicity, inhibition of PDEs showed a neuroprotective profile via the suppression of pro-apoptotic caspase-3 activity (Chen et al. 2007). Stimulation of cGMP signaling via cGMP analogs and selective inhibition of cGMP-specific PDE5 protected motor and non-motor neurons to acute reactive oxygen species-induced neurotoxicity in vitro (Nakamizo et al. 2003; Urushitani et al. 2000). Neuroprotection is thus a second mechanism by which PDE-Is can induce pro-cognitive effects.

6.1.4 Blood Flow and Glucose Metabolism

Effects of PDE-Is on blood flow and glucose metabolism provide a third modus of action (Paterno et al. 1996; Dundore et al. 1993). However, such a mechanism is likely to be important when the cognitive impairment arises from vascular insufficiency, e.g. vascular dementia. Of note, it has been found that improved memory performance in rats was achieved with a dose that did not consistently affect blood flow or glucose utilization in the brain (Rutten et al. 2009). This rules out cerebrovascular effects as a (sole) mechanism for cognition enhancement after PDE inhibition and advocates a role for plasticity changes, e.g. LTP and/or neurogenesis, and neuroprotective effects.

6.2 Localization

Table 6.2 provides a short overview of the localization of the different PDEs in the brain of rodents and humans based on mRNA expression and situ histochemistry (Lakics et al. 2010; Pérez-Torres et al. 2000).

Only clear expression levels are taken into consideration. Note that this table does not provide information with respect to the level of expression (protein or mRNA) of the different PDEs.

6.3 Translational Data on Cognition Enhancement

The chapter will continue with an overview of the translational data per PDE family. Mostly, preclinical studies have been conducted investigating the cognition enhancing effects of different families of PDEs, using healthy, pharmacologically impaired and transgenic animals (for a review see Reneerkens et al. 2009; Heckman et al. 2015b). The main behavioral tasks used in animals are described in Table 6.3.

Table 6.2 Localization of the different phosphodiesterases (PDEs) in the brain of rodents and humans in adulthood (adapted from Prickaerts 2010; based on Lakics et al. 2010; Pérez-Torres et al. 2000)

PDE	Localization in the human brain
PDE1A-C	Hippocampus, cortex, olfactory bulb, striatum (highest expression levels), thalamus, amygdala, cerebellum; Expression levels are in general highest for 1A and lowest for 1C
PDE2A	Hippocampus, cortex, striatum, hypothalamus, amygdala, midbrain
PDE3A-B	Throughout the brain low expression levels
PDE4A-D	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum; Expression levels are in general highest for 4A-4D (differs per brain structure) and lowest for 4C
PDE5A	Hippocampus, cortex, cerebellum
PDE6A-C	Pineal gland
PDE7A-B	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, midbrain; Expression levels are in general highest for 7B
PDE8A-B	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, midbrain; Expression levels are in general highest for 8B
PDE9A	Hippocampus, cortex, olfactory bulb, striatum, thalamus, hypothalamus, amygdala, midbrain, cerebellum
PDE10A	Hippocampus, cortex, striatum (highest expression levels), midbrain, cerebellum
PDE11A	Throughout the brain low expression levels

Clinical studies, by contrast, have been far less frequently conducted. However, the NO/cGMP pathway as well as the cAMP/PKA pathway is known to be altered in aged brains (Francis et al. 2011; Domek-Lopacinska and Strosznajder 2010; Blokland et al. 2006) and have also been linked to AD (Jancic et al. 2009; Chen et al. 2007). Additionally, the downstream target of both pathways (CREB) is also affected in AD patients (Lu and Hawkins 2002; Saura and Valero 2011).

Increased PDE activity is assumed to reduce cAMP and/or cGMP signaling in pathways important for brain plasticity and cognition and is therefore considered to be causal, while a decrease in PDE activity might be considered as compensatory (Bollen and Prickaerts 2012; Gurney et al. 2015). In general, PDE expression is assumed to decrease with aging. Whether this is an age-related decrease or a compensatory mechanism is not known (Richter et al. 2013). The expression of specific PDE isoforms in the brains of AD patients is, however, not clear as it has only sporadically been investigated, i.e. case studies or only one gene (Heckman et al. 2015b). From a therapeutic perspective, plasticity and cognition deficits resulting from impaired cyclic nucleotide signaling might be improved by inhibiting specific PDE isoforms.

We will discuss both preclinical as well as clinical studies per PDE family into cognition enhancement in the field of aging and AD. For the wealth of preclinical studies investigating memory enhancing effects of PDE-Is we will limit our discussion to rodent models of aging and AD. Preclinical studies in healthy animals or other neuropsychiatric disorders will therefore not be discussed, although abundantly

Table 6.3 Main behavioral rodent tasks used in memory research related to aging and AD including the type of memory involved

Behavioral task	Brief description	Main brain structures involved	References
Object recognition task (ORT)	Test to study object memory	Hippocampus and rhinal cortex	Ennaceur and Delacour (1988), Winters and Bussey (2005), and Mumby (2001)
Social recognition task	Measures social memory (exploration of another previously seen rat)	Hippocampus and rhinal cortex	Boess et al. (2004)
Object location task (OLT)	Assesses spatial memory	Hippocampus and rhinal cortex	Ennaceur et al. (1997)
Y-maze	Measures spatial working memory by recording spontaneous alternation behavior	Hippocampus and cerebellum	Itoh et al. (1993)
Passive avoidance test	Test of general memory	Hippocampus	Hiramatsu and Inoue (2000)
Morris water maze (MWM)	Test of spatial learning and memory	Hippocampus	Morris (1984)
Barnes circular maze	Assesses spatial learning and memory	Hippocampus	Barnes (1979)
Adapted elevated plus maze	Measures spatial learning and memory	Hippocampus	Patil et al. (2004)
Radial arm water maze	Test for spatial working memory	Hippocampus	Olton and Samuelson (1976)
Contextual fear conditioning	An aversive learning task for memory of the spatial context as well as memory for electric shock stimulus	Hippocampus/Amygdala	Sanders et al. (2003)

existent. In contrast, we will discuss the effects of specific PDE-I families on human cognition in the broadest sense since the number of clinical studies is limited and specific data of studies with AD patients is only sparsely available.

6.3.1 Phosphodiesterase 1 Inhibition

PDE1 is a dual substrate enzyme hydrolyzing both cAMP and cGMP (Table 6.1) (Medina 2011). Vinpocetine is the classical inhibitor of PDE1 (Vereczkey 1985) and has already shown to improve memory function in rodents more than 20 years ago (DeNoble 1987). Vinpocetine has shown to facilitate LTP (Molnar and Gaal 1992; Molnar et al. 1994) as well as to enhance the structural dynamics of dendritical spines (Lendvai et al. 2003). In addition to the effects through enhanced plasticity, it was recently demonstrated that vinpocetine also has strong anti-inflammatory effects (Jeon et al. 2010), although this mechanism is independent of PDE1 inhibition. Only one study investigated the effects of PDE1 inhibition in an AD rodent model (Deshmukh et al. 2009). In rats treated with intracerebroventricular streptozocin, a model for Alzheimer-like cognitive problems, PDE1-I treatment was able to restore performance in the water maze and the passive avoidance test. To our knowledge, no further PDE1-I has been tested in preclinical models of aging or AD.

In clinical trials vinpocetine made it up to Phase IV trials for the treatment of memory impairment (ClinicalTrials.gov Identifier: NCT00719953). In the phase IV study, vinpocetine, as the nutritional supplement Cognitex, was tested on memory impairments in elderly showing a positive effect on memory performance (Richter et al. 2011). However, this was not a placebo-controlled open label study and Cognitex was a mixture of vinpocetine and some other ingredients. In addition, vinpocetine was ineffective in improving cognitive impairments in AD patients (Thal et al. 1989; Szatmari and Whitehouse 2003). In contrast, vinpocetine did improve memory in healthy female volunteers (Subhan and Hindmarch 1985). Vinpocetine was never approved by the Food and Drug Administration (FDA) for treatment of memory impairment, although it is still widely used as a supplement for vasodilatation and as a nootropic for the improvement of memory, including Mild Cognitive Impairment (MCI) (Valikovics et al. 2012), organic psychosyndromes (Hindmarch et al. 1991) and elderly with chronic cerebral dysfunction (Balestreri et al. 1987). The latter effect is likely to be related to vasodilatation. However, the relevance of the possible therapeutic effect of vinpocetine can be questioned as it has not been shown to be of real benefit on memory loss in the clinic.

More recently, a novel PDE1-I has emerged. In a series of Phase I single and multiple ascending dose studies performed in the US and Japan, the PDE1-I ITI-214, given orally and once-a-day was shown to be safe and well-tolerated, with a linear PK profile. This study has been reported in a press release (http://www.intracellulartherapies.com/products-technology/pde-inhibitor-platform.html), where the

company concludes that "these studies represent a significant milestone as the first demonstration of the safety of a potent and highly specific PDE1-I in humans" (ClinicalTrials.gov Identifier: NCT01900522).

6.3.2 Phosphodiesterase 2 Inhibition

PDE2 is a dual substrate enzyme hydrolyzing both cAMP and cGMP (Table 6.1). Preclinical evidence for the efficacy for PDE2 inhibition is substantially more elaborate than for PDE1. The first available selective PDE2-I, BAY 60-7550, improved memory acquisition and consolidation in the ORT in both healthy rats and mice, and improved consolidation in the social recognition task in rats (Boess et al. 2004; Domek-Lopacinska and Strosznajder 2008; Rutten et al. 2007; Rutten et al. 2009). When administered before learning, BAY 60-7550 improved acquisition in 3 and 12-month old rats and, when administered immediately after learning, it improved consolidation in 3, 12 and 24-months old rats (Domek-Lopacinska and Strosznajder 2008). This improvement of memory is caused by the enhancement of neuronal NOS activity in all age groups after administration of BAY-7550. Additionally, the study by Sierksma et al. (2013) found that chronic PDE2-I treatment improved memory performance in the ORT and Y-maze of APPswe/PS1dE9 mice, a transgenic model of AD. However, no changes in plaque load, phosphorylated CREB (pCREB), BDNF concentrations, or presynaptic density in the hippocampus were observed.

Despite the promising preclinical results, to our knowledge BAY 60-7550 never made it to clinical trials. Another PDE2-I, Exisulind (Aptosyn), did make it up to Phase III trials. Exisulind also has PDE5-inhibiting activity. This drug induces apoptosis in a broad range of cancer cell lines and inhibits the formation and growth of cancer in several animal models. Presently, this compound has been tested in clinical Phase III trials for breast, lung, prostate, and colon tumors (ClinicalTrials.gov Identifier: NCT00041054, NCT00078910, NCT00026468, NCT00037609), however not in CNS disorders. PF-05180999 is a PDE2-I tested in two Phase I trials for the treatment of migraine, of which one was terminated prematurely due to safety concerns and the other trial was withdrawn prior to participant enrollment (ClinicalTrials.gov Identifier: NCT01981486 and NCT01981499).

6.3.3 Phosphodiesterase 3 Inhibition

PDE3 hydrolyzes both cAMP and cGMP (Table 6.1). Hiramatsu and his group (Hiramatsu et al. 2010) showed that intracerebroventricular injections of $A\beta_{25\text{-}35}$ led to an impairment in memory performance as evidenced by decreased spontaneous alternations in the Y-maze and shortened step-down latency in the passive avoidance task. Repeated administration of the PDE3-I cilostazol after $A\beta_{25\text{-}35}$ treatment

attenuated these symptoms. On the other hand, acute treatment or treatment before $A\beta_{25\text{-}35}$ administration of cilostazol did not change impairments in memory. Therefore, the effects of cilostazol may be attributed to neuroprotective effects (the prevention of oxidative damage caused by Aβ accumulation in the hippocampus) rather than neuroplasticity effects. Further support is provided by a study reporting that repeated administration of cilostazol strongly attenuated Aβ accumulation in the brain of $A\beta_{25\text{-}35}$-injected mice and significantly improved spatial learning and memory as assessed with the MWM task (Park et al. 2011).

Clinically, cilostazol has already been approved by the FDA for the treatment of intermittent claudication. However, it has been or is being investigated for several other indications as well. Firstly, in two Phase IV studies as a prevention of stroke recurrence (ClinicalTrials.gov Identifier: NCT00216749). Both studies are completed, though no results have been disclosed. Secondly, cilostazol has been tested for cognition enhancing effects. One study (open-label pilot study) was conducted in six schizophrenia patients (Shirayama et al. 2011). One memory task and six cognitive tasks assessing prefrontal functioning were performed. Results on the Trial Making Test showed a significant decrease after 8 weeks of cilostazol treatment as compared to baseline. This suggests improved visuo-motor search skills, simple attention and processing speed. Of note, patients were medicated with drugs that are known to have pro-cognitive effects. Since the cilostazol promotes the effects of neurotransmitter systems affected by the drugs the patients were receiving, it might be possible that the resulting improvement is an interaction effect. On the other hand, in the future PDE-Is may be used as add-on therapy in real-life.

In AD, three clinical trials have been performed with cilostazol. In a first pilot trial of Arai and Takahashi (2009), ten mild to moderate AD patients received 100 mg/day cilostazol as add-on to donepezil (5 mg/day) for a variable period of time, ranging between 1 and 13 months. This study was an open-label, uncontrolled trial. In this small group, a statistically significant improvement on the Mini Mental State Examination (MMSE) was reported during the first 6 months of follow up.

Secondly, Sakurai et al. (2013) describe a sample of 11 patients with mild to moderate AD and cerebrovascular disease who received cilostazol 100 μg for 6 months. AD patients in the control group received clopidogrel or aspirin and showed cognitive decline over this 6-month period. The AD patients treated with cilostazol for 6 months did not show this cognitive decline. This might indicate that cilostazol prevents AD progression. However, in addition to stable cognitive performance, cilostazol increased regional cerebral blood flow in the right anterior cingulate lobe inducing increased supply of oxygen and brain specific nutrients. The latter may, at least in part, be responsible for the positive effects of cilostazol. The cerebral blood flow increasing ability of cilostazol in humans has been confirmed before in different studies in chronically treated patient groups (Kai et al. 2011; Mochizuki et al. 2001). Remarkably, this effect was not found in acute treatment in healthy volunteers (Birk et al. 2004), which suggests that longer term treatment is necessary to exert effects on cerebral blood flow.

A third similar study was initiated in 2011 by the Seoul National University Hospital (Lee (personal communication); ClinicalTrials.gov identifier: NCT01409564).

In total, 36 mild to moderate AD patients treated with donepezil were included. Subjects were equally divided over a cilostazol (100 mg BID) group and placebo group and treated for a period of 24 weeks. However, no difference between groups was found for cognitive measures which included the MMSE and the cognitive scale of the cognitive portion of the Alzheimer's Disease Assessment Scale.

6.3.4 Phosphodiesterase 4 Inhibition

PDE4 is cAMP-specific (Table 6.1). The effects of PDE4-Is have been extensively studied (Richter et al. 2013). Cognition enhancing effects of PDE4-Is have been found in healthy, age- and pharmacologically-impaired, and AD animal models (Reneerkens et al. 2009; Richter et al. 2013). However, we will limit our discussion to rodent aging and AD studies. Rolipram has been most extensively investigated in several CNS disorders including aging and AD. For instance, positive effects on spatial learning in the Barnes circular maze are found in age-impaired mice after daily rolipram treatment (Bach et al. 1999). Rolipram recovered ORT deficits associated with aging in rats (de Lima et al. 2008). Interestingly, high doses of rolipram impaired prefrontal cognitive function in aged, but not young monkeys, likely due to overstimulation of the already disinhibited cAMP/PKA signaling pathway in the aged prefrontal cortex (Arnsten et al. 2005; Ramos et al. 2003). It is expected that plasticity and cognition deficits resulting from impaired cAMP/PKA signaling might be improved by PDE4 inhibition. However, PDE inhibition might have negative effects on cognition and plasticity when PDEs are already downregulated and cAMP levels and PKA activity are high. In this scenario, elevated cAMP levels might go over a physiological level and disrupt signaling. This indicates that it is essential to find the optimal dose of a PDE inhibitor in order to restore disrupted signaling.

Acute as well as chronic treatment of rolipram in the Tg2576 transgenic mouse model of AD showed cognition enhancing effects in contextual fear conditioning (Comery et al. 2005). In an APP/PS1 transgenic mouse model of AD rolipram in improved contextual fear condition as well as spatial working and spatial long-term memory in the radial arm maze and MWM task, respectively (Gong et al. 2004). Another study showed positive effects of rolipram in PS1/PDAPP transgenic AD mice in the radial arm maze (Costa et al. 2007). Although rolipram exerts memory enhancing effects and reverses decreased CREB phosphorylation in APP/PS1 mice (Gong et al. 2004), no effects have been observed on Aβ levels of plaque load in this AD mouse model (Costa et al. 2007) or Tg2576 mice (Comery et al. 2005). Therefore, it could be argued that activation of the pCREB pathway would make the synapses more resistant to the damaging effects of Aβ. Intrahippocampal injection of the $A\beta_{25\text{-}35}$ or $A\beta_{1\text{-}40}$ was found to impair the memory performance in the MWM task and the passive avoidance test in rats while hippocampal pCREB levels were decreased (Cheng et al. 2010). The same effects were found for the more toxic $A\beta_{42}$ peptide (Wang et al. 2012). In both studies, chronic treatment of at least 1 week with

rolipram reversed both the memory deficit in the MWM task and the passive avoidance test and the biochemical deficits (Cheng et al. 2010; Wang et al. 2012).

Next to rolipram other PDE4-Is have been developed and proved effective as cognition enhancers in rodent models of aging and AD, e.g. HT-0712 (Peters et al. 2014), MK-0952 (Gallant et al. 2010), GEBR-7b (Bruno et al. 2011) and roflumilast (Vanmierlo et al. 2016). However, as promising as the cognition enhancing effects of PDE4-Is may be, aversive side effects such as emesis and nausea hamper PDE4-Is reaching the market as a treatment for memory-related disorders. The main idea is that these side effects are caused by the inhibition of PDE4D in particular (Robichaud et al. 2002) and most PDE4-Is are non-selective and inhibit all fiur different gene products (PDE4A-D). Of note, more recently developed PDE4-Is do show increased efficacy and better side-effect profiles. For instance, GEBR-7b was 10 times more effective than rolipram in improving memory performance in healthy rodents, yet its emetic potential was greatly reduced (Bruno et al. 2011). However, this may not be sufficient to completely solve issues related to emesis. New strategies have been explored, like small-molecule allosteric modulators that do not completely inhibit enzymatic activity, and have proven successful (Burgin et al. 2010).

Of note, PDE4 inhibition causes peripheral vasodilatation by elevating cAMP levels (Paterno et al. 1996) which could be seen as an alternative explanation for their beneficial effects on memory performance. Effects through such a mechanism may be present when the cognitive impairment arises from vascular insufficiency, e.g. vascular dementia. However, it has been found that improved ORT memory performance in rats was achieved with a dose that did not consistently affect blood flow or glucose utilization in the brain (Rutten et al. 2009). This does not support the notion that cerebrovascular effects underlie the cognition enhancement of PDE4 and PDE5 inhibition. On the other hand, it advocates a role for plasticity changes, e.g. LTP and/or neurogenesis (Bollen et al. 2014; McGirr et al. 2015).

Clinically, several PDE4-Is have been tested for memory enhancement up to Phase II studies. One of these studies involves a Phase II trial investigating whether MK-0952 improves cognition in patients with mild to moderate AD. This study was completed in 2008, however its results have not been disclosed (ClinicalTrials.gov Identifier: NCT00362024). Same holds for results of the Phase I clinical trial by Roche for MEM1414 (http://www.wikinvest.com/stock/Memory_Pharmaceuticals_(MEMY)/Mem_1414_Treatment_Alzheimers_Disease). Additionally, HT-0712 was tested in age-associated memory impairment and reported on the internet in 2008 to improve long-term memory (http://www.dartneuroscience.com/press_releases/july_22_2008.pdf). Recently, a similar follow-up Phase 2 study has started and first results are expected in 2016 (ClinicalTrials.gov identifier: NCT02013310). In 2011, roflumilast was approved as an anti-inflammatory drug for the treatment of Chronic Obstructive Pulmonary Disease (COPD) (Izquierdo and Aparicio 2010; Puhan 2011). Subsequently, roflumilast was tested in a Phase I study to determine whether a scopolamine-induced cognitive impairment is attenuated by the administration of roflumilast in combination with donepezil in healthy adults (ClinicalTrials.gov identifier: NCT02051335). Roflumilast (dosage unknown) in combination with donepezil 10 mg significantly improved memory function when compared to

placebo or roflumilast alone. Recently, a Phase II study was finished investigating whether roflumilast improves memory, attention, information processing and executive function in healthy humans (no study results provided; ClinicalTrials.gov Identifier: NCT01433666).

In the field of dementia, studies have also been performed in larger groups. Denbufylline is a xanthine derivate with PDE4 inhibitory activity (Miyamoto et al. 1994). In total, 336 patients with different types of dementia received denbufylline for 16 weeks (Treves and Korczyn 1999). Patients were assigned to one of four treatment groups (placebo, 25, 50 or 100 mg BID). Every 4 weeks patients were tested on a cognitive battery consisting of the MMSE, digit symbol substitution subtests of the Wechsler Adult Intelligence Scale (WAIS), and the vocabulary subtest of the WAIS. Patients on denbufylline showed a 3% increase on the MMSE, which was statistically different from the 4% decrease in the placebo group. However, the clinical meaning of the increase needs to be determined.

Saletu et al. (1992) performed a study in which 96 mildly to moderately demented patients were assigned to a 12-week treatment period of either denbufylline (100 mg BID) or placebo. Patients were assessed on the Clinical Global Impression, the Mini-Mental State (Folstein et al. 1975), the SCAG (Shader et al. 1974) and the Digit-Symbol Substitution Test (Wechsler 1956). Secondary target variables were the Trail-Making Test and the Digit Span Test (Wechsler 1956). In addition, electrophysiological correlates were included. In both groups, patients showed treatment induced improvements on all tasks, with significantly stronger increases in the denbufylline group as compared to the placebo group. Clinical global impression was reduced with one point in the denbufylline group, based on which the authors concluded that the denbufylline induced changes were clinically relevant.

In addition to cognition in aging and AD, the PDE4-I roflumilast was tested as a treatment for cognitive impairment related schizophrenia. Takeda conducted a Phase I proof of mechanism study in schizophrenia patients to determine whether cognitive impairment associated with schizophrenia is attenuated by add-on roflumilast administration to second generation antipsychotics (ClinicalTrials.gov Identifier: NCT02079844). No results have been disclosed yet.

Finally, PDE4-Is have also been tested in several clinical studies for their therapeutic effects beyond the cognitive domain in CNS disorders like depression, schizophrenia, Huntington's disease, pain, and drug abuse, however discussion of these studies is beyond the scope of this chapter.

6.3.5 Phosphodiesterase 5 Inhibition

PDE5 is characterized by specificity for cGMP hydrolysis (Table 6.1). PDE5-Is have been tested quite elaborate in animals and humans to investigate the cognition enhancing potential of this subfamily of PDE-Is. In rodents, several different PDE5-Is have been examined. Zaprinast was the first drug to be tested. Acute treatment of 3, 12 and 24-month old rats with zaprinast showed efficacy, i.e. improving acquisition and consolidation, but only in the 3-month old animals (Domek-Lopacinska

and Strosznajder 2008). Next to PDE5, zaprinast also weakly inhibits PDE1, PDE9, PDE10 and PDE11, though, in low concentrations like in this study, it should only inhibit PDE5 (Domek-Lopacinska and Strosznajder 2008). In contrast to the restricted effects on young animals, Patil *et al*. showed improvement in memory function in age-impaired mice in an adapted version of elevated plus maze and the passive avoidance task after zaprinast treatment as well as after sildenafil treatment (Patil et al. 2004). In fact, the improvement in memory functioning was more pronounced in the aged animals compared the young animals. Additionally, positive effects after chronic sildenafil treatment were observed in the ORT and MWM in age-impaired mice (Palmeri et al. 2013). Sildenafil also restored phosphorylation of hippocampal CREB in these aged mice. Moreover, administration of sildenafil to hippocampal slices reversed the age-related impairment of L-LTP. Lastly, chronic treatment with sildenafil ameliorated cognitive deficits and tau pathology in a senescence-accelerated mouse model (senescence-accelerated mouse prone-8 (SAMP8)) (Orejana et al. 2012).

The PDE5-I sildenafil has also been tested in AD mouse models (Puzzo et al. 2009; Cuadrado-Tejedor et al. 2011; Zhang et al. 2013). For instance, chronic administration of sildenafil improved synaptic function and CREB activity, memory deficits and Aβ load in APP/PS1 mice (Puzzo et al. 2009). In addition, impairments of L-LTP in hippocampal slices were reversed after administration of sildenafil. Comparable results were found by Zhang and co-workers who showed that sildenafil lowered Aβ levels and improved cGMP/PKG/pCREB signaling and cognitive performance in the ORT in these same APP/PS1 mice (Zhang et al. 2013). In vitro studies showed that the NO/cGMP pathway is capable of altering APP activity and expression, thereby influencing Aβ production (Austin et al. 2010; Kwak et al. 2011). This latter protective mechanism could possibly explain the results of both sildenafil studies in APP/PS1 mice. However, in a different preclinical AD model, the Tg2576 transgenic mice, chronic treatment with sildenafil only improved memory deficits, though no effects on Aβ levels were observed (Cuadrado-Tejedor et al. 2011). Tadalafil is another PDE5-I which was tested in AD mouse models with partially contradicting results (Puzzo et al. 2009; Garcia-Barroso et al. 2013). Even though tadalafil reversed the reduction of LTP in APP/PS1 mice slices, it was unable to induce any behavioral effects in these mice (Puzzo et al. 2009). The latter was attributed to the inability of tadalafil to cross the blood-brain barrier (Prickaerts et al. 2004). In contrast, Garcia-Barroso *et al*. found that tadalafil can cross the blood-brain barrier (Garcia-Barroso et al. 2013). They even showed that chronic sildenafil treatment in J20 AD transgenic mice improved MWM performance and reduced tau phosphorylation in the hippocampus. No effects on Aβ were levels were found.

Of note, just like PDE4-Is, PDE5-Is can cause peripheral vasodilatation (Paterno et al. 1996). While PDE4-Is exert their effects through cAMP, PDE5-Is function via cGMP. Again, this could be seen as an alternative explanation for their beneficial effects on memory performance. Indeed, sildenafil (~1 mg/kg) has been found to dilate the middle meningeal artery (Kruuse et al. 2012) and to increase local cerebral blood flow in anesthetized rats (Zhang et al. 2002). However, tadalafil and vardenafil, both considered to be the most potent PDE5-Is (Patil et al. 2004; Yuan et al. 2013),

have not shown any effects on blood flow (Rutten et al. 2009; Kruuse et al. 2012). These findings argue against a cerebrovascular mechanism of action underlying the cognition-enhancing effects of PDE5-Is.

In humans, PDE5 inhibition causes relaxation of smooth muscles in blood vessels, hence its importance for the treatment of erectile dysfunction (ED) (Zusman et al. 1999). All three above mentioned PDE5-Is (sildenafil, tadalafil and vardenafil) are approved by the FDA for treatment of ED. Sildenafil is also approved by the FDA under the name of Revatio for the treatment of hypertension of the pulmonary artery. For the same indication, tadalafil and vardenafil have also been investigated; trials completed in 2008 and 2010, respectively (ClinicalTrials.gov Identifier: NCT00125918 and NCT00718952). No results were disclosed so far. Recently, sildenafil has been evaluated in a Phase I study for its neuroprotective properties in the treatment for stroke (ClinicalTrials.gov Identifier: NCT00452582). However, in 2011 this study was terminated because of a failure to recruit in the expected time period.

Several clinical studies investigating the effects of PDE5 inhibition on cognition have been conducted. In the field of dementia, Grass et al. (2001) studied the effects of 100 mg sildenafil on a range of cognitive functions. Sildenafil enhanced performance in a simple reaction time test when given before testing. However, no effects were found on short-term memory, divided attention and other psychomotor tasks. In addition, in a study by Schultheiss et al. (2001) it was shown that 100 mg sildenafil induced no direct cognition enhancing effects on auditory attention and word recognition. Yet, sildenafil changed certain components of event-related potentials (ERPs), indirectly indicating improved focused attention. Also, a reduced negativity in the electroencephalogram (EEG) was found in the word recognition experiment after sildenafil treatment. The latter may indirectly indicate an effect on information processing (Schultheiss et al. 2001). In two recent studies, Reneerkens et al. (2013a, d) investigated the effects of vardenafil on information processing (sensory gating), reaction time responding, executive function and memory performance (e.g. word learning). Memory and executive functioning were tested while EEG activity was recorded. Both 10 and 20 mg vardenafil induced no prominent effects on information processing, reaction time responding, cognition or EEG measures.

With regard to treatment of cognitive symptoms in schizophrenia, Goff et al. (2009) showed that sildenafil, in addition to antipsychotic treatment, did not affect cognition in schizophrenia patients. Another study investigating the effects of repeated dosing of the PDE5-I udenafil in patients suffering from ED, demonstrated that this treatment improved performance of these patients on the Korean version of the MMSE, and on an assessment battery measuring frontal executive function (Shim et al. 2011, 2014).

Of note, possible ceiling effects in healthy volunteers may have limited the effects of a single dose of PDE5-I. Future studies with healthy subjects are therefore encouraged to test either low-cognitive performers or use deficit models to assess cognition enhancing effects of PDE5-Is in healthy volunteers. It would even be better to proceed to (sub)chronic PDE5-I treatment using a patient population.

PDE5-Is have also been tested in clinical studies for their therapeutic effects beyond the cognitive domain. For instance in schizophrenia for their effects on

negative symptoms (Akhondzadeh et al. 2011) and the treatment of dyskinesias in Parkinson's disease (ClinicalTrials.gov Identifier: NCT02162979).

6.3.6 Phosphodiesterase 6 Inhibition

PDE6 is cGMP-specific (Table 6.1) and exclusively expressed in the pineal gland and as a photoreceptor PDE. No preclinical or clinical studies have therefore been performed in the cognitive or any other domain (Bender and Beavo 2006).

6.3.7 Phosphodiesterase 7 Inhibition

Like PDE4 and PDE8, PDE7 is highly specific for cAMP (Table 6.1). Preclinical research into PDE7-Is is currently starting to emerge though no studies have reached clinical trials yet (Morales-Garcia et al. 2015; Banerjee et al. 2012; Perez-Gonzalez et al. 2013). So far, these preclinical studies have shown pro-cognitive and neuroprotective effects. Perez-Gonzalez et al. (2013) found that chronic treatment with the PDE7-I S14 significantly decreased the memory impairments in APP/PS1 mice in the ORT supported by decreased Aβ deposition, enhanced astrocyte-mediated Aβ degradation and a decrease in tau phosphorylation. Additionally, inhibition of PDE7 has shown to exert neuroprotective effects (Morales-Garcia et al. 2011; Perez-Gonzalez et al. 2013), making PDE7 a promising target for future studies.

6.3.8 Phosphodiesterase 8 Inhibition

PDE8 is cAMP-specific (Table 6.1). Recently, the first PDE8-Is have been disclosed (DeNinno et al. 2011). Preclinical behavioral analysis of PDE8B KO mice demonstrated an enhancement in contextual fear, spatial memory, performance in an appetitive instrumental conditioning task, motor-coordination, and an attenuation of age-induced motor coordination decline (Tsai et al. 2012). In addition, basal anxiety levels increased. These findings indicate that selective antagonism of PDE8B may be an attractive target for improvement of cognitive and motor functions. Since preclinical studies with PDE8-Is (e.g. PF-04957325; Tsai and Beavo 2012) are just starting to emerge, PDE8-Is are currently not under clinical investigation for CNS treatments.

6.3.9 Phosphodiesterase 9 Inhibition

The PDE9 family has the highest affinity for cGMP (Table 6.1). Several studies have been conducted with PDE9-Is. The PDE9-I BAY 73-6691 had no effect on basal synaptic transmission in hippocampal slices prepared from young adult

(7- to 8-week-old) Wistar rats (van der Staay et al. 2008). However, BAY 73-6691 increased basal synaptic transmission and enhanced early LTP after weak tetanic stimulation in hippocampal slices prepared from very old (31- to 35-month-old) FBNF1 rats. Additionally, BAY 73-6691 enhanced acquisition, consolidation, and retention of long-term memory in a social recognition task and tended to enhance long-term memory in the ORT. Also, it attenuated the scopolamine-induced retention deficit in a passive avoidance task and the MK-801-induced short-term memory deficits in a T-maze alternation task (van der Staay et al. 2008). Another PDE9-I, PF-04447943, significantly increased neurite outgrowth and synapse formation (as indicated by increased synapsin 1 expression) in cultured hippocampal neurons (Hutson et al. 2011). Additionally, PF-04447943 significantly facilitated hippocampal slice LTP evoked by a weak tetanic stimulus. Also, PF-04447943 significantly improved cognitive performance in a mouse Y maze model of natural forgetting, a mouse social recognition memory model of natural forgetting and a rat ORT with a scopolamine deficit. Pro-cognitive effects of PF-04447943 were also shown in the conditioned avoidance attention task (CAAT) (Vardigan et al. 2011). Finally, PF-04447943 improved responses to cholinergic and monoaminergic perturbations in a range of related behavioral tasks (Kleiman et al. 2012). Interestingly, it has been reported that chronic dosing of PF-04447943 demonstrated synaptoprotective effects in Tg2576 transgenic mice, although this did not translate into an improvement in fear conditioning (Kleiman et al. 2010).

In 2009, PF-04447943 entered a Phase II study to evaluate its effects compared to placebo on cognitive symptoms in AD (Schwam et al. 2014). Recently, the data have been disclosed and 25 mg dosing (BID) during 12 weeks had no effects on cognition in patients with mild to moderate AD. Two possible explanations for this outcome are suggested by the authors. Firstly, treatment duration may not have been long enough and secondly, neurodegeneration in the target population may have been too extensively. Prodromal AD patients or age-associated cognitive impaired subjects may be a better choice for future studies. Recently, Boehringer Ingelheim has investigated the safety, tolerability, and relative bioavailability of a tablet and oral solution of their PDE9-I BI 409306 in healthy male subjects. The safety and pharmacokinetics were compared in extensive and poor metabolizers of cytochrome P450 (CYP)-2C19 (Moschetti et al. 2016). Efficacy studies in the memory domain are expected in the near future.

6.3.10 Phosphodiesterase 10 Inhibition

PDE10 is a dual-substrate enzyme hydrolyzing both cAMP and cGMP (Table 6.1). PDE10-Is have been extensively developed and investigated as antipsychotics (Menniti et al. 2007; Schmidt et al. 2008; Kehler and Nielsen 2011) and cognition enhancers in the field of schizophrenia (Reneerkens et al. 2013b, c; Rodefer et al. 2012; Grauer et al. 2009). However, no interest is shown in PDE10-Is as cognition enhancers in aging and AD. The mechanism of action of PDE10 inhibition was

attributed to be modulation/normalization of dopaminergic fronto-striatal function (Menniti et al. 2007). The most widely used PDE10-I is papaverine, though more selective PDE10-Is have been developed, including, MP-10, PQ-10, TAK-063, THPP-1 and TP-10.

Several clinical studies have been conducted by Pfizer, Takeda, Hoffmann-La Roche and Amgen testing the efficacy of PDE10-Is as a treatment for schizophrenia (ClinicalTrials.gov Identifier: NCT00570063, NCT02477020, NCT02019329 and NCT01568203). Recently, pharmaceutical companies are also redesignating their compounds to Huntington's disease (ClinicalTrials.gov Identifier: NCT02197130, NCT02074410 and NCT02061722). To our knowledge, no PDE10-Is have been tested in clinical models of aging and AD.

6.3.11 Phosphodiesterase 11 inhibition

PDE11 is a dual-substrate enzyme hydrolyzing both cAMP and cGMP (Table 6.1). PDE11 is the most recently identified member of the PDE superfamily. Especially in the brain, little is understood of its exact function. Interestingly, PDE11A KO mice showed subtle psychiatric-disease-related deficits, including hyperactivity in an open field, increased sensitivity to the glutamate N-methyl-D-aspartate receptor antagonist MK-801, as well as deficits in social behaviors (social odor recognition memory and social avoidance) (Kelly et al. 2010). In addition, PDE11A KO mice showed enlarged lateral ventricles and increased activity in CA1 (as per increased Arc mRNA), phenotypes associated with psychiatric disease.

Currently, the first PDE11-Is have been developed (Ceyhan et al. 2012), though, to the best of our knowledge, no clinical studies are or have been conducted.

6.4 Discussion

6.4.1 Preclinical Conclusions

Table 6.4 provides an overview of the outcome of preclinical studies with different PDE-Is in aged rodents and rodent models of AD. The effects of PDE inhibition on memory performance in healthy adult rodents are also included for comparison. Functional recovery of memory is the main therapeutic effect attainable in aging and AD. Therefore, in this chapter, we focused on the memory enhancing effects of different PDE families. Concluding, the most effective PDE-Is in preclinical models of aging and AD seem to be PDE2-Is, PDE4-Is and PDE5-Is. They all improved memory performance in both aged animals and rodent models of AD after chronic treatment, and incidentally already after acute treatment (Comery et al. 2005) (see Table 6.3). When focusing on reversal of pathological alterations, PDE5-Is, again,

Table 6.4 Overview of the preclinical studies of the effects of different PDE-Is on memory performance and bio- and pathological markers in healthy adult, aged and AD rodent models

Phosphodiesterase	Healthy adult	Aged		AD model			
Type	Memory	Memory	pCREB	Memory	pCREB	Aβ	Tau
PDE1	+	TBD	TBD	TBD	TBD	TBD	TBD
PDE2	+	+	TBD	+	–	–	–
PDE3	TBD	TBD	TBD	+	TBD	TBD	TBD
PDE4	+	+	+	+	+	–	TBD
PDE5	+	+/–	+	+	+	+/–	+
PDE7	TBD	TBD	TBD	+	TBD	+	+
PDE8	TBD	TBD	TBD	TBD	TBD	TBD	TBD
PDE9	+	TBD	TBD	–	TBD	TBD	TBD
PDE10	+	TBD	TBD	TBD	TBD	TBD	TBD
PDE11	TBD	TBD	TBD	TBD	TBD	TBD	TBD

+ positive effect reported; – no effect reported; TBD to be determined. Of note, PDE6 is not mentioned/relevant since it is only expressed in the pineal gland and retina

show best results. PDE3-Is and PDE7-Is have already shown to be effective as cognition enhancers in two distinct mouse models of AD after chronic treatment (Hiramatsu et al. 2010; Park et al. 2011; Perez-Gonzalez et al. 2013).

From Table 6.3, it may be concluded that when a PDE-I is effective on memory in aged rodents, it is also effective in healthy adult animals. Unfortunately, this does not work in the opposite direction as PDE5 inhibition which is effective in healthy adult rats did not always improve memory in aged rats (Domek-Lopacinska and Strosznajder 2008). The same holds for the comparison of healthy adult animals to AD rodent models (Kleiman et al. 2010). Extrapolating results between aged rats and AD models is more difficult as PDE5 inhibition has always been consistently efficacious in AD models, though not always in aged rats (Domek-Lopacinska and Strosznajder 2008). Obviously, more studies have to be done to draw more generalizing conclusions regarding extrapolation of results from one brain state to the next (healthy, aged, and diseased).

PDE1-Is, PDE8-Is and PDE10-Is have not yet been tested in preclinical models of aging and AD. PDE1 and PDE10 are highly expressed in the fronto-striatal circuits (especially the striatum) and are therefore considered to be more interesting targets for the treatment of schizophrenia and movement disorders like Parkinson's disease and Huntington's disease. PDE8-Is are just emerging and are currently not tested for CNS disorders.

6.4.2 Clinical conclusions

In Table 6.5, an overview is given of the effects of PDE-Is on memory in healthy adults and patients with AD. Clinical trials in *healthy adults* have tested three different families of PDE-Is, being PDE1, PDE4 and PDE5 (see Table 6.5). The PDE1-I

Table 6.5 Overview of the clinical studies on the effects of different PDE-Is on memory performance in healthy adults and AD patients

Phosphodiesterase Type	Healthy adult Memory	Aged Memory	AD Memory
PDE1	+	+?	–
PDE2	TBD	TBD	TBD
PDE3	TBD	TBD	+?
PDE4	+	+?	Phase II
PDE5	+/–	TBD	TBD
PDE7	TBD	TBD	TBD
PDE8	TBD	TBD	TBD
PDE9	TBD	TBD	–
PDE10	TBD	TBD	TBD
PDE11	TBD	TBD	TBD

+ positive effect reported; – no effect reported; +? questionable positive effect due to drug constraints (PDE1) or conflicting data (PDE3) or data not being peer-reviewed (PDE4); TBD to be determined. Of note, PDE6 is not mentioned/relevant since it is only expressed in the pineal gland and the retina.

vinpocetine showed positive effects in healthy female volunteers (Subhan and Hindmarch 1985). The Phase I trial testing the effects of the PDE4-I roflumilast combined with donepezil 10 mg in a scopolamine-deficit model also showed positive results. The PDE5-Is sildenafil and vardenafil are tested in four different studies in healthy volunteers, though none of these studies found any cognition enhancing effects of PDE5-Is (Grass et al. 2001; Schultheiss et al. 2001; Reneerkens et al. 2013a, d).

Only two types of PDE-Is have been investigated in *aged participants*. Both studies showed positive results on memory but are of questionable nature. The first study investigated the effects of Cognitex on memory function in elderly. Cognitex is a mixture of several ingredients of which one is the PDE1-I vinpocetine. Although Cognitex showed positive effects on memory functioning, results of this study are questionable since the study was uncontrolled and the purity can be questioned (Richter et al. 2011). The second study tested the PDE4-I HT-0712, which showed positive results in a study including age-associated memory impaired subjects. Results were, however, mentioned on the internet but never peer reviewed published elsewhere.

Most clinical studies into the effects of PDE-Is as cognition enhancers in aging and AD have been conducted in *AD patients*. In total 4 families of PDE-Is have been investigated (see Table 6.5). The PDE1-I vinpocetine was shown to be ineffective in improving cognitive impairment (Thal et al. 1989; Szatmari and Whitehouse 2003). Three studies have been investigating the PDE3-I cilostazol, which has shown mixed results. A first pilot study showed positive results (Arai and Takahashi 2009). The second study by Sakurai *et al*. showed positive effects, though these effects may be explained by increased cerebral blood flow and concomitant increase in supply of oxygen and brain nutrients (Sakurai et al. 2013). The third study, initiated by the

Seoul National University Hospital, found no effect of cilostazol on memory. All three studies were conducted in patients with mild to moderate AD. Additionally, the PDE4-I MK-0952 has been tested in AD, although the outcome has not yet been disclosed. Finally, the PDE9-I PF-04447943 was reported to have no effects on cognition (Schwam et al. 2014).

6.4.3 Translational Aspects

From the previous sections, it may already have become clear that there is a clear discrepancy between the results from preclinical and clinical studies. Promising preclinical results have not yet been translated into clinical efficacy. However, the PDE3-I cilostazol is being evaluated in patients for (co)treatment in AD after initial positive results in a mouse model of AD (after central Aβ injection) (Hiramatsu et al. 2010; Park et al. 2011). Interest for PDE3 is, however, somewhat surprising due to the relative low expression of PDE3 in memory-related hippocampal and cortical areas in humans (Lakics et al. 2010). Additionally, PDE9 inhibition did not influence cognition in mild to moderate AD patients (Schwam et al. 2014). This is in agreement with data of a mouse AD model showing that the same PDE9-I was not effective on memory (Kleiman et al. 2010). Most promising are the preclinical results of PDE5 inhibition. Whether it be healthy animals, aged animals or animal models of AD, PDE5-Is consistently induced cognition enhancing effects (Heckman et al. 2015b; Palmeri et al. 2013; Orejana et al. 2012; Puzzo et al. 2009; Cuadrado-Tejedor et al. 2011; Zhang et al. 2013). In contrast, acute treatments with PDE5-Is in healthy volunteers did not clearly improve cognitive functions (Grass et al. 2001; Schultheiss et al. 2001; Reneerkens et al. 2013a). However, a chronic study with the PDE5-I udenafil improved cognitive functions in ED patients (Shim et al. 2014). Actually, PDE5 inhibition displays the best therapeutic profile in animals compared to the other types of PDE-Is already tested in models of AD, improving most behavioral and pathological measures (see Table 6.4). This urges the need to be cautious in translating findings from preclinical studies into expectations for clinical studies.

The following should be considered when explaining the apparent discrepancies in results between animal and human studies:

1. There are translational differences between animals and humans in: (i) PDE-I pharmacokinetics: more specifically the half-life of the inhibitors. PDE-Is have a short half-life in animals (e.g. Rutten et al. 2007; Krause and Kuhne 1988; Reneerkens et al. 2012), whereas in humans, who have a slower metabolism than for instance rodents, the half-life of PDE-Is is in general extended (e.g. Schultheiss et al. 2001; Rabe 2011). (ii) Differential expression of the several PDE subfamilies in the brains of animals and humans. For instance, PDE9 has a high mRNA brain expression in rodents (Van Staveren et al. 2003), whereas its mRNA expression in human brains is relatively low (Lakics et al. 2010).

Interestingly, the expression levels of PDE9 mRNA did not change in AD patients (Reyes-Irisarri et al. 2007) so it cannot be ruled out as a potential target to treat cognitive dysfunction in AD. In contrast, PDE5 mRNA levels (Reyes-Irisarri et al. 2007) and PDE8 mRNA levels (Perez-Torres et al. 2003) may be so low in the brain of AD patients that the lack of enzyme availability could result in inefficacy of the corresponding inhibitors. (iii) The model and test validity: this relates to the deficit model that reflects a disease model or the test model in which the behavior is interpreted in terms of learning and memory.

2. Most animal studies evaluate the acute effects of cognition-enhancing drugs, whereas in human disease states, chronic treatments are considered to be more relevant. Eventually, chronic drug treatment is usually required for treating patients. However, chronic effects of drugs may differ from acute effects of drugs. Acute treatment is assumed to affect memory via an LTP-like mechanism improving signal transduction between neurons. On the other hand, chronic treatment might act predominantly via a neuroprotective effect by promoting synaptogenesis and/or neurogenesis. Eventually, this will also improve communication between neurons and thus memory performance. PDE5-Is have only been tested after acute treatment in healthy adults (Grass et al. 2001; Schultheiss et al. 2001; Reneerkens et al. 2013a). Perhaps (sub)chronic treatment with a PDE5-I would have been effective, assuming the main effects of PDE5 inhibition are expressed through neuroprotective mechanisms in humans (cf. Shim et al. 2011). Likewise, for the PDE9-I PF-04447943 the treatment duration may have been insufficient to exert positive effects (Kleiman et al. 2012).
3. The selection of the target population in clinical studies. Testing PDE5-Is in aged subjects or aged-associated cognitive impaired subjects might prove more effective. Actually, this has also been suggested as an explanation for the lack of a PDE9-I effect on cognition in mild to moderate AD patients (Kleiman et al. 2012).

6.4.4 Overall Conclusions

Based on expression levels in memory-related brain structures like the hippocampus and cortex, PDE1, PDE2, PDE4 and PDE8 seem to be the most interesting targets. Especially, if we take into account the favorable low peripheral expression. PDE1-Is have shown to be effective in healthy animals and humans, though show no efficacy in aging or AD. Inhibition of PDE2 or PDE4 has been shown to improve memory performance in aged rodents and AD rodent models. Clinical trials for PDE2 have not yet been conducted, whereas PDE4-Is have been tested in five clinical trials including healthy subjects, aged subjects as well as AD patients. However, results for PDE4 have not been disclosed (except for a non-peer-reviewed press release on aged participants and a positive result in a scopolamine-deficit study in healthy participants). PDE8-Is have not yet been tested in preclinical models of aging and AD. When considering PDE inhibition for treatment of AD, in particular PDE4D

and PDE8B are interesting targets since they have shown AD related changes in mRNA expression in the hippocampus (McLachlan et al. 2007; Perez-Torres et al. 2003). Expression of PDE2 mRNA was not changed in the hippocampus of AD patients, though, as for PDE9, this does not rule out PDE2 as a potential target for cognition enhancement in aging and AD. Finally, in contrast to the above mentioned interesting targets due to brain expression, PDE7 may be interesting based on behavioral results as it improves memory performance in an AD mouse model (Perez-Gonzalez et al. 2013) next to observed changes in PDE7A mRNA expression in the AD brain (Perez-Torres et al. 2003).

By now, it may be apparent that selectivity of a PDE-I for one specific subfamily determines its usefulness for aging and AD through expression of that particular PDE subtype in the hippocampus and cortical areas related to memory. However, this selectivity implies that all isoforms of a particular subfamily will be inhibited. Just like the different subfamilies, individual isoforms are differentially expressed in the brain and some are specifically related to unwanted side effects. Therefore, more selective PDE-Is are needed to induce more specific biological activity without unwanted side effects. A clear example is provided for the PDE4 family. A PDE4-I will inhibit about 25 isoforms (Gurney et al. 2011), of which some can induce unwanted side effects. This resulted in the recent development of more selective PDE4-Is for only one of the different gene products (PDE4D). Especially, emesis (vomiting) and nausea are linked to PDE4 inhibition. There are now PDE4D-specific inhibitors which are devoid of emetic effects (Burgin et al. 2010) or have at least greatly reduced emetic effects (Bruno et al. 2011). In the future, this has to be continued to the level of splice variant- or isoform-specific PDE-Is. Further support for this notion is provided by brains of AD patients, which show mRNA expression of the different PDE4D subtypes to be specifically and differently affected (McLachlan et al. 2007). Selective inhibition of one of the PDE4D isoform subtypes may thus yield the best therapeutic outcome. Increasing the selectivity of PDE-Is poses a major challenge which has to be achieved by influencing compound-enzyme interactions most likely outside the catalytic domain of the PDE enzymes (Gurney et al. 2011).

In summary, several hurdles still have to be overcome in PDE research in the field of aging and AD. First, the development of isoform selective compounds will increase the safety margin for early proof of concept studies into human cognition enhancement in healthy and aged subjects or AD patients. Additionally, several translational issues have to be addressed, i.e. dosing regimen, model and test validity, description and selection of target population. Together this will enhance translation of currently observed positive preclinical results into clinical efficacy providing clinical proof of concept for cognition enhancing effects of PDE-Is for the treatment of age-associated cognitive decline or cognitive dysfunction in AD.

Conflict of Interest Arjan Blokland and Jos Prickaerts have a proprietary interest in the PDE4 inhibitor roflumilast. In addition, Jos Prickaerts has a proprietary interest in selective PDE4D inhibitors, including GEBR-related compounds.

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