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# Phosphodiesterases: CNS Functions and Diseases

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# Phosphodiesterases: CNS Functions and Diseases

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# Foreword

The cyclic nucleotides, cAMP and cGMP, have long been known as important second messengers that can modulate a multitude of central nervous system (CNS) functions. It also has long been known that one of the most effective ways to regulate cyclic nucleotide tone in various compartments in the cell is to inhibit or activate the activity of one or more cyclic nucleotide phosphodiesterases (PDEs). In fact, many natural products that have as part of their function the ability to inhibit cAMP or cGMP PDE activity have been used as mood and disease ameliorating agents for centuries. Probably the best known of these is theophylline, a major component of tea.

We now know that eleven different gene families of PDEs exist, many of which contain several genes. In addition, most of these genes code for more than one mRNA by virtue of multiple start sites and/or alternative splicing events. In more recent years, our knowledge of the cellular and subcellular expression, localization, and functions of the various PDE variants has begun to be sorted out. Some are modified by myristyl, palmitoyl, or isoprenyl groups thereby altering their subcellular localization. Others bind to specific AKAPS (A Kinase Anchoring Proteins) that in turn can alter their subcellular localization. Still others bind to different scaffolding proteins. Ultimately, each cell type has evolved mechanisms to express a distinct subset of PDEs in discrete functional compartments in the cell. The aggregate effect of these expression patterns is to allow the PDEs to control the amplitude and duration of the cAMP and cGMP signals in specific regions of the cell. Ultimately these expression patterns work together to allow the cyclic nucleotide to coordinate the regulation of many different processes in the cell.

PDE localization and cyclic nucleotide coordination is particularly important in the CNS where different combinations of PDEs are expressed for example in the presynaptic space, while others are present in the postsynaptic area. Similarly, the PDEs that control the cyclic nucleotides in the vicinity of the nucleus are likely to be different than those in the vicinity of the Golgi, or ER, or mitochondria. Up to now, as can be seen in the various chapters of this book, most of our knowledge of PDE function in the brain is based on localization studies and empirical observations using PDE family-selective inhibitors or mice having targeted disruptions of

specific PDE subtypes. A few studies have started to explore in a non-biased manner the roles for specific combinations of different PDEs on specific functions in the cell (Golkowski et al., Cellular Signaling 28:764–778, 2016); however, for the most part these types of approaches have not yet taken place for CNS functions.

In this book, cutting-edge chapters by experts in the field explore what currently is known about many of the CNS functions postulated to be controlled by cyclic nucleotide PDEs. As mentioned most of these descriptions come from studies using family-selective PDE inhibitors or whole animal PDE knockout models. After an initial introduction on the cyclic nucleotide signaling circuitry and its implications for CNS function and disease, in Chap. 1, Neves-Zaph provides a detailed current view for PDE diversity in the CNS and the associated cAMP signaling networks. This is followed by an excellent chapter by Schulke and Brandon on the role(s) for PDE10A in the basal ganglia. This PDE has long been known to be highly expressed in this region of the brain and is thought to act largely as a cAMP-inhibited cGMP in these cells. Clinical trials for PDE10 inhibitors in several diseases including Huntington's disease, Schizophrenia, and Parkinson's disease are either in process or recently completed.

In Chap. 3, Hu, Pan, and Zhang discuss the possible interactions of cell division kinase 5 (CDK5) and PDE modulated cyclic nucleotide levels in CNS cell function and neuropsychiatric and neurodegenerative diseases. Interestingly, it was found recently in a phosphoproteomic study in another tissue that many different predicted CDK substrates were altered in response to PDE inhibitors (Golkowski et al., Cellular Signaling 28:764–778, 2016).

Rolipram, the prototype for PDE4 inhibitors, was originally developed as a possible antidepressant agent. Since that time our understanding of the multiple isozymes in this gene family has increased enormously. The next several chapters discuss the status of PDE4 inhibition on cognitive function and diseases. In Chap. 4, Bolger describes our current understanding for the role(s) of PDE4s in the regulation of depression and more recently their use as possible targets for memory enhancement and affective disorders. In the next chapter Clapcote discusses the possibilities for targeting the specific isozyme of PDE4, PDE4B, for treatment of cognitive impairment and possibly also obesity-related diseases. In Chap. 6, Heckman, Blokland, and Prickaerts discuss not only the possible roles for PDE4 inhibitors but also of PDEs 1 and 2 as a treatment for age-related cognitive decline, and in Chap. 7 Hansen and Zhang give their views on the role(s) of PDE4 in the same processes.

Chapter 8 by Kelly switches to a discussion of the possible role(s) of PDE11A in regulation of social function. This PDE will catalyze the hydrolysis of both cAMP and cGMP and is expressed widely in the brain.

Chapter 9 by Dorner-Ciossek, Kroker, and Rosenbrock changes the focus to cGMP and the cGMP-selective PDE, PDE9. The original localization studies of this PDE suggested a possible role in cognitive function and this aspect is discussed. It is thought that this PDE may be particularly important in controlling the downstream output of the nitric oxide (NO) signaling pathways, particularly at the synapse.

Chapter 10 by Padovan-Neto and West and Chap. 11 by Fusco and Paldino discuss the roles for PDEs in Parkinson's and Huntington's diseases, respectively. Parkinson's disease has been a target of PDE10 inhibitors and the results of recent trials are discussed in that chapter. PDE10 is highly implicated in DOPA function as it is most highly expressed in the striatum. The possible role(s) of PDE inhibitors as treatments for Huntington's disease are discussed largely in the context of PDE4, PDE5, and PDE10 inhibition.

Chapters 12, 13, and 14 discuss the roles of PDE inhibitors as modulators of psychiatric disorders. In Chap. 12, Zhang, Leuptow, Zhang, O'Donnell, and Xu give us more details on the possible roles of PDE2 in psychiatric and neurodegenerative disorders. This PDE is also called the cGMP-stimulated PDE by virtue of a high-affinity binding site on its N-terminal GAF domain. Its high expression in the limbic system has directed interest to its possible roles in these disorders. In Chap. 13, Wennogle, Hoxie, Peng, and Hendrick discuss in more detail the possible unique role for PDE1s in degenerative and cognitive function. PDE1s are activated by calcium and calmodulin. The authors also briefly discuss early clinical results with selective PDE1 inhibitors with regard to safety and function. In Chap. 14, Snyder and Vanover discuss their views on the roles for PDE inhibitors in the treatment of schizophrenia.

In Chap. 15, Wen, Liang, and Zhang discuss the targeting of PDEs for treatment of substance abuse. The roles for dopamine and cyclic nucleotide in the reward circuitry of the brain have made PDE inhibition a logical target for investigation in this area.

Finally, in Chap. 17, Wang and Ke discuss the crystal structures of PDEs in relation to the architecture of the binding pockets for substrate and inhibitors. Crystal structures for many of the PDE catalytic subunits with their selective PDE inhibitors have been solved and have provided much needed information on the physical basis for selectivity among PDE inhibitors. With the more recent realization that in order to see appreciable changes in function more than one PDE must be inhibited; this information will become even more crucial to our understanding of how to design selective PDE inhibitors on the basis of both their binding and functional effects.

Overall, this book provides a current up-to-date view of the status of research on the roles for cyclic nucleotide PDEs in CNS function and also the use of PDE isozyme selective inhibitors as approaches to treatment of several different CNS-mediated diseases. As expected, some approaches have proven more successful than others, but the current studies clearly set the stage for further advances in our understanding of CNS signaling and likely also in the treatment of CNS disorders.

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# Preface

Phosphodiesterases (PDEs), a superfamily of enzymes catalyzing the hydrolysis of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), have been studied for half a century. This area has been developed quickly during the recent two decades, particularly since the discovery of sildenafil (Viagra®), a selective PDE5 inhibitor, for treatment of male sexual dysfunction in 1998; additional PDE5 inhibitors were approved later (tadalafil, Cialis®; vardenafil, Levitra®). Other family-selective PDE inhibitors have been developed for clinical use since then, i.e., roflumilast (Daxas®) approved by the US FDA in 2011 for treating chronic obstructive pulmonary disease (COPD) and apremilast (Otezla®; 2014) for psoriasis and psoriatic arthritis; both are selective PDE4 inhibitors. Nevertheless, while many of the 11 PDE families are involved in the mediation of intracellular signaling in the central nervous system (CNS) and could be targets for CNS diseases, PDE inhibitors are not available yet for treating neurological or psychiatric conditions. This has been due to the difficulty of overcoming significant side effects (e.g., emesis for PDE4 inhibitors), challenges encountered in synthesizing promising inhibitors (e.g., inhibitors that are selective for a subtype within a PDE family), and an incomplete understanding of the role of the various PDEs in cellular function in the brain. Therefore, it is necessary and important to understand the roles of PDEs in CNS functions, CNS diseases, and the utility of novel PDE inhibitors. To address this, we have published a special issue entitled "Targeting Phosphodiesterases (PDEs) for Treatment of CNS Diseases" in *Current Pharmaceutical Design* (Volume 21, Issue 3, 2015); the review articles have received relatively high citations since publication. We decided to publish a book focusing on the similar topic in order to have comprehensive discussions from experts with a broad expertise in PDE research. The purpose of this book is to characterize the contributions of PDEs to brain functions and identify PDEs and their isoforms as potential targets for treatment of CNS diseases, including psychiatric diseases such as depression, anxiety, and schizophrenia, and neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, and Parkinson's disease, as well as other CNS disorders such as stroke, alcoholism, and drug abuse.

We are very grateful to the authors, who are internationally recognized experts in the PDE research area, for their contributions of excellent chapters, which cover almost all aspects of the CNS diseases described above. We sincerely appreciate the efforts of the reviewers who made many helpful suggestions to improve the book. We gratefully acknowledge Linda Nguyen and Yongxu Huang for their assistance in editing the manuscripts. We know that, despite the hard work of the dedicated team, it is inevitable to miss some points, papers, and any issues related to the subject of this book. Any feedback from readers would be appreciated and important for improving our next edition.

Morgantown, WV, USA — Han-Ting Zhang
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# About the Editors

**Han-Ting Zhang** received his M.D. from Southern Medical University (formerly the First Military Medical University) in Guangzhou and M.S. and Ph.D. of pharmacology from Beijing Institute of Pharmacology and Toxicology, the Military Academy of Medical Sciences, China, in 1995. He worked as a postdoctoral fellow at Louisiana State University Health Sciences Center in 1998 and then at the University of Tennessee Health Science Center in 2000. In 2005, Dr. Zhang joined the Departments of Behavioral Medicine and Psychiatry and Physiology and Pharmacology as a faculty at West Virginia University School of Medicine, Morgantown, WV, USA. He has been a tenured associate professor there since 2012. Dr. Zhang is also a "Taishan scholar" overseas distinguished expert and professor of pharmacology at Taishan Medical University (adjunct), China.

Dr. Zhang's major research interests focus on intracellular signaling in the mediation of neurodegenerative and psychiatric disorders. More specifically, he is interested in exploring the roles of phosphodiesterase (PDE)-mediated cyclic nucleotide (cAMP, cGMP) signaling in depression, anxiety, alcohol dependence, drug abuse, and cognition deficits associated with neurodegenerative disorders such as Alzheimer disease. Development of novel drugs for treating these disorders is also one of his research foci.

Dr. Zhang has published more than 80 research papers and review articles and 18 book chapters, most of which focuses on PDEs, in particular the PDE4 enzyme family. He has been awarded the NARSAD Young Investigator Award twice (2006 and 2008). Dr. Zhang has been served as the guest chief editor of Current Pharmaceutical Design and currently serves as the deputy chief editor/associate editor for Metabolic Brain Disease, Frontiers in Pharmacology, Frontiers in Aging Neuroscience, Translational Neuroscience Review, eNeuroscience, and Austin Psychiatry and editorial board member for Scientific Reports and several other international journals.

**Ying Xu** received her Ph.D. degree from Peking University in 2006. She has been a research assistant professor in the School of Pharmacy and Pharmaceutical Sciences at the State University of New York at Buffalo since 2013. Dr. Xu has long been working in drug discovery focusing on treatment of neuropsychiatric disorders. Her research includes the effects and molecular mechanism of natural polyphenols and target synthetic drugs on neurodegenerative and psychiatric disorders, such as age-related cognitive disorders, depression, anxiety, and neuropathic pain. Her recent work has focused on development of small molecule drugs, particularly phosphodiesterase-2 and phosphodiesterase-9 inhibitors, on central nervous system disorders. Dr. Xu was the winner of the 2015 New Investigator Award (NIA) in the American Association of Colleges of Pharmacy. She is serving for seven peer-reviewed journals as an editorial board member and serving as the deputy chief editor or associate editor for Metabolic Bain Disease and Frontiers in Aging Neuroscience. Dr. Xu published more than 60 peer-reviewed research papers, reviews, and book chapters.

**James M. O'Donnell** received his B.S. in psychology from Carnegie Mellon University and Ph.D. in pharmacological and physiological sciences from the University of Chicago; he completed postdoctoral training in neuropsychopharmacology at the University of Pennsylvania. He was appointed the 11th dean of the University at Buffalo School of Pharmacy and Pharmaceutical Sciences in 2013, where he also holds academic appointments as professor of pharmaceutical sciences and professor of pharmacology and toxicology (adjunct). Previously, he held research or faculty positions at Los Alamos National Laboratory, Louisiana State University, University of Tennessee, and West Virginia University, where he served as associate dean for research in the School of Medicine and assistant vice president for health sciences research.

Dr. O'Donnell's research has focused on the relationship between the neurochemical and behavioral effects of drugs, primarily those used to treat neuropsychiatric illnesses. This work has been supported by the NIH, primarily the National Institute of Mental Health, and has involved collaborations with scientists at other universities and biotech and pharmaceutical companies. He has been active in teaching professional and graduate students in the areas of pharmacology and neuroscience and served as director of an NIGMS-supported, T32 predoctoral training grant at the interface of behavioral and biomedical sciences.

He has served on NIH review panels in the neuroscience and drug discovery areas, including as founding chair of the Pathophysiological Basis of Mental

Disorders and Addictions study section, and is associate editor for the Journal of Pharmacology and Experimental Therapeutics. He is a member of a number of scientific and professional societies, was elected fellow of the American College of Neuropsychopharmacology and the American Association for the Advancement of Science, and cochaired the Gordon Research Conference on Cyclic Nucleotide Phosphodiesterases.

# Part I
# PDEs and Signaling, Circuitry, and Implications of CNS Functions and Disorders

# Chapter 1
# Phosphodiesterase Diversity and Signal Processing Within cAMP Signaling Networks

**Susana R. Neves-Zaph**

**Abstract** A large number of neuromodulators activate G-protein coupled receptors (GPCRs) and mediate their cellular actions via the regulation of intracellular cAMP, the small highly diffusible second messenger. In fact, in the same neuron several different GPCRs can regulate cAMP with seemingly identical timecourses that give rise to distinct signaling outcomes, suggesting that cAMP does not have equivalent access to all its downstream effectors and may exist within defined intracellular pools or domains. cAMP compartmentalization is the process that allows the neuron to differentially interpret these various intracellular cAMP signals into cellular response. The molecular mechanisms that give rise to cAMP compartmentalization are not fully understood, but it is thought that phosphodiesterases (PDEs), the enzymes that degrade cAMP, significantly contribute to this process. PDEs, as the sole mechanism of signal termination for cAMP, hold great promise as therapeutic targets for pathologies that are due to the dysregulation of intracellular cAMP signaling. Due to their diverse catalytic activity, regulation and localization each PDE subtype expressed in a given neuron may have a distinct role on downstream signaling.

**Keywords** cAMP • Protein kinase A • Phosphodiesterase • PDE • GluA1 • AMPAR trafficking

## 1.1 Introduction

Cyclic adenosine monophosphate (cAMP), the classical second messenger, is a critical intracellular mediator of the actions of most neuromodulators in the brain. The original studies that elucidated classical cAMP signaling described it as a straightforward linear pathway, where neuromodulator-activated G-protein coupled receptors (GPCRs) activate membrane adenylyl cyclases (ACs) inducing the synthesis of cAMP. This increase

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in cAMP activates cAMP-dependent protein kinase (PKA) leading to a series of phosphorylation events that result in the regulation of ion channels, enzymes, and changes in transcriptional and translational activities. The termination of the signal is due to the activity of 3′-5′ cyclic nucleotide phosphodiesterases (PDEs), enzymes that degrade cAMP into 5′AMP.

Studies in the last 20 years have highlighted the underappreciated complexity of this once considered simple signaling pathway. First, PKA is not the sole direct effector of cAMP. Additional cAMP targets, such as Exchange Protein Activated by Cyclic AMP (EPACs, guanine nucleotide exchange proteins that activate small GTPases Rap1 and Rap2), and cyclic nucleotide gated channels have been identified and are also involved in relaying cAMP downstream action into cellular outcomes. Additionally, the abundance of highly regulated isoforms responsible for the production and degradation of cAMP further complicates the picture. There are nine genes coding for G-protein activated ACs, with variants expressed from each gene. The large number of possible cyclase isoforms pales in comparison with the vast multiplicity of PDE isoforms identified so far. The PDE superfamily consists of 11 gene families, with most families containing several genes giving rise to a total of 21 coding PDE genes and potentially generating close to 100 isoforms variants. These variants display diverse enzymatic characteristics, regulation and localization, and any given cell may express tens of these different isozymes, creating a cell type-specific cAMP processing profile.

PDEs have garnered a tremendous amount of attention due to their role as the exclusive degradation activity for cyclic nucleotides. Several studies using specific pharmacological inhibitors and genetic ablation approaches have identified PDEs as key contributors to normal neuronal function. Despite all this, the identity of downstream signaling controlled by each PDE isoform is still poorly understood. The fundamental questions that remain are: if all PDEs have the same termination role in cAMP signaling, why is there such a large number of distinct PDEs expressed in a given neuron? And what is the identity of the intracellular signaling derived from the cAMP pool controlled by each of these PDEs? In this chapter we will review how the diversity of PDEs found in neurons contributes to the fine tuning the amplitude and duration of cAMP signaling, and how regulation of these activities can further modulate cAMP processing in neurons.

## 1.2 The Range of Kinetic Characteristics of PDE Isoforms Fine-Tunes cAMP Levels

A great deal of attention is paid to the expression level of each PDE in specific neuron types or brain regions. Yet, in order to gauge the actual contribution of these enzymes in cAMP signaling other factors must also be taken into account, such as their affinity and catalytic activity for cAMP, as these kinetic features can range widely for each PDE family. For instance, a PDE with modest cellular expression but possessing high affinity and catalytic activity may have a substantial role in cAMP dynamics. These enzymes act on a substrate whose levels are dynamically

regulated as cellular cAMP levels can vary up to 100-fold upon receptor-mediated cyclase stimulation. At rest, most neurons exhibit cAMP levels in the low to mid nanomolar range and receptor activation of cyclases increases cAMP levels up to micromolar range, with the specific cAMP cellular concentrations dependent on the identity of ACs/PDEs expressed, and GPCRs activated (Bacskai et al. 1993; Mironov et al. 2009). Additionally, cAMP is not homogenously distributed throughout the neuron, leading to the possibility that not all PDEs have equal access to their substrate (Bacskai et al. 1993; Li et al. 2015; Neves et al. 2008). The nonlinear nature of all these factors makes it challenging to intuit the contribution of each PDE to cyclic nucleotide homeostasis and signaling.

Neurons express a multitude of PDEs, and each PDEs may have a precise role depending on the signaling status of the cell and the resulting cellular levels of cAMP. For instance, medium spiny neurons (MSNs), the principal neurons of the striatum that receive dopaminergic stimulation express several PDE activities with a wide range of kinetic properties. MSNs are particularly enriched in PDE1B, PDE2A, PDE4B, PDE7A/B, PDE8B and PDE10A (Heiman et al. 2008; Kelly et al. 2014; Stephenson et al. 2012; Erneux et al. 1981; Martins et al. 1982; Repaske et al. 1993; Van Staveren et al. 2003). To illustrate the diverse kinetic profile of the PDEs expressed, the Michaelis Menten constant (Km) value for cAMP for each MSN-expressed PDE is plotted in Fig. 1.1. It is striking that all these MSNs-expressed PDE subtypes cover three orders of magnitude of cAMP levels, with PDEs displaying high affinity (PDE7A/B, PDE8A and PDE10A), mid-affinity (PDE4B) or lower affinity (PDE1B and PDE2A) for cAMP degradation. Based solely on these Km values, it is easier to appreciate how each PDE activity may have a distinct function during the induction and maintenance of basal and receptor-mediated cAMP levels. The high affinity PDEs may be maximally active even during basal conditions to

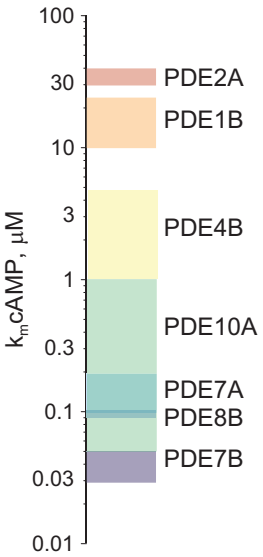


**Fig. 1.1** PDEs enriched in MSNs display a wide range of affinities for cAMP. Michaelis Menten constant (Km) for each PDE gene variant. Each box depicts the range of experimental Km values reported in the literature for the various isoforms of each gene

regulate the basal tone of cAMP. Whereas the lower affinity PDEs may be responsible for the amplitude and duration of the receptor activated cAMP levels. To further illustrate this complexity, we explore the relationship between varying cAMP levels due to different cellular states (basal and DA stimulation), and the kinetic properties of PDEs on downstream cAMP signaling by employing an ordinary differential equations (ODEs)-based model of a simplified cAMP signaling scheme (Fig. 1.2a). The percent contribution of each PDE subtype for the degradation of cAMP dramatically changes with increasing cAMP levels due to dopamine stimulation (Fig. 1.2b). At low DA concentrations, the main PDE acting on cAMP is PDE10A, accounting for 80% of all degradation activity. These simulations results are in agreement with reported contributions of PDEs in MSNs. In MSNs, PDE10A accounted for the majority of the degradation activity acting on basal cAMP (Russwurm et al. 2015). Additionally, inhibition of PDE10A activity has a more robust effect on MSNs expressing D2 (Gi-coupled—dopamine receptor) than in D1-MSNs, suggesting that this high affinity PDE may have more significant role in neurons that display a decreased basal cAMP tone (Polito et al. 2015). As dopamine concentration is increased, cAMP levels rise to the high nanomolar/low micromolar range and the contribution of PDE4B becomes more prominent (Fig. 1.2b). At supersaturating concentrations of dopamine, cAMP levels accumulate to the micromolar range and the activity of PDE2 predominates. The kinetic characteristics of PDEs must be taken under consideration when testing the contribution of

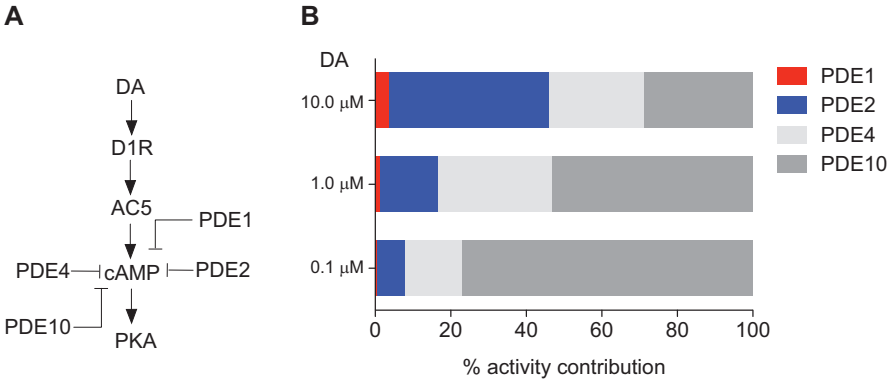


**Fig. 1.2** The contribution of each PDE to cAMP levels is dependent on cellular state. (**a**) The model is based on our work (Song et al. 2013) and contains a depiction of dopamine-induced cAMP signaling in MSNs including: dopamine D1-receptor mediated activation of AC5, PKA activation, and detailed representations of cAMP degradation activities by PDE1B, PDE2A, PDE4B, and PDE10A with their appropriate reported kinetic parameters. For illustration purposes, the assumption is that these four PDE activities have access to the same local cAMP pool. PDE7 and PDE8 were not included for clarity sake as they cover the same cAMP range as PDE10A. To simplify the interpretation of the simulations, the initial concentrations of each PDE was kept equimolar, and total PDE concentration was constrain to achieve the reported basal and receptor activated cAMP levels in neurons. (**b**) integral of the velocity of each PDE reaction plotted as percent of each PDE subtype over total PDE activity of the simulation

each PDE. For instance, inhibiting a low affinity PDE under basal conditions may not produce a change in cAMP signaling reflective of the true intracellular role of such PDE. Thus, the strength of the extracellular stimuli and the catalytic activity of the defined PDEs are variables that must be taken into consideration when interpreting the function of each PDEs in downstream signaling.

## 1.3 Regulation of PDEs Activities Expands Their Catalytic Capabilities to Allow Rapid Signal Modulation and Integration

PDE activities are modulated by a number of regulatory mechanisms that allow the rapid and transient control of the intensity of cAMP signaling. These regulatory mechanisms may also be points of intracellular signaling integration, where modulation of the activity of PDEs may result in redirection of signals between different downstream targets. The PDE superfamily displays a variety of mechanisms that result in dynamic activity control such as allosteric binding by cyclic nucleotides, competitive inhibition and post-translational modifications (Leroy et al. 1996; Noyama and Maekawa 2003; Omori and Kotera 2007). An example of allosteric regulation of a PDE is the activation of PDE2A by cGMP. This allosteric regulation occurs due to cGMP binding to GAF-domain present in the N-terminus of PDE2A isoforms, resulting in a conformational change that enhances substrate access to the catalytic site (Martinez et al. 2002; Martins et al. 1982; Noyama and Maekawa 2003; Pandit et al. 2009; Rosman et al. 1997). This regulation results in significant degradation of cellular cAMP levels upon increases in cGMP in MSNs and has functional consequences to downstream targets (Lin et al. 2010; Polito et al. 2013; Wykes et al. 2002). Additionally, this type of cGMP regulation of cAMP levels may result in significant crosstalk interactions between PDEs, where inhibiting the activity of a PDE with cGMP activity may result in the unintended regulation of PDE2 activity (Zhao et al. 2015; Zhao et al. 2016).

The best-characterized example of PDE activity regulation by phosphorylation is observed in the PDE4 family as certain isoforms are regulated by a number of kinases (Mika and Conti 2016). Isoforms that contain the two conserved N-terminal regions, called “upstream conserved-regions” (UCR1 and UCR2), are classified as long isoforms and can form autoinhibitory domains that control PDE4 oligomerization and enzymatic activity (Cedervall et al. 2015; Xie et al. 2014). It is thought that phosphorylation events within the interface of these domains modulate their stability and can result in activation (enhanced degradation of cAMP) or inactivation (diminished degradation of cAMP) (Bender and Beavo 2006; Conti et al. 2003; Richter and Conti 2002). For instance, PDE4 activity can be regulated acutely by PKA phosphorylation within the UCR1 region, inducing conformational changes that increase PDE4 activity above basal levels (Lim et al. 1999). This PKA-phosphorylation is conserved in all PDE4 long isoforms and results in a twofold enhancement of degradation activity (Hoffmann et al. 1999; Sette and Conti 1996). It is believed that this regulation creates

a negative feedback loop mechanism that results in signal attenuation or termination of cAMP signaling (Fig. 1.3). A number of other protein kinases can also stimulate the phosphorylation of PDE4 resulting in the bidirectional control of the degradation of cAMP, making PDE4 a key node of signaling crosstalk. For instance, regulation of PDE4 activity by ERK phosphorylation results in a reduction of degradation activity (Hoffmann et al. 1999). Simultaneous phosphorylation by PKA, cancels the inhibitory effect of ERK regulation, returning activity to basal levels. More recently the identification of Cdk5 and CaMKII as additional regulators of PDE4 activity have highlighted the central role PDE4 in the integration of cAMP/$Ca^{++}$ signaling (Mika et al. 2015; Plattner et al. 2015). In particular, in D1-expressing MSNs Cdk5 can synergize with PKA activity to fully potentiate PDE4 hydrolytic activity (Plattner et al. 2015). Cdk5 phosphorylation of PDE4 induces a modest increase in basal activity, but in combination with PKA phosphorylation there is a 2.5-fold increase in cAMP degradation capability. Thus, these phosphorylation events can regulate the directionality and magnitude of PDE4 activity and dramatically modulate downstream signaling (Fig. 1.3).

Despite the extensive insight into the regulation of PDE4 activity, the identity of the intracellular signaling derived from the cAMP domain controlled by each PDE4 isoform is still limited. GluA1, a subunit of α-amino3-hydroxy-5-methy-4-isoxazolepropionic acid receptors (AMPAR), is a target of PDE4-regulated PKA activity (Nishi et al. 2008; Song et al. 2013). This phosphorylation event is of particular importance for AMPAR trafficking, as PKA phosphorylation of S845 promotes AMPAR membrane insertion at extra-synaptic and peri-synaptic sites, and primes GluA1-containing AMPAR for synaptic insertion (Esteban et al. 2003; Serulle et al. 2007; Snyder et al. 2000). DARPP32, a highly enriched striatal protein that is also a major target of PDE4-regulated PKA (Nishi et al. 2008), is a potent inhibitor of protein phosphatase-1 (PP1) when phosphorylated at T32 DARPP32 (Hemmings et al. 1984; Ouimet et al. 1984; Svenningsson et al. 2004). PP1 dephosphorylation of Ser845 induces the endocytosis of GluA1 (Shen et al. 1999; Snyder et al. 2000). Therefore, the balance between PKA and PP1 activities determines the phosphorylation state of AMPAR, and is tightly coupled to the dendritic levels of cAMP and the activity of PDEs.

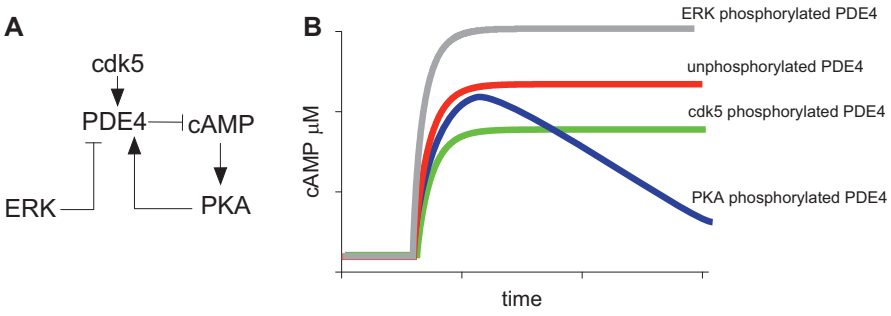


**Fig. 1.3** Acute regulation of PDE4 activity by phosphorylation produces diverse cAMP dynamics. (**a**) PDE4 activity is the regulated by a number kinases. (**b**) The phosphorylation of PDE4 results in distinct cAMP dynamics

We have studied the role of PDE4 and its regulation by ERK on dopamine-induced AMPAR trafficking in MSNs. Dopamine stimulation increases active ERK levels resulting in the phosphorylation and inhibition of PDE4 (Song et al. 2013). We found that ERK, by inhibiting PDE4 activity, amplifies dopamine-induced GluA1 phosphorylation, and GluA1 membrane insertion by altering the balance between PKA and PP1, as the ERK mediated increase in PKA-phosphorylation of DARPP32 prevents PP1 from dephosphorylating GluA1. Blocking this ERK-mediated regulation of PDE4 activity results in a decrease in cAMP levels, GluA1-S845 and DARPP32-T34 phosphorylation. This leads to a robust decrease in GluA1-containing AMPAR surface expression. Conversely, co-treatment of dopamine and brain-derived neurotrophic factor, a neurotrophin that activates ERK independently of cAMP, enhances ERK-phosphorylation of PDE4, resulting in an increase of GluA1 phosphorylation, and GluA1 insertion by tipping the PKA/PP1 balance in favor of PKA. It is possible that other stimuli that activate ERK, such $Ca^{++}$-activated Ras, may modulate PDE4 activity and result in GluA1 trafficking changes, making PDE4 a point of integration for AMPAR trafficking regulation. Similarly, cdk5 regulation of PDE4 also resulted in significant decrease in cAMP signaling. Inhibition of cdk5 induces an increase in the PKA-mediated phosphorylation of DARPP32 at T32, and GluA1 at S845. Interestingly, one must note that cdk5 also directly phosphorylates DARPP32 at T75, resulting in a form of DARPP32 that is inhibitory to PKA. Thus cdk5 mediates the phosphorylation and activation of PDE4, along with the simultaneous phosphorylation of DARPP32 to inhibit PKA, resulting in the synergistic dampening of cAMP signaling. Whether this regulation of PDE4 by cdk5 also affects surface expression of GluA1 remains to be confirmed.

## 1.4 The Diversity of PDE Subcellular Localizations Promotes Signal Specificity

Different GPCRs expressed in the same neuron can increase cAMP with similar temporal dynamics, but resulting in distinct cellular outcomes, raising the question of how the cell differentially decodes signals from these receptors. This concept, called cAMP compartmentalization where gradients of the second messenger are localized within defined domains that are functionally distinct, remains poorly understood. The advent of novel imaging technologies has allowed the examination of the non-homogenous nature of cAMP signaling in neurons (Bacskai et al. 1993; Li et al. 2015; Neves et al. 2008). Since the intracellular diffusion of cAMP is fast, studies have focused on determining the mechanisms that permit cAMP to accumulate within these domains to target downstream signaling with high specificity (Saucerman et al. 2014). There is some evidence that physical constrains, such as cell shape, can affect cAMP diffusion and may play a role in cAMP compartmentalization. In fact, neurons display significantly higher concentration of cAMP in dendrites versus cell body, pointing to surface-to-volume ratio-driven accumulation of cAMP (Bacskai et al. 1993; Li et al. 2015; Neves et al. 2008). It is thought that since

dendrites are high surface to volume ratio regions, cAMP accumulates due to the mostly plasma membrane location of cyclases (surface), and the predominant cytoplasmic localization of PDEs (volume) (Neves et al. 2008). Also, in these regions diffusion of cAMP may be hindered, further promoting accumulation of cAMP (Meyers et al. 2006).

PDEs also significantly play a role in the formation and maintenance of cAMP compartmentalization to ensure precise spatial and temporal signal propagation to downstream effectors (Perino et al. 2012, Sample et al. 2012; Taylor et al. 2013; Tsvetanova and von Zastrow 2014). Although the precise manner by which PDEs contribute to compartmentalization is still a matter of debate, there is strong evidence that points to PDEs acting as enzymatic barriers. This is supported by the observation that irrespective of cell type, inhibition of PDEs results in an increase in the spatial range of cAMP signaling (Zaccolo and Pozzan 2002; Neves et al. 2008). Two possible barrier-mechanisms have been proposed to explain such data (reviewed in Conti et al. 2014): (1) PDEs function to keep cAMP from leaving signaling compartments; or (2) PDEs maintain cAMP to minimal levels within subdomains to prevent activation of downstream signaling (Terrin et al. 2006).

PDEs exhibit a wide range of subcellular locations that may contribute to their role in cAMP compartmentalization, with many displaying signal-driven translocations and interactions with macromolecular complexes. Thus, even low expressing PDEs targeted to the appropriate location may play a significant role in downstream signaling. In this context, subcellular localization of PDEs adds an additional layer of complexity to cAMP cellular action by directing a degradation activity to specific location of downstream effectors. Thus, not only will each PDE isoform act on specific ranges of cellular cAMP concentration but also at a defined location, and only affecting a subset of PKA or EPAC activities.

PDEs employ various localization mechanisms, and this very much depends on isoform identity as even splice variants derived from the same PDE gene may utilize diverse subcellular targeting strategies. For instance PDE2A1 is cytosolic, while splice variant PDE2A3 contains myristoylation sites in its N-terminal region that allow association with synaptic membrane regions, whereas PDE2A2 associates directly with the plasma membrane via a hydrophobic motif (Russwurm et al. 2009; Yang et al. 1994). A great number of PDEs exhibit specific subcellular targeting by interacting with scaffolds. In particular, members of the PDE4, PDE7 and PDE10 families interact with scaffolds to bring them in close proximity to other signaling intermediates, such as protein kinases, protein phosphatases, and GTPases allowing further means of efficient crosstalk and downstream regulation. Several members of the A Kinase Anchoring Proteins (AKAP) scaffold family bind to both PKA and PDEs (Carlisle Michel et al. 2004; Dodge et al. 2001; Terrenoire et al. 2009). Moreover, some interactions with scaffolds can be dynamic and signal-driven, as seen with the interaction of PDE10A with AKAP150. PDE10A is enriched in membranes of spines and dendrites and found mostly palmitoylated and in association with AKAP150 (Charych et al. 2010; Kotera et al. 2004; Xie et al. 2006). Upon PKA phosphorylation of PDE10A, the affinity of PDE10A for AKAP150 is reduced, promoting its dissociation from the AKAP150 complex (Russwurm et al. 2015).

Unlike PKA phosphorylation of PDE4, PDE10 phosphorylation by PKA has no effect on its catalytic activity. Hence, this additional spatial dimension to PDEs places their specific range of catalytic activity within a local cAMP pool and neighboring downstream effectors.

## 1.5 Conclusions

The contribution of each PDE isoform to cAMP signaling is context specific: the role of a PDE under basal conditions may be significantly different than in receptor activated conditions due to their kinetic properties. Regulation of PDE activity and localization demonstrate the complex series of controls that serve not only to tune the intensity of local cAMP signaling but also how this signaling can be effectively funneled to different downstream intracellular targets.

Significant advances in cyclic nucleotide imaging have started to elucidate the spatial aspect of cAMP signaling controlled by PDEs. However, much work remains to be done. Although the identity of local cAMP functional domains controlled by PDEs can be discerned by monitoring downstream signaling, current experimental methods lack the resolution to image these domains raising into question their nature. Computational studies have also provided mechanistic detail into the role of PDEs in cAMP signaling and highlighted their significant potential for signaling integration across time and space.

Understanding the diversity of cellular PDEs may provide novel mechanistic insight into designing therapeutic strategies for psychiatric disorders involving dopaminergic dysregulation, such as drug addiction, Parkinson's disease and schizophrenia.

**Conflict of Interest** The author declares no conflicts of interest.

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# Chapter 2
# Current Understanding of PDE10A in the Modulation of Basal Ganglia Circuitry

**Jan-Philip Schülke and Nicholas J. Brandon**

**Abstract** The basal ganglia are a forebrain network of interconnected nuclei that are involved in action selection, reward circuits and coordinating movement. PDE10A inhibition has been proposed as a novel way to modulate basal ganglia circuitry and to ameliorate symptoms in Huntington's disease, Parkinson's disease and Schizophrenia. However, despite encouraging results from pre-clinical models, PDE10A inhibitors failed to show efficacy as an antipsychotic in several clinical trials. PDE10A is expressed in the medium spiny neurons of the striatum and works to limit cyclic nucleotide signaling in response to modulatory neurotransmitters like dopamine. In this chapter, we will review the current literature on PDE10A and discuss how inhibition of PDE10A will result in alterations of the basal ganglia circuitry at the biochemical, physiological and behavioral level.

**Keywords** PDE10A • Basal ganglia • Schizophrenia • Huntington's Disease

## 2.1 Introduction

Cyclic nucleotides are second messengers that serve as signal transduction molecules mediating intracellular adaptive changes to extracellular cues. They are involved in the integration of multiple signals and regulate a variety of physiological processes throughout the whole body (Beavo and Brunton 2002; Hardman et al. 1971). In the central nervous system (CNS), cyclic nucleotides are involved in transducing neurotransmitter signals that are modulating neuronal activity and ultimately behavior (Xu et al. 2011). Synthesis of the two major cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) is catalyzed

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by adenylyl- and guanylyl-cyclases respectively, which convert ATP or GTP to their respective 3′,5′-cyclic nucleotide monophosphate and pyrophosphate. GPCRs serve as the signaling input and can control the activity of adenylyl-cyclases, which catalyze the production of cyclic adenosine monophosphate (cAMP) (Vassilatis et al. 2003). Levels and subcellular distribution of cAMP and cGMP are tightly regulated by phosphodiesterases (PDEs) that degrade cyclic nucleotides by hydrolyzing their 3′-phosphodiester bond and PDE inhibitors have been shown to be effective drugs in various indications (Maurice et al. 2014). For example the PDE3 inhibitors Enoximone, Imamrinone and Milrinone are used to treat heart failure and hypertension and Cilostazol is used to treat thrombosis (Endoh and Hori 2006). PDE4 inhibitors are approved to treat inflammatory conditions like Apremilast that is approved for psoriasis and psoriatic arthritis, Roflumilast for chronic obstructive pulmonary disease (COPD) and Ibudilast, a moderately selective PDE4 inhibitor that is used to treat bronchial asthma (Gavaldà and Roberts 2013; Huang et al. 2006). Selective PDE5 inhibitors like sildenafil, vardenafil, avanafil, udenafil, mirodenafil, tadalafil are recognized for its clinical and commercial success in treating erectile dysfunction.

Selective PDE5 inhibitors like Sildenafil, Vardenafil, Avanafil, Udenafil, Mirodenafil, Tadalafil are recognized for its clinical and commercial success in treating asthma, chronic obstructive pulmonary disease (COPD), pulmonary hypertension, cardiac failure, autoimmune diseases and erectile dysfunction (Maurice et al. 2014).

PDEs are a large superfamily of isozymes that differ in their substrate specificity, domain organization, tissue distribution and subcellular localization (Beavo et al. 2010). Among the 11 PDE family members are the cAMP selective PDEs 4, 7 and 8, the cGMP selective PDEs 5, 6 and 9, as well as dual specific PDEs 1, 2, 3, 10 and 11. Gene duplication, alternative transcriptional start sites and alternative splicing give rise to a wide variety of isoforms that further contribute to the complexity of regulating cyclic nucleotide signaling on multiple levels (Beavo et al. 2010). These isoforms display unique expression patterns across different tissues, which potentially allows targeting cyclic nucleotide levels in selective physiologically processes (Francis et al. 2011). Since all 11 PDE family members have been detected in neuronal tissues, PDE inhibitors are being pursued as potential treatments for psychiatric and neurodegenerative diseases (Kelly 2014; Kelly et al. 2014; Menniti et al. 2006). The dual specific phosphodiesterase 10 A (PDE10A) is highly enriched in a brain structure called the striatum that is part of the basal ganglia (Seeger et al. 2003).

Basal ganglia circuits are involved in a variety of neurological diseases like the neurodegenerative diseases Parkinson's disease and Huntington's disease and also psychiatric conditions like Schizophrenia, Autism-Spectrum disorders, Tourette syndrome and addiction (DeLong and Wichmann 2007). Therefore, based simplistically on distribution, PDE10A has been considered a potential therapeutic target for diseases of the basal ganglia (Wilson and Brandon 2015). Pfizer's PDE10A inhibitor MP-10 (PF-2545920) has been tested in several phase II clinical trials for schizophrenia, both as a monotherapy and an adjunctive treatment (ClinicalTrials.gov: NCT01175135, NCT01939548). In all studies it failed to meet its primary endpoint (DeMartinis et al. 2012; Verhoest et al. 2009). The fact that the preclinical data for MP-10 was supporting the hypothesis that PDE10A inhibition would prove antipsychotic, challenges our use of behavioral models to test novel mechanisms for psychiatric and behavioral disorders

(Dunlop and Brandon 2015). Besides Pfizer, many other pharmaceutical companies are pursuing the development of PDE10A inhibitors for a variety of disease indications (Chappie et al. 2012). In this chapter, we will review studies of PDE10A biology and relate the findings to the basal ganglia circuitry and its diseases.

## 2.2 The Role of the Striatum as Part of the Basal Ganglia

### *2.2.1 Anatomy and Circuitry of the Basal Ganglia*

The basal ganglia are a large subcortical structure of the forebrain, which is involved in action selection and coordination of movements (DeLong and Wichmann 2007; Graybiel 2000; Redgrave et al. 1999). The majority of cortical layer 5 pyramidal neurons project to the striatum, collectively comprised of the caudate and putamen in primates. This is the main input area of the basal ganglia, while output nuclei of the basal ganglia project to the ventral anterior nucleus (VA) and ventral lateral nucleus (VL) of the thalamus and also to the superior colliculus and the pedunculopontine nucleus (PPN) of the brainstem (Wilson 1994). In general terms, these cortico-striatal-thalamic connections can be viewed as a filter in which cortical outputs are modulated based on additional contextual information. It is important to note that the basal ganglia in general and also the striatum in particular are topographically and functionally segregated. For example, different cortical areas that get filtered through the basal ganglia terminate in specific thalamic regions, which project back to the same areas of the cortex forming segregated reentrant loops. In addition, the striatum receives modulatory stimuli from distinct midbrain regions that function to influence different behavioral domains (DeLong and Wichmann 2007; Simpson et al. 2010). The majority (~90–95%) of the cells in the striatum, that receive glutamatergic input from the cortex and also from the thalamus, are GABAergic medium spiny neurons (MSN). The MSNs are interconnected by aspiny interneurons that form microcircuits in the striatum and can be categorized into medium-sized GABAergic and large cholinergic interneurons (Kreitzer 2009). MSNs are subdivided based on their connectivity to other basal ganglia nuclei. Direct pathway (striatonigral) neurons project directly to the main output regions of the basal ganglia, the internal segment of the globus pallidus (GPi), which project to the VA/VL nuclei of the thalamus and the substantia nigra pars reticulata (SNr) that project to the superior colliculus. Indirect pathway (striatopallidal) neurons project indirectly to the output nuclei through connections in the external (lateral) segment of the global pallidus (GPe) and the subthalamic nucleus (STN) (DeLong and Wichmann 2007; Graybiel 2000).

The activity of the MSN can be modulated by dopaminergic inputs from midbrain regions. As indicated above, the striatum can be anatomically divided into functionally distinct regions that differ in their connectivity and modulatory inputs. The nucleus accumbens is the ventral most part of the striatum and sometimes considered a subdivision of the striatum that receives dopaminergic inputs from the ventral tegmental area (VTA) in the mesencephalon/midbrain (mesolimbic pathway).

This region is often associated with attention and reward behavior and shows a degree of functional segregation from dorsal parts of striatum that are often described to be involved in coordination of motor function. In contrast to the NAc that receives input from the VTA, the MSN neurons in the dorsal striatum are modulated by the activity of the dopaminergic neurons projecting from the substantia nigra (nigrostriatal pathway), which are the neurons degenerating during Parkinson's disease (Nicola 2007; Simpson et al. 2010). The dorsal striatum can further be subdivided into the dorsomedial striatum (caudate nucleus in primates) and the dorsolateral part (putamen in primates) that receives inputs from functional different regions of the cortex (Kreitzer 2009).

### *2.2.2 Properties of Striatal Medium Spiny Neurons*

The striatonigral and striatopallidal MSNs of the striatum can not only be distinguished by their axonal projection targets, but also by their biochemical and physiological properties and their impact on behavior. Striatonigral MSNs are classically characterized by expression of the GPCRs dopamine 1 receptor (D1R) and muscarinic M4 receptor (Chrm4), and by the neuropeptides substance P and dynorphin. In contrast, striatopallidal neurons express the dopamine 2 (D2R) and adenosine A2A receptors as well as the neuropeptide enkephalin (Gerfen 1992; Hersch et al. 1995; Ince et al. 1997; Schiffmann and Vanderhaeghen 1993). Other studies further characterized these neuronal populations on the transcriptional level by separating differential labelled MSN of the direct and indirect pathway by FACS sorting (Ena et al. 2013; Heiman et al. 2008; Lobo et al. 2006).

The striatum is heavily innervated by dopaminergic efferents from different midbrain regions as described above (Prensa and Parent 2001). But based on expression of the different receptor subtypes, dopamine has a differential effect on the activity of the striatonigral and striatopallidal neuron population. D1Rs, expressed in the striatonigral MSN, are coupled to the stimulatory α-subunit of G-proteins (Gαs/Gαolf), whereas the D2Rs are coupled to inhibitory Gαi proteins that activate or block adenylate cyclase activity (Zhuang et al. 2000). Therefore, dopamine release increases cAMP and excitability in direct pathway MSNs while decreasing cAMP and reducing activity of the indirect pathway. The balance between direct and indirect pathway activity plays a pivotal role in motor control (Graybiel 2000). Activation of direct pathway neurons disinhibits the striatonigral pathway by reducing the activity of inhibitory GABAergic neurons in the substantia nigra pars reticulata (Chevalier et al. 1985; Deniau and Chevalier 1985). Consistent with its documented role in controlling movement, optogenetic stimulation of the direct pathway neurons increased locomotor activity while activation of indirect pathway neurons resulted in inhibition of movement (Kravitz et al. 2010). Conversely, cell type specific knockout of the dopamine mediator DARPP-32, resulted in similar conclusions with regard to the roles of the MSN population in controlling locomotion (Bateup et al. 2008). Hence, direct

activation of direct pathway neurons facilitates intended movement (go pathway), while activation of the indirect pathways suppresses unwanted movement (no-go pathway). Besides its well-recognized role in controlling motor function, the direct and indirect pathways in the striatum have been shown to be involved in regulating reward circuitry and addiction. Especially the nucleus accumbens, which is a ventral region of the striatum regulated by mesolimbic pathways, is believed to be an integrator of motor and limbic inputs and involved in mood disorders (Krishnan and Nestler 2008; Nicola 2007; Russo and Nestler 2013). It has been shown that modulating activities of the direct striatonigral MSN is associated with reward related tasks like conditioned place preference, whereas indirect striatopallidal MSN are mediating aversive behaviors. Furthermore, activating indirect pathway neurons counteracted cocaine self-administration and therefore promotes resilience towards compulsive reward seeking behavior (Bock et al. 2013; Hikida et al. 2010; Kravitz et al. 2012). Akin to the simple analogy of the *go* and *no-go* pathway in motor function, the direct pathway could therefore also being considered as the *reward* and the indirect pathway as the *punishment* pathway.

What are the actitvites of these pathways under basal conditions? The dopaminergic neurons of the midbrain are spontaneously active at low frequencies (Schultz 2007). D2 receptors have higher affinity for dopamine than the D1 receptor, and are therefore activated at lower threshold levels of dopamine release (Beaulieu and Gainetdinov 2011; Richfield et al. 1989). Since D2 receptors are Gi coupled, and D1 receptors Gs coupled, one might assume that low dopamine levels would lead to less active indirect pathway neurons than those in the direct pathway. However, the opposite seems to be the case. Striatopallidal/indirect MSN show increased excitability compared to striatal-nigral neurons. D2 positive indirect pathway neurons have higher average firing rates than D1-positive direct pathway neurons, when stimulated optogenetically or in response to current injections (Kravitz et al. 2010; Kreitzer and Malenka 2007). What accounts for these physiological differences of the MSNs? D1 neurons have a longer average total dendrite length and a more complex arborization compared to D2 expressing indirect pathway neurons (Gertler et al. 2008). This difference in length, while still having the same spine density, will result in 50% more glutamatergic input to the direct pathway than to the indirect pathway. Therefore, these differences could account to indirect MSN being more excitable than direct pathway neurons (Gerfen and Surmeier 2011). Besides its structural differences, direct and indirect pathway neurons can also be divided by their biochemical properties and expression of signaling molecules, which are likely contributing to these physiological differences (Ena et al. 2013; Lobo et al. 2006). As indicated above, cyclic nucleotide levels are an integral part of the cell signaling cascade and directly influence excitability of the MSNs. In the following section we will discuss the role of PDEs of the basal ganglia in general and in particular the contributions of PDE10A, which is the major cyclic nucleotide degrading enzyme in the striatum.

## 2.3 Expression of Phosphodiesterases in the Basal Ganglia

### *2.3.1 Expression of the Striatal Enriched PDEs*

Kelly and colleagues have published two comprehensive studies comparing the expression levels of PDEs in the rodent brain and their changes during aging (Kelly 2014; Kelly et al. 2014). This study revealed that mRNA of all PDE family members (except PDE6) can be detected in the brain. PDE10A, PDE1B, PDE7B are the phosphodiesterases that are most highly enriched in striatum (Lakics et al. 2010). Moderate mRNA levels of PDE4B and PDE8B and low levels of PDE1A, 1C, 2A, 4A and 9A are detected as well and may contribute to cyclic nucleotide metabolism in the striatum and also in other brain regions where these PDEs are expressed as well (Kelly et al. 2014; Menniti et al. 2006). Interestingly, the expression levels of several PDEs change during normal aging and during disease progression, suggesting that adaptive mechanisms are responding to changes in cyclic nucleotide metabolism during aging and disease. For example, the expression of PDE1C and PDE8B in the striatum increases during aging (Kelly et al. 2014). While PDE10A levels remain relatively stable during aging, it is now evident that a loss of PDE10A enzyme levels precedes symptomatic manifestations in Parkinson's and Huntington's disease (Hebb et al. 2004; Ooms et al. 2014; Wood 2015).

Since each cell expresses a number of different phosphodiesterases, it is plausible that one phosphodiesterase can compensate for a loss or inhibition of another to restore baseline cyclic nucleotide levels. PDE10A and PDE1B both are dual specific phosphodiesterases, which are enriched in the striatum and could therefore have overlapping functions. In studies comparing the relative effect of these striatal PDEs on cyclic nucleotide levels, it was found that PDE10A is the major degrading enzyme for cAMP whereas PDE1B inhibition shows the strongest effect on cGMP hydrolysis among the tested enzymes (Russwurm et al. 2015). Although probably expressed at lower levels compared to PDE10A and PDE1B, the dual specific phosphodiesterase PDE2 has been shown to be present in synaptic preparations alongside PDE10A and might contribute to the degradation of cAMP and cGMP levels in the striatum as well (Lin et al. 2010; Russwurm et al. 2015; Xie et al. 2006). Supporting the hypothesis that PDE10A and PDE1B have complementary functions, knockout mice for each of these phosphodiesterases show opposing phenotypes. PDE10A knockout mice show decreased exploratory activity whereas PDE1B knockout mice show a hyperlocomotor phenotype (Reed et al. 2002; Siuciak et al. 2006b, 2007).

### *2.3.2 Expression of PDE10A in MSNs of the Striatum*

While PDE10A is mostly expressed in the CNS, moderate to low levels have also been detected in a variety of peripheral tissues like testis, pineal-gland, retina, pancreatic islets and supraclavicular brown adipose tissue (Coskran et al. 2006; Fujishige et al. 1999; Hankir et al. 2016; Heimann et al. 2010; Seeger et al. 2003;

Soderling et al. 1999; Spiwoks-Becker et al. 2011; Wolloscheck et al. 2011). Within the CNS, PDE10A expression is highest in the caudate nucleus, nucleus accumbens, substantia nigra, globus pallidus and the olfactory tubercle that lays ventral to the NAc and contains a component of GABAergic MSNs. Even though PDE10A has been shown to be expressed outside the basal ganglia in the cortex, hippocampus and granule cell layer of the cerebellum, PDE10A seems to be primarily positioned to modulate basal ganglia circuitry (Coskran et al. 2006; Meyer et al. 1989; Seeger et al. 2003).

In the basal ganglia PDE10A expression has been shown to localize to fibers and terminals while expression in other brain regions appear to be primarily nuclear (Coskran et al. 2006; Seeger et al. 2003). In synaptic preparations from striatal tissues, PDE10A is enriched together with synaptic proteins like PSD-95 and Synapsin. Furthermore, PDE10A has been shown to be associated with PSD-95, AKAP150 and the NMDA receptor subunits NR2A and NR2B indicating that PDE10A is part of a signaling complex in the postsynaptic density (PSD) (Russwurm et al. 2015). Electron microscopy studies further support that PDE10A is located close to the PSD in synaptic spines (Xie et al. 2006). The PDE10A gene gives rise to expression of a number of gene products (PDE10A1-PDE10A18) due to alternative transcriptional start sites and alternative splicing (Fig. 2.1) (Strick et al. 2006). These variants primarily differ in their N-terminal amino-acid sequence, which can result in alterations in their subcellular localization (Charych and Brandon 2014). Preparations from striatal tissues have consistently shown that the PDE10A protein is enriched in membrane preparations (Charych et al. 2010; Kotera et al. 2004; Schülke et al. 2014). This membrane insertion is dependent on a particular N-terminal amino acid sequence containing a CFRRLT motif, which is present in particular PDE10A variants (Fig. 2.1). It has been shown that PDE10A is palmitylated on Cys11 of the CFRRLT motif, which serves as an anchor for the membrane localization. Furthermore, membrane insertion is dependent on the phosphorylation status of Thr16 (Charych et al. 2010; Kotera et al. 2004). It is important to note that the nomenclature of the variant describing the membrane associated form is not

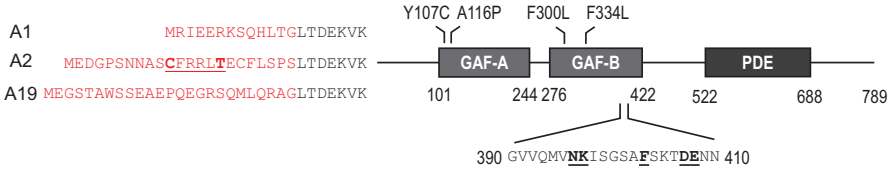


**Fig. 2.1** Schematic representation of PDE10A. The N-terminal sequence variation in the different isoforms PDE10A1, PDE10A2 and the novel, primate specific isoform PDE10A19 (MacMullen et al. 2016) are highlighted in red. Numbering of the amino acid residues refers to the human PDE10A2 isoform (Uniprot Q9Y233-2) that contains the N-terminal CFRRLT sequence (underlined). Cysteine 11 residue that can be palmitylated and the threonine 16 phospho-residue within the CFRRLT sequence are highlighted in bold (Charych et al. 2010). Movement disorder causing mutations in the GAF-A (Diggle et al. 2016) and GAF-B (Mencacci et al. 2016) domains of PDE10A gene are indicated

consistent between the different resources. The uniprot database (www.uniprot.org) refers to the isoform containing the CFRRLT motif as isoform 2 (PDE10A2) in human (Q9Y233-2), isoform 1 in rat (Q9QYJ6-1) and isoform 3 in mouse (Q8CA95-3), whereas the literature generally refers to PDE10A2 as the membrane bound variant across species. Regardless of nomenclature, the variant containing the N-terminal CFRRLT sequence, has been shown in many studies to be the major isoform and suggests that primarily membrane-bound PDE10A contributes to cyclic nucleotide metabolism in the striatum (Charych et al. 2010; Kotera et al. 2004). A recent paper reports the observation of a novel PDE10A gene product with a unique N-terminal sequence only identified in primates (Fig. 2.1) (MacMullen et al. 2016). This isoform, named PDE10A19, lacks the N-terminal cysteine residue for membrane localization and was shown to be localized in the cytosol. Based on the number of next-generation sequencing reads, the PDE10A19 isoform is expressed to similar levels than PDE10A2, while RT-PCR experiments in the same study showed PDE10A19 to be expressed at lower levels compared to PDE10A2. Therefore, further studies are needed to evaluate the relative contribution of the different PDE10A isoforms on cyclic nucleotide metabolism in the human brain.

## 2.4 Role of PDE10A in Modulating Basal Ganglia Circuitry

### *2.4.1 Cellular Effects of PDE10A on Cyclic Nucleotide Signaling*

As a dual specific phosphodiesterase PDE10A has been shown to modulate signaling of the cyclic nucleotides cAMP and cGMP. GPCRs coupled to stimulatory G proteins (Gs) activate adenylate cyclases that catalyze the formation of cAMP from ATP (Vassilatis et al. 2003). In contrast, the formation of cGMP is catalyzed by guanylate cyclases (GC) that are activated by a variety of stimuli. Particulate GCs are membrane bound and activated by natriuretic peptides and soluble GCs most notably by nitric oxide (NO) (Lucas et al. 2000). These cyclic nucleotides influence a number of downstream signaling events as part of the adaptive process to extracellular stimuli. cAMP binds and activates protein kinase A (PKA), exchange proteins directly activated by cAMP (Epacs) and cyclic nucleotide-gated ion channels (CNG) which in turns modulates a wide variety of cellular processes (Beaulieu and Gainetdinov 2011). Epacs have been identified as activators of guanine nucleotide exchange factor Rap1 and shown to be involved in synapse remodeling upon dopamine signaling (Kawasaki et al. 1998; de Rooij et al. 1998; Woolfrey et al. 2009). CNGs are known for their role in mediating cyclic nucleotide signaling in photoreceptor and olfactory receptor neurons but are now also recognized to regulate other functions in the CNS (Podda and Grassi 2014). However, the impact of PDE10A influencing cyclic nucleotide signaling through these pathways is less

well understood. In the striatum, the dopamine and cyclic AMP-regulated phosphoprotein (DARPP-32) is highly expressed in all MSNs and is a major target of PKA-mediated phosphorylation upon DA stimulation. DARPP-32 is also phosphorylated by the cGMP-activated protein kinase G (PKG) and positioned to integrate a variety of signals (Svenningsson et al. 2004). When phosphorylated by PKA or PKG on Thr34, DARPP-32 is converted into a potent inhibitor of protein phosphatase-1 (PP-1) that, when active, counteracts PKA activity by dephosphorylating PKA targets. This positive feedback loop serves to amplify the activation of downstream signaling cascades to cyclic nucleotides and allows that transient changes in the cyclic nucleotide levels are translated into a defined cellular response. Cyclic nucleotide signaling also leads to phosphorylation changes of a variety of other cellular targets and activation of the MAPK/ERK pathway through disinhibition of MEK by PP-1. Among the most studied downstream effects of cyclic nucleotide activation are phosphorylation of GluR1-Ser845, Erk1-Thr202/Tyr204, Erk2-Thr185/Tyr187, CaMKII-Thr286 and increased expression changes of immediate early genes like cFos mediated through phosphorylating Histone-H3-Ser10 and the transcription factor CREB-Ser133 (Greengard 2001; Nishi et al. 2011). In line with the function of PDE10A in regulating cyclic nucleotide levels in MSNs, inhibition of PDE10A increases cellular cAMP and cGMP levels and leads to increases in phosphorylation of downstream substrates. In vivo administration of the potent and selective PDE10A inhibitor TP-10 increased cAMP and cGMP levels in a dose dependent manner with the cyclic nucleotide signal reaching its maximum 1 h post injection (Schmidt et al. 2008). Phosphorylation of CREB upon PDE10A inhibition reached its maximum after 30 min supporting the mechanism that signal transduction through DARPP-32 potentiates the effect on downstream substrates (Schmidt et al. 2008). The clinical PDE10A inhibitor MP-10 was also shown to increase phosphorylation of the PKA substrates DARPP-32, CREB and GluR1 (Grauer et al. 2009). Furthermore, TP-10 also significantly induced expression of a CREB reporter gene in vivo and increased phosphorylation of H3-Ser10, pERK and pMEK (Kleiman et al. 2011). The effect on cAMP-PKA substrates was also shown ex vivo by using another tool inhibitor of PDE10A. Papaverine applied to striatal slices induced phosphorylation of DARPP-32-Thr34, GluR1-Ser845 and Erk-Thr202/Tyr204 at sub-micromolar concentrations. In contrast, the presynaptic targets of cyclic nucleotide signaling, tyrosine hydroxylase (TH)-Ser40 and Synapsin 1-Ser9, were only marginally increased at high concentrations of the inhibitor whereas inhibition of PDE4, which is localized in synaptic terminals, lead to a robust increase of TH-Ser40 and Synapsin 1-Ser9 phosphorylation (Nishi et al. 2008). Thus, this evidence supports a role for PDE10A in modulating cyclic nucleotide signaling in MSNs of the striatum from a primarily postsynaptic localization.

### 2.4.2 *Differential Effects of PDE10A Inhibition Between Striatonigral and Striatopallidal Neurons*

PDE10A is expressed at similar levels in both, striatonigral and striatopallidal neurons. This suggests that PDE10A inhibition will mediate similar cellular effects on both neuronal populations. However, given the biochemical and physiological differences between direct and indirect pathway neurons, the physiological effects caused by PDE10A inhibition might be a result of a more complex interplay of signaling pathways. A study by Polito and colleagues analyzed differential effects of PDE10A inhibition using FRET-based biosensors (Polito et al. 2015). Using the cAMP sensitive EPAC-FRET sensor, they showed that bath application of the PDE10A inhibitor PQ-10 resulted in the same dose dependent increase of the FRET signal in direct and indirect pathway neurons, suggesting that PDE10A regulates cAMP levels in both MSN populations similar. However, using the AKAR3-FRET biosensor to monitor PKA-dependent phosphorylation, the authors found that PDE10A inhibition results in an increased PKA activity only in A2A-receptor expressing neurons. This indicates that despite similar expression levels and regulation of cAMP, the downstream effects of these increased cyclic nucleotide levels through the PKA-pathway are only reflected in striatopallidal neurons. In fact there have been several reports that suggest that the changes mediated by PDE10A inhibition are more pronounced in striatopallidal than in striatonigral MSNs. Nishi et al. (2008) used mice in which the cAMP mediator DARPP-32 was tagged with FLAG or Myc in direct and indirect pathway neurons respectively, to analyze differential pathway activation upon phosphodiesterase inhibition. They observed, that upon treatment with the PDE10A inhibitor Papaverine, the increase in phosphorylation on DARPP-32-Thr34 was greater in D2R-expressing indirect pathway neurons than in direct pathway neurons expressing the D1R. Furthermore, blockade of Papaverine-mediated DARPP-32 T34 phosphorylation is reduced to a greater extent by ZM241385 (A2A receptor antagonist, reducing cAMP in indirect pathway neurons) than by SCH23390 (D1 antagonist, reducing cAMP in direct pathway neurons) indicating a preferential effect of Papaverine in striatopallidal MSNs. However, in the same study, Papaverine also potentiated cAMP downstream effects (DARPP-32-Thr34 phosphorylation) of both, a D1R agonist (SKF-81297) and of an A2A-receptor agonists (CGS21680) suggesting that PDE10A inhibition affects both pathways but perhaps threshold levels to activate downstream signaling events differ between the two MSN populations (Nishi et al. 2008). Papaverine was also shown to induce phosphorylation of Erk1/2. This effect was potentiated when combined with the D2R-anatagonist Sulpiride, but phosphorylation was affected to a lesser degree when combined with the D1R-agonist SKF-38393 (Hsu et al. 2011). Interestingly, this effect was only present in male or in ovariectomized female rats, but not in control female rats suggesting that circulating levels of estrogen, which is a known inducer of synaptic plasticity (Liu et al. 2008), regulate sensitivity of the MSNs to activation of the cyclic nucleotide signaling cascade.

The preferential effect on indirect pathway neurons was also observed during electrophysiological recordings in the dorsal striatum. Threlfell et al. (2009) used antidromic stimulation to identify striatonigral MSNs. They showed that the PDE10A inhibitor TP-10 increased cortically evoked activity only in MSNs that did not show an antidromic response and concluded that TP-10 effects primarily the activity of indirect pathway MSNs (Threlfell et al. 2009). In another study, PDE10A inhibition alone did not change output of MSN to the substantia nigra but the authors showed that these direct pathway neurons are responsive if this treatment is combined with a D1R agonist (Mango et al. 2014). This suggests that PDE10A inhibition effects also the direct pathway but the threshold of response is greater in these striatonigral neurons. Also, reports looking at downstream transcriptional targets of cyclic nucleotide signaling indicate that PDE10A inhibition acts through both pathways. Systemic administration of the clinical PDE10A inhibitor MP-10 increased transcript levels of both, enkephalin and substance P, while haloperidol only increased expression of enkephalin, which is indicative of the D2R antagonistic effect of this antipsychotic drug in indirect pathway neurons (Gentzel et al. 2015; Grauer et al. 2009; Strick et al. 2010). Interestingly, this differential effect of PDE10A inhibition between direct and indirect pathway neurons seems to depend on the striatal sub-region and its specific in vivo connectivity. Serine 10 phosphorylation of histone H3 (H3-Ser10) can be indicative of cAMP pathway activation since this residue can be phosphorylated by PKA (Taylor 1982). In line with a preferential effect of PDE10A inhibition on indirect pathway neurons, in vivo administration of TP-10, resulted in an increased phosphorylation of histone H3-Ser10 in striatopallidal neurons. However, this selective activation of H3-Ser10 was only seen in the dorsomedial striatum. In the dorsolateral striatum, both MSN populations showed increased H3-Ser10 phosphorylation upon PDE10A inhibition (Polito et al. 2015). Other reports, investigating the expression of the cyclic nucleotide-dependent immediate early gene c-Fos, found a greater number of c-Fos, arc and egr-1 positive neurons in the dorsolateral striatum compared to the dorsomedial striatum after PDE10A inhibition (Gentzel et al. 2015; Wilson et al. 2015). Even though the authors did not differentiate specifically between direct and indirect pathway neurons using cell-type specific labels, it is intriguing to speculate that the greater number of positive nuclei in the dorsolateral striatum is a result of increase in sensitivity of direct pathway neurons in response to activation of cyclic nucleotide signaling.

What causes this differential effect? Thus, while it is evident that PDE10A inhibition raises cAMP levels in both MSN populations, it is plausible that differences in the excitability of striatopallidal and striatonigral MSNs are driving the differences in the downstream activation of the cAMP pathway by PDE10A. Differences regarding direct and indirect pathway neuron activation might also be attributed to the way this activation was measured. If measured with a downstream marker (c-FOS, P-CREB etc) there is a significant amount of amplification of the signal. This is also observed through the fact that TP-10 treatments leads to a peak in the P-CREB signal preceding that of the cAMP peak, even though P-CREB is considered (“downstream”) of cAMP (Schmidt et al. 2008). Polito and colleagues suggest

that a lower phosphorylation of DARPP-32 at Thr34 could mitigate the downstream effects of the cAMP signal in direct pathway neurons compared to indirect pathway neurons. However, this effect seems to be specific to PDE10A inhibition since the cAMP-PKA response is functional to similar levels in both MSN populations in the experiments using the FRET-based PKA sensor because FSK/IBMX increased PKA activity in both neuronal populations (Polito et al. 2015). Indeed, DARPP-32 is a signal integrator of multiple pathways and also necessary to mediate many of the physiological effects of dopamine (Fienberg et al. 1998). Its activity is modulated by phosphorylation on different residues and DARPP-32 can serve as a potent inhibitor of PP1, when phosphorylated at Thr34, or as an inhibitor of the cAMP-PKA pathway when phosphorylated at Thr75. Phosphorylated Ser102 and Ser137 on DARPP-32 residues potentiate Thr34 phosphorylation (Svenningsson et al. 2004). These residues are phosphorylated at high levels under basal conditions, which indicates that DARPP-32 should be sensitive for Thr34 phosphorylation and therefore a responsive cAMP effect. The psychostimulants cocaine, caffeine, clozapine and haloperidol have a differential effect on Thr34 and Thr75 phosphorylation in D1R- or D2R expressing neurons. Intriguingly, haloperidol shows a selective increase of DARPP-32-Thr34 phosphorylation and confirming that PDE10A inhibition mimics the effect of D2R-antagonism on this particular residue (Bateup et al. 2008). Even though the induction of DARPP-32 phosphorylation on the Thr34 residues upon PDE10A inhibition was shown to be more pronounced in D2R-expressing neurons, basal phosphorylation levels on the Thr34 and Thr75 residues seems to be similar. (Bateup et al. 2008; Nishi et al. 2008). It is interesting to note that in striatal slices, while PDE10A inhibition could increase DARPP-32-Thr34 phosphorylation and the D2R-agonist Quinpirole could decrease it, antagonizing D2Rs using Raclopride alone, or in combination with Papverine, could not increase basal phosphorylation of DARPP-32-Thr34 (Nishi et al. 2008). This suggests that in the absence of dopamine ex vivo, PDE10A inhibitors exert their stimulatory effect independent of concomitant dopamine stimulation, in contrast to classical D2R-antagonist antipsychotics that modulate endogenous dopamine signals. Therefore, this disconnect of the PKA activation between in direct and indirect pathway neurons seems to be specific for PDE10A and might reflect differences in subcellular localization and/or incorporation into signaling complexes of PDE10A in these neuronal populations.

PDE10A2, the major splice variant of PDE10A, contains a threonine residue at position 16 (Thr16) that can be phosphorylated by protein kinase A (PKA) and dephosphorylated by PP2A and/or PP1 but not PP2B (calcineurin) (Kotera et al. 1999; Russwurm et al. 2015). Under basal conditions, PDE10A2 phosphorylation on Thr16 is low but can be induced through activating PKA by increasing cAMP levels. Phosphorylation at Thr16 does not change its enzymatic activity to hydrolyze cAMP, but prevents membrane localization of newly synthesized PDE10A2 and association with synaptic proteins AKAP150, NR2A, NR2B and PDS95 (Charych et al. 2010; Russwurm et al. 2015).

The membrane localization of PDE10A also seems to play a particular role in reinforcement plasticity of synaptic connections in MSNs. Yagishita and col-

leagues showed that the enlargement of synaptic spines by dopamine as part of reinforcement structural plasticity in MSNs only occurs if the concomitant dopamine stimulation occurred in a defined time window after glutamatergic stimulation (Yagishita et al. 2014). This effect was dependent on PKA activation and the downstream disinhibition of CamKII, as well as adenylate cyclase 1 stimulation through NMDA mediated $Ca^{2+}$ influx and protein synthesis. PDE10A counteracts this activation through degradation of cAMP necessary for the plasticity response. Due to its membrane localization, the authors hypothesize that PDE10A is particularly effective in the thin distal dendrites because of a high surface to volume ratio. In MSNs, synaptic spines are only present in the distal dendrites and spine enlargement is only observed if the dopamine and NMDA-mediated cAMP increase overcome the activity of PDE10A (Yagishita et al. 2014). Therefore, PDE10A ensures that structural plasticity upon dopamine release only occurs in a defined time-window. Indeed, inhibition of PDE10A using Papaverine, disrupted this time dependent structural plasticity, which suggests that PDE10A impairs the encoding of a dopamine mediated modulation of a NMDA mediated stimuli in reward and reinforcement circuits (Yagishita et al. 2014). This work looked at the effect of dopamine in direct pathway neurons and the strong inhibitory effect of PDE10A in distal spines, together with a longer average dendritic length and pronounced arborization might provide an explanation why direct pathway neurons are less responsive to NMDA evoked potentials. It remains to be seen if PDE10A serves a similar role in indirect pathway MSNs. However, it has been shown previously that induction of long term potentiation (LTP) at glutamatergic terminals in the striatum is dependent on D1R receptors in direct pathway neurons and on A2AR-signalling in the indirect pathway neurons, suggesting that increases in the intracellular cAMP concentrations are needed in both MSN population to mediate adaptive changes to reinforcement stimuli.

### *2.4.3 Contribution of cGMP Signaling*

PDE10A inhibition has been shown to increase the levels of cAMP and cGMP in vitro and in vivo according to its documented function as a dual specific phosphodiesterase, while having a lower affinity for cGMP (Fujishige et al. 1999; Kotera et al. 1999; Loughney et al. 1999; Soderling et al. 1999). Furthermore, the relative increase of these cyclic nucleotides upon systemic PDE10A inhibition is greater for cAMP than for cGMP (Schmidt et al. 2008). While PDE10A hydrolyzes cGMP in vitro, PDE1 and PDE2 seem to be the major cGMP hydrolyzing enzymes in striatal homogenates (Russwurm et al. 2015). However, PDE10A inhibition has been shown to increase responsiveness of MSNs to excitatory corticostriatal transmission driven by stimulation of the frontal cortex (Threlfell et al. 2009). Cyclic GMP elevation was shown to be involved in this effect since TP-10 does not change the firing rate when the neuronal nitric oxide synthase (nNOS) was genetically inactivated or guanylyl cyclase was blocked using the inhibitor

ODQ (Padovan-Neto et al. 2015). Furthermore, PDE10A could potentially also regulate cGMP levels produced by particulate GCs downstream of natriuretic peptides as has been shown for PDE2, 5 and 9 (de Vente et al. 2006). However, PDE10A inhibition does not affect cGMP levels in striatal slices and also the guanylyl cyclase inhibitor QDQ did not block a Papaverine-mediated increase in DARPP-32 Thr34 phosphorylation. This suggests that without the activity of NO releasing interneurons in the striatum and a basic tone of soluble GC activation in the MSN PDE10A inhibition can not increase cGMP and subsequently activate PKG. (Nishi et al. 2008; Polito et al. 2015).

## 2.5 The Physiological Role of PDE10A and Its Relevance in Basal Ganglia Diseases

### *2.5.1 The Role of PDE10A in Regulating Motor Function*

PDE10A has been shown to be involved in the regulation of motor function and coordinated movement. There have been several lines of evidence suggesting altered PDE10A levels and function associated with disease pathology in a number of movement disorders (Wilson and Brandon 2015). A recent genome-wide association study conducted on sporadic Parkinson's disease patients found a SNP (kgp8130520) in proximity of the PDE10A gene on chromosome 6 (SNP location: 166,068,329), associated with an OR of 3.72 (2.75–5.04, 95% CI) (Hu et al. 2015). In another study looking at PD patients, the authors found that loss of PDE10A levels associated with disease progression and severity as demonstrated with a PDE10A specific PET ligand (Niccolini et al. 2015). PDE10A levels were also altered in an animal model for PD. In line with clinical observations, PDE10A transcripts were reduced after elimination of dopaminergic midbrain neurons through injection of 6-hydroxydopamine (Giorgi et al. 2011). This suggests that MSNs respond to alterations in cAMP levels by downregulation of cyclic nucleotide hydrolyzing enzymes. Alterations in cyclic nucleotide levels are also associated with Huntington's disease (HD). Reduced levels of cAMP were found in both animal models of HD and in post mortem brain samples and lymphoblasts from patients with HD (Cramer et al. 1984; Gines et al. 2003). Furthermore, similar to the effects observed in PD, several studies now show that PDE10A levels are downregulated during HD as well. In the transgenic R6/1 and R6/2 animal models of HD that are expressing the mutant form of exon 1 and also in knock-in models of HD, like the Q175 mice, PDE10A transcript and protein levels are significantly reduced even prior detection of motor symptom phenotypes (Hebb et al. 2004, 2008; Hu et al. 2004; Langfelder et al. 2016). Furthermore, this reduction in PDE10A levels are also detected in patients with HD using the PET ligand $^{11}$C–IMA107 derived from the clinical PDE10A inhibitor MP-10 (Ahmad et al. 2014; Plisson et al. 2014). Importantly, in HD patients, the loss of PDE10A was detected before the onset of

pathology and the reduction of PDE10A correlated with disease progression and severity (Niccolini et al. 2015; Russell et al. 2016; Russell et al. 2014).

Two recent studies further highlight the importance of PDE10A in the regulation of coordinated movement (Diggle et al. 2016; Mencacci et al. 2016). These studies independently found evidence that non-synonymous mutations in the human *PDE10A* gene underlie a hyperkinetic movement disorder, which resembles some of the pathological features of early HD. In the study by Diggle and colleagues (Diggle et al. 2016), the authors describe two separate families which carry mutations in a conserved region in the GAF-A domain of PDE10A2 (Tyr107Cys in family 1 and Ala116Pro in family 2; see Fig. 2.1), which were inherited in a recessive fashion. Both mutations were shown to lead to dramatic reductions in PDE10A protein levels and loss of protein was confirmed in one patient from family 1 using a PDE10A PET ligand. The reduction in protein levels and the associated reduction in cyclic nucleotide metabolism were recapitulated in a transgenic knock-in mouse expressing the PDE10A-Tyr107Cys mutation. Importantly, the transgenic mice also displayed impairments in motor function providing strong evidence that alterations in the levels of PDE10A can cause disturbances in motor function. The second study by Mencacci and colleagues (Mencacci et al. 2016) identified three unrelated individuals that carried heterozygous mutations in conserved amino-acid residues in the GAF-B domain (Phe300Leu in two individuals and Phe334Leu in one individual; see Fig. 2.1). Similar to the mutations identified in the GAF-A domain these GAF-B domain mutations lead to a hyperkinetic movement disorder. Interestingly, while no overt changes on overall brain structure could be identified in the MRI scan of an individual with the GAF-A mutation, patients harboring the dominant mutations in the GAF-B displayed symmetrical striatal bilateral lesions similar to those observed in childhood chorea. Furthermore, while the reported mutations did not change the intrinsic activity of PDE10A, the full capacity to hydrolyze cyclic nucleotides is impaired in case of the GAF-A mutations due to the loss in protein levels and for the GAF-B mutation at least due to a loss of the cAMP-mediated activation of the cyclic nucleotide hydrolyzation activity via the GAF-B domain (Diggle et al. 2016; Mencacci et al. 2016).

Behavioral tests further support a direct influence of PDE10A in regulating motor function. Genetic deletion of PDE10A in mice showed a decrease in spontaneous locomotor activity (Siuciak et al. 2006b, 2007). Furthermore, administration of the PDE10A inhibitor Papaverine also resulted in perturbations in motor function (Hebb et al. 2008; Siuciak et al. 2006a). However, another study that independently generated PDE10A knockout mice in a C57BL/6 background strain did not observe a significant effect on locomotor activity but an increase in social interactions, even though the authors observed an increase of striatal cAMP and CREB phosphorylation, suggesting impaired cyclic nucleotide metabolism in these mice (Sano et al. 2008). However, in a subsequent study Siuciak and colleagues verified their initial findings of a locomotor phenotype in C57BL/6 mice, even though the effect size on some locomotor findings were less pronounced than in the KO mice on a DBA background (Siuciak et al. 2008).

According to the simplified go and no-go pathway paradigm, a decrease in locomotor activity suggests that impaired PDE10A activity shifts the balance between the MSN populations towards the indirect no-go pathway. This is supported by findings in which extrapyramidal side effects (akathisia-like behavior) induced by the PDE10A inhibitor MP-10 or by an indirect pathway activating D2R antagonist, can be reversed through A2A antagonism, which counteracts the effects of elevated cAMP in the indirect pathway (Bleickardt et al. 2014). However, the effect of PDE10A on motor behavior are likely to be more complex, since the activation state of the direct and indirect striatal output pathways (measured by inhibiting D1R or D2R) influences whether PDE10A inhibition can stimulate or inhibit motor behavior (Megens et al. 2014a).

The effect of PDE10A inhibition to reduce stimulant-induced hyperlocomotion has been shown in a variety of paradigms. NMDA-receptor blockers like phencyclidine (PCP) and MK-801 are believed to mimic a NMDA-receptor hypofunctional state and model aspects of the positive, negative and cognitive symptoms of Schizophrenia (Jentsch and Roth 1999; Jones et al. 2011). Megens and colleagues compared the effect of four different PDE10A inhibitors on stimulant-induced hyperlocomotion and found that the inhibitors were able to reduce hyperlocomotion in the PCP model but also in paradigms that stimulate locomotion through mACh antagonism (scopolamine) and by mimicking an hyperdopaminergic state using d-amphetamine (Megens et al. 2014b). The effect of PDE10A inhibition on reducing hypoglutamatergic-mediated hyperlocomotion was also confirmed using MK-801 and a variety PDE10A inhibitors (Siuciak et al. 2006a; Suzuki et al. 2016). Furthermore, the effects of PCP and MK-801 to induce hyperlocomotion were also blunted in PDE10A KO mice, confirming the crucial role of PDE10A in regulating locomotor activity (Siuciak et al. 2006a, 2008). Apomorphine induced climbing and stereotypic behavior is a preclinical model of antipsychotic efficacy, which can differentiate between mesolimbic pathways (climbing) and the striatonigral pathway (stereotypy) (Marquis et al. 2007). MP-10 showed a preferential inhibitory effect on climbing activity, which is a profile observed with atypical antipsychotics (Grauer et al. 2009). Another study investigating the effect in d-amphetamine induced hyperdopamine paradigm found that MP-10 not only reduced hyperlocomotion, but also reduced dopamine efflux in the VTA upon d-amphetamine treatment, which is surprising because PDE10A is not expressed in the dopaminergic neurons of the VTA (Sotty et al. 2009). D-amphetamine and other psychostimulants are known to cause a dopamine efflux (increase) in the NAc due to a block of DA reuptake and an increase of DA release. On the other hand, amphetamine also reduces the spontaneous activity of dopaminergic neurons in the substantia nigra and VTA (amphetamine-induced depression), an effect that is reversed by antipsychotics like haloperidol and reserpine. This effect known as dopamine mediated feedback inhibition is believed to be a result of DA autoreceptors and long feedback loops from DA innervated areas. Alternatively, dopamine can also inhibit glutamate release and subsequent MSN activation by acting on presynaptic D2 receptors (Dani and Zhou 2004). Interestingly, it was found that

PDE10A inhibition potentiated the inhibitory effect of high doses of d-amphetamine on VTA cell firing. This effect might be mediated through a feedback loop involving D1R-expressing direct pathway neurons, because administration of the D1R-agonist SCH23390 had a similar effect on VTA cell firing than MP-10 (Sotty et al. 2009). These findings support the idea that the physiological effects observed after PDE10A inhibition are mediated through an effect on cyclic nucleotide metabolism in direct and indirect neurons even though certain biochemical measures only identify effects of PDE10A inhibition on indirect pathway neurons.

### *2.5.2 PDE10A Inhibition to Treat Neurodegenerative Motor Disorders*

As described above, impairment of cyclic nucleotide signaling has been observed during disease progression of Parkinson's disease and Huntington's disease and restoration of this signaling pathway is under investigation as a potential treatment paradigm for these conditions (Threlfell and West 2013). For example, activation of CREB has been suggested to mediate the resistance to cell death in particular neuronal populations in animal models of HD (Giampà et al. 2006). Supporting the hypothesis of a beneficial effect of elevated cAMP levels on cell survival, DeMarch and colleagues found that activation of CREB using the PDE4 inhibitor Rolipram, reduces striatal neuro-degeneration in both the quinolinic acid and R6/2 genetic models of HD (DeMarch et al. 2007, 2008). Similarly, PDE10A inhibition using the selective inhibitor TP-10 also resulted in reduced pathology, less striatal lesions and an increase in striatal volume in both models of HD. These effects are possibly mediated through increased CREB activation and an increase in the expression of BDNF (Giampà et al. 2009, 2010). In fact impairment of BDNF signaling through tyrosine-related kinase B receptor (TrkBR) has been shown to underlie plasticity deficits seen in HD (Plotkin et al. 2014). Several clinical trials to test the efficacy of the PDE10A inhibitor MP-10 in HD patients are currently under way (www.clinicaltrials.gov: NCT01806896, NCT02197130, NCT02342548). This inhibitor was reported to reverse a hyperexcitable state of the MSN and to restore the reduced corticostriatal connectivity in transgenic mouse models of HD (Zaleska 2013). Recent clinical trial data presented at "CHDI's 11th Annual HD Therapeutics Conference" further indicate a potential beneficial effect of MP-10 on motor coordination and motivation. During the 28 day trial (NCT01806896), measuring the safety, tolerability and exploratory efficacy, patients receiving MP-10 showed a significant improvement in their physical effort in response to incentive motivation during a grip-strength test (Cléry-Melin et al. 2011; Delnomdedieu 2016). Unfortunately, in the 26 weeks "Amaryllis" phase II clinical trial (NCT02197130) MP-10 did not meet its primary endpoint by improving Huntington's disease symptoms and as a result, another open-label extension study has been terminated (NCT02342548).

Besides its role as a potential target in HD, PDE10A has been discussed as a therapeutic target in PD (García et al. 2014). This might seem to be counterintuitive since PDE10A inhibition elevates cAMP in both, direct and in-direct pathway neurons and a loss of dopamine innervation during disease progression leads to a less active D1-expressing neurons and a disinhibition of D2-neurons (Surmeier et al. 2007). However, it has been proposed that PDE inhibition could ameliorate treatment-induced dyskinesias in PD patients. In fact, raising cyclic nucleotide levels through inhibition of PDE5 has been reported to reduce L-DOPA-induced dyskinesias (Picconi et al. 2011). Furthermore, a study report on the Michael J. Fox Foundation website indicated that MP-10 could reduce levodopa-induced dyskinesias, even though only in a narrow dose range (Ellenbroek et al. 2010). However, another study using the PDE10A inhibitor Papaverine, among other PDE inhibitors, did not verify that raising cyclic nucleotide levels ameliorate levodopa-induced dyskinesias in the 6-OHDA model of PD (Sancesario et al. 2014). Even though changes in PDE10A levels are observed in PD, it is not clear if loss of PDE10A is part of the underlying pathology that is causative for the development of the disease or if it is an adaptive change due to reduced cyclic nucleotide levels. Further studies are needed to evaluate a potential beneficial effect of PDE10A inhibition in PD.

### *2.5.3 Involvement of PDE10A in the Pathophysiology of Schizophrenia and Related Disorders*

Through the realization that the principal activity of antipsychotics is to inhibit D2Rs, dopaminergic signaling has been recognized to be critically involved in the pathophysiology of Schizophrenia and possibly other conditions, like bipolar disorder, that share symptomology in a variety of behavioral domains. In line with previous suggestions, the landmark GWAS study from the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC) found a variant near the *DRD2* gene to be significantly associated with disease (Dolgin 2014). In this same study, the *PDE10A* gene did not reach genome wide significance to be associated with Schizophrenia. However in a more focused study, in which balanced chromosomal abnormalities were analyzed, PDE10A was significantly associated with late-onset psychiatric diseases, together with other genes that were previously identified in association with neurodevelopmental disorders (Talkowski et al. 2012). Furthermore, other studies found a rare SNP in the vicinity of the *PDE10A* gene on chromosome 6q27 and additional PDE10A variants were found to be associated with certain forms of bipolar disorder (Kerner et al. 2011; McDonald et al. 2012).

The various classical animal models of schizophrenia aim to model the spectrum of positive, negative and cognitive symptoms of the disease. The conditioned avoidance response (CAR) model is considered a sensitive test to detect the ability of an antipsychotic medication to reduce aberrant attribution of salience to a stimuli (Kapur et al. 2000; Wadenberg and Hicks 1999). PDE10A inhibition shows a clear

dose-dependent effect in reducing the conditioned avoidance response in multiple studies (Grauer et al. 2009; Schmidt et al. 2008). PDE10A target occupancy and the effect on downstream signaling events like CREB phosphorylation showed a clear correlation and the effect was absent in PDE10A-KO mice, providing proof of specificity of the effect (Helal et al. 2011; Li et al. 2016; Megens et al. 2014b; Schmidt et al. 2008). However, when assessing extrapyramidal side effects by measuring catalepsy, there was no clear relationship between PDE10A target occupancy and cataleptic response observed, in contrast to D2R-antagonist antipsychotics like haloperidol, that show a clear positive correlation in this paradigm (Grauer et al. 2009; Kapur et al. 2000; Li et al. 2016; Schmidt et al. 2008).

Deficits in sensorimotor gating that can result in an aberrant attribution of salience to stimuli and is amongst the best characterized symptoms in Schizophrenia patients. Prepulse inhibition (PPI) is commonly used to test gating deficits in both humans and in animal models for schizophrenia. Pharmacological administration of the NMDA-antagonist MK-801 induces deficits in PPI. This effect can be reversed by antipsychotics like risperidone, but the PDE10A inhibitor TP-10 failed to rescue the MK-801 induced deficits in PPI (Schmidt et al. 2008). Another study also showed, that while Papaverine could reduce amphetamine-induced hyperlocomotion it failed to rescue impairments in PPI induced by either apomorphine or amphetamine (Weber et al. 2009). However, a later study revealed that while TP-10 administration alone still did not rescue apomorphine-induced PPI deficits, it could rescue PPI deficits induced by the D2R agonist Quinpirole. Furthermore, TP-10 was shown to attenuate apomorphine-induced disruptions in PPI when D1R signaling was inhibited (Gresack et al. 2014). This finding suggest that the activation of direct pathway neurons by inhibiting PDE10A might counteract its antipsychotic efficacy. Effects of PDE10A inhibition on cognitive symptoms are likely mediated through the indirect pathway as well.

PDE10A inhibition was shown to reverse cognitive deficits induced by either the muscarinic acetylcholine receptor antagonist scopolamine, or by MK-801 (Reneerkens et al. 2013). Nikiforuk and colleagues found that the pro-cognitive effect of the PDE10A inhibitor MP10 as measured in the attention set-shifting paradigm in rats are not blocked by the D1 antagonist SCH-23390, which indicates that the effect do not depend on D1 activation and is likely not mediated through the direct pathway (Nikiforuk et al. 2015).

As part of the spectrum of negative symptoms, patients suffering for Schizophrenia also show behavioral features, which are reminiscent of depression and reduced social interactions. Chronic MK-801 treatment was shown to lead to increased immobility in the forced swim test. It also induced hypersensitivity to D1 agonists suggesting hypofunction of D1 pathway. Interestingly in the same study, PDE10A inhibition reversed MK801 induced immobility whereas haloperidol did not, suggesting that MP-10 exerts its effect on negative symptoms through the direct pathway (Langen et al. 2012). Furthermore, a study found that PDE10A KO mice showed an increase in social interactions, which suggests that PDE10A inhibition might be beneficial in ameliorating part of negative symptom spectrum in Schizophrenia (Sano et al. 2008).

### *2.5.4 Involvement of PDE10A in the Reward System*

The nucleus accumbens, as part of the ventral striatum is well characterized for its influence mediating reward circuitry and addiction-related behavior (Russo and Nestler 2013). Thus, because of its specific expression in the MSNs of the striatum, PDE10A inhibition has been suspected to influence reward behavior and proposed to be beneficial in the management of substance abuse and addictions. While inhibition of PDE9 showed a potential to accelerate the extinction of a cocaine-induced conditioned place preference (CPP), the authors found no effect in this paradigm when using the PDE10A inhibitor Papaverine (Liddie et al. 2012). However, a later study found that inhibition of PDE10A may have therapeutic potential for opioid addiction since it reduces morphine induced conditioned place preference. Interestingly in this study, MP-10 administration inhibited the acquisition of cocaine induced conditioned place preference and also accelerated the extinction but it did not alter the expression of the CPP. However, this effect did not show a strict dose response since higher concentrations of the inhibitor (5, 10 mg/kg) did not show the same effect (Mu et al. 2014). In another paradigm, systemic administration of TP-10 resulted in reduced alcohol and saccharin self-administration suggesting that PDE10A regulates reward pathways related to reinforcing substances (Logrip et al. 2014). Interestingly, another study found that genetic deletion and pharmacological inhibition of PDE10A also reduces caloric intake due to induction of hypophagy (Nawrocki et al. 2014). Even though, the weight loss could be a consequence of an increased energy expenditure, as has been suggested in a recent study that shows reduction of diet-induced obesity upon PDE10A inhibition (Hankir et al. 2016), this effect could also be interpreted that PDE10A inhibition results in a disruption of pairing with a rewarding cue. This hypothesis is supported by a study that identified impaired attribution of incentive salience measured by instrumentally conditioned reinforcement task after genetic deletion of PDE10A. The PDE10A KO animals show increased responding in a non-directed manner. While WT animals do not show increased nose pokes when a stimulus (tone) is not paired with a reward, KO animals showed increased nose pokes just to the tone alone without the subsequent reward suggesting a misattribution of salience to cues (Piccart et al. 2014).

## 2.6 Discussion

Our understanding of PDE10A function in the striatum is rapidly increasing through a convergence of data, principally from the use of selective PDE10A inhibitor compounds, through the characterization of mouse models and also more recently the identification of human mutations in the PDE10A gene. As the precise physiological role of PDE10A is understood it should allow us to better consider how we might manipulate its activity for the treatment of a range of diseases. In addition the availability of high quality PET ligands for PDE10A enzyme occupancy studies and

the quantification of other PDE10A driven responses and behaviours will allow us to confidently assess the therapeutic utility and safety of these PDE10A targeted molecules.

**Conflict of Interest** Nicholas J. Brandon and Jan-Philip Schülke were both full-time employees and shareholders in AstraZeneca at the time of writing.

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# Chapter 3
# Interaction of Cdk5 and cAMP/PKA Signaling in the Mediation of Neuropsychiatric and Neurodegenerative Diseases

**Yafang Hu, Suyue Pan, and Han-Ting Zhang**

**Abstract** Both cyclin-dependent kinase 5 (Cdk5) and cyclic AMP (cAMP)/protein kinase A (PKA) regulate fundamental central nervous system (CNS) functions including neuronal survival, neurite and axonal outgrowth, neuron development and cognition. Cdk5, a serine/threonine kinase, is activated by p35 or p39 and phosphorylates multiple signaling components of various pathways, including cAMP/PKA signaling. Here, we review the recent literature on the interaction between Cdk5 and cAMP/PKA signaling and their role in the mediation of CNS functions and neuropsychiatric and neurodegenerative diseases.

**Keywords** cyclin-dependent kinase 5 • cyclinc AMP • protein kinase A

## 3.1 A Brief Introduction

Cyclin-dependent kinase 5 (Cdk5) and cyclic AMP (cAMP)/protein kinase A (PKA) signaling are two extensively studied and important mediators in fundamental central nervous system (CNS) functions. Accumulating evidences suggest crosstalk between Cdk5 and cAMP/PKA signaling in several physiological and disease

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conditions. Their specific relationship and interaction, however, remains to be elucidated. In this review, we summarize the phosphorylation of the elements in the cAMP signaling pathway by both Cdk5 and cAMP/PKA. In addition, we highlight the Cdk5 regulation of phosphodesterase-4 (PDE4), a critical regulator of intracellular cAMP levels, in memory and learning and responses to stress exposure.

## 3.2 Cdk5 and Cdk5 Cofactors

Cdk5 belongs to the Cdk family of serine/threonine kinases (Lew et al. 1992; Meyerson et al. 1992). Unlike most Cdks, Cdk5 is activated by one of two noncyclin cofactors, p35 (Tsai et al. 1994) and p39 (Tang et al. 1995). Cdk5 is ubiquitously expressed in all cells and tissues, while p35 is highly expressed in embryonic neurons and p39 prominently in synapses of the postnatal brain (Humbert et al. 2000) and oligodendroglia (Bankston et al. 2013). Accordingly, the highest activity of Cdk5 is identified in the brain (Su and Tsai 2011). Mice deficient in Cdk5 ($Cdk5^{-/-}$) display perinatal lethality associated with abnormal corticogenesis and cerebellar defoliation due to neuronal migration deficits and impaired axonal transport of neurofilaments (Ohshima et al. 1996). Notably, $p35^{-/-}$ animals exhibit less severe cortical lamination defects compared to Cdk5 null mice and suffer from sporadic adult lethality and seizures (Chae et al. 1997). Mice with p39 deficiency do not result in overt detrimental phenotypes, (Ko et al. 2001) but exhibit impaired remyelination (Bankston et al. 2013). However, p35 and p39 double knockout mice exhibit nearly identical phenotypes to Cdk5-null mice (Ko et al. 2001). Thus, Cdk5/p35 appears to play a major role in neurons, (Su and Tsai 2011) whereas Cdk5/p39 may be critical for oligodendroglia differentiation (Bankston et al. 2013).

p35 is prominently located in perimembrane due to its N-terminal myristoylated region. It is rapidly degraded by the proteasome. Under neurotoxic conditions, p35 is converted into N-terminal p10 and C-terminal p25 fragments. p25 is characterized by predominant cytoplasm and nucleus location and retains the Cdk5 binding site. Compared to p35, p25 is more resistant to proteasome degradation and has fivefold longer half-life and, consequently, extends Cdk5 activity (Patrick et al. 1999). More than sixty Cdk5 substrates have been identified (Su and Tsai 2011). By targeting on a myriad of downstream substrates, Cdk5/p35 play an essential role in brain development, neuronal survival, synaptic plasticity, learning and memory formation, (Su and Tsai 2011; Dhavan and Tsai 2001) whereas Cdk5/p25 have been considered to be involved in neurotoxicity and neurodegeneration (Patrick et al. 1999; Cruz et al. 2003; Nguyen et al. 2001). With mislocation, p25, unlike p35, may target not only physiological substrates, but also disease-associated substrates (Lew 2013).

## 3.3 Cdk5 Phosphorylates Multiple Substrates of the cAMP Signaling Pathway

cAMP, together with cGMP, are two important second messengers that mediate numerous CNS functions, including cell signaling, synaptic transmission, neuronal survival, neuron development, and cognition. The levels of both cAMP and cGMP are tightly controlled to maintain the specificity and integrity of the intracellular signal propagation (Hebb and Robertson 2007). cAMP synthesis is catalyzed by adenylyl cyclase (AC), and breakdown is carried out by the enzyme PDE4. cAMP activates PKA, which phosphorylates the transcription factor cAMP-response element binding protein (CREB) and PDE4 at Ser 133; the latter forms a negative feedback loop. Cdk5 modulates cAMP/PKA signaling at multiple steps by directly phosphorylating several downstream substrates, including PDE4, dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32), protein phosphatase 1 (PP1), and tyrosine hydroxylase (TH), which are summarized at the end of section 3.3.

### *3.3.1 PDE4*

PDE4 has four subtypes (PDE4A-D), which are encoded by four distinct genes, consisting of at least 25 splice variants (Li et al. 2011). Their differential distributions in the brain indicate different roles of individual PDE4 subtypes in CNS functions (Perez-Torres et al. 2000). With the exception of PDE4C, which is primarily expressed in peripheral tissues and has limited expression in the human brain (cortex, thalamic nuclei, and cerebellum), all other PDE4 subtypes (PDE4A, B, and D) are widely and differentially distributed in the brain (Perez-Torres et al. 2000). Specifically, PDE4A and PDE4D are the major subtypes expressed in the hippocampus, while PDE4B is prominent in the striatum, including the nucleus accumbens (NAc). Functionally, PDE4A is involved in anxiety (Hansen et al. 2014); PDE4B is associated with schizophrenia (Millar et al. 2005; Liu et al. 2016), anxiety (Zhang et al. 2008), and depression (Plattner et al. 2015); and PDE4D is important for antidepressant activity (Zhang et al. 2002), memory (Giorgi et al. 2004), and synaptic plasticity (Rutten et al. 2008). Long-form PDE4s which containing the upstream conserved regions (UCR1 and UCR2) in the N-terminus are most important in cAMP hydrolysis (Baillie et al. 2000). It has been found that Cdk5 efficiently phosphorylates PDE4B1 at Ser145 located in the UCR1 domain and results in activation of PDE4 (Plattner et al. 2015). The Cdk5 site (Ser 145) and PKA site (Ser 133), which is also located in the UCR1 domain, synergistically activate PDE4 (Plattner et al. 2015). Since UCR1 is conserved in all long PDE4s, whether Cdk5 can phosphorylate all long-form PDE4 isoforms remains to be determined.

### 3.3.2 DARPP-32 and PP1

DARPP-32, a postsynaptic protein highly expressed in striatal medium-size spiny neurons, was identified initially as a major target for dopamine in the striatum. Dopamine binding to D1 receptors increases cAMP and subsequently activates PKA, which phosphorylates DARPP-32 at Thr34 and converts it into a potent inhibitor of PP-1 (Hemmings et al. 1984). PP-1 is a Ser/Thr phosphatase which controls the phosphorylation status and activity of a variety of downstream effector molecules including CREB. Cdk5 phosphorylates DARPP-32 at Thr75, which in turn inhibits PKA (Bibb et al. 1999). In other words, DARPP-32 acts as an inhibitor of either PP-1 by PKA phosphorylation or PKA by Cdk5 phosphorylation. In cortical neurons, Cdk5 phosphorylates PP1 at T320 which suppresses PP1 activity. Under synaptic N-methyl-D-aspartate (NMDA) receptor stimulation, p35 degradation leads to a loss of Cdk5 activity and activation of PP1 (Hou et al. 2013). The complex network of positive and negative feedback is indicated as in Fig. 3.1.

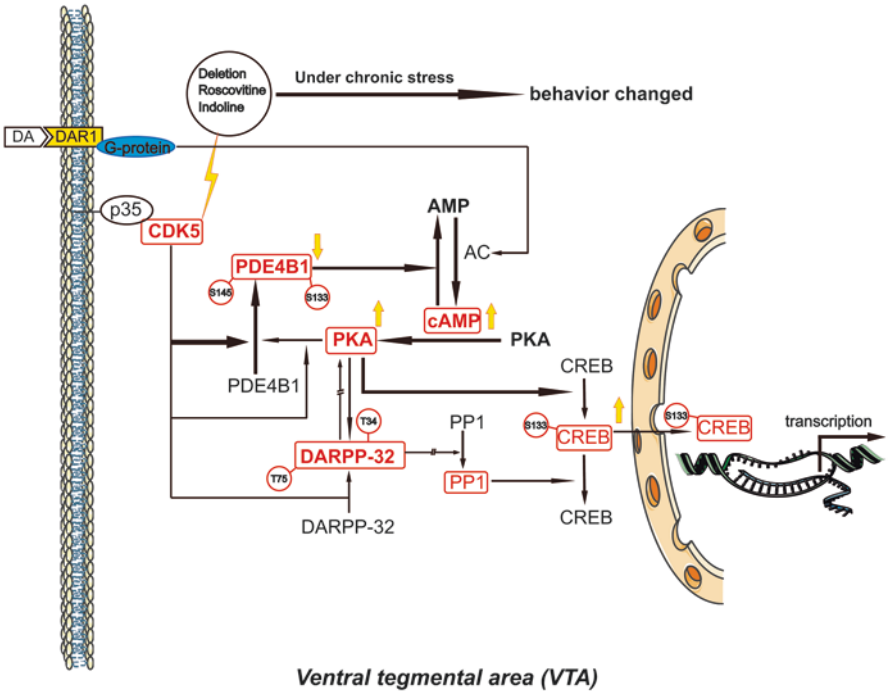


**Fig. 3.1** Cdk5 negatively regulates PDE4 signaling in the VTA of the striatum. *Red* fond representing phosphorylated protein with the phosphorylated site labeled. *Arrows* indicate improving, *blocked arrows* indicate inhibiting. *T* Thr, *S* Ser

### 3.3.3 TH

TH is the rate-limiting enzyme for dopamine synthesis in presynaptic terminals. When phosphorylated, it has an increased activity. This can be accomplished by several kinases, including PKA at Ser 40 and $Ca^{2+}$-calmodulin-dependent protein kinase II (CaMKII) at Ser19 (Haycock et al. 1998). Cdk5 also plays a critical role in the regulation of TH activity; it phosphorylates TH at Ser 31 (Moy and Tsai 2004). In addition, transgenic mice with increased Cdk5 activity display increased TH Ser 31 phosphorylation in neurons of the substantia nigra, which is enriched with TH-positive neurons (Moy and Tsai 2004). On the other hand, Cdk5 deficiency reduces TH levels. TH is also phosphorylated at the same site by extracellular signal-regulated kinases 1/2 (ERK1/2) (Moy and Tsai 2004). Cdk5 phosphorylation at Ser 31 modulates ERK1/2-dependent phosphorylation of TH through the phosphorylation of mitogen-activated protein kinase 1 (MEK1), providing another route by which Ckd5 regulates TH activity (Kansy et al. 2004).

### 3.3.4 Coronin 1

Coronin 1 belongs to the family containing WD repeat, which is a structural motif comprising approximately 40 amino acids usually ending with the amino acid sequence tryptophan (W) and aspartic acid (D). It is expressed in leukocytes and neurons, particularly in excitatory neurons (Ferrari et al. 1999). Increase copy numbers in the genomic region of coronin 1 located in chromosome 16 causes varying degrees of cognitive impairment (Horev et al. 2011). Upon cell surface stimulation, coronin 1 assembles with the G protein subunit Gαs to increase cAMP production. Being an upstream modulator, Cdk5 can phosphorylate coronin 1 in T lymphocytes (Pareek et al. 2010). In human melanoma cells (Mel JuSo), Cdk5 phosphorylates coronin 1 at Thr 418 and 424. The Cdk5-dependent phosphorylation of coronin 1 is essential but not sufficient for Gαs-mediated cAMP production, suggesting additional mechanisms upstream of coronin 1 to activate the coronin 1-dependent cAMP/PKA pathway (Liu et al. 2016).

### 3.3.5 Disrupted-in-Schizophrenia 1 (DISC1)

The Disrupted-in-Schizophrenia 1 (DISC1) is a susceptibility factor for multiple mental disorders, including schizophrenia, mood disorders, and autism. It is expressed in both neuronal progenitor cells and postmitotic neurons in the developing cerebral cortex (Ishizuka et al. 2011). DISC1 can be phosphorylated at two sites, Ser58 and Ser710, by PKA and Cdk5, respectively. Cdk5-mediated phosphorylation

of DISC1 at Ser710 acts as a molecular switch from maintaining proliferation of mitotic progenitor cells to activating migration of postmitotic neurons (Kamiya et al. 2008). The function of phosphorylation of Ser58 by PKA is unclear.

### *3.3.6 Synapsin III*

Synapsin III (SynIII) is an atypical member of the synapsin family of neuron-specific phosphoproteins associated with synaptic vesicles (SVs). Among the three Synapsins (I, II, III), SynIII is the earliest expressed Syn isoform during development (Porton et al. 1999; Porton et al. 2004). In addition to a highly conserved phosphorylation site (Ser 9) for PKA shared by all Syn isoforms, SynIII has a specific domain J containing a phosphorylation site for Cdk5 at Ser404 (Perlini et al. 2015; Piccini et al. 2015). Cdk5 and SynIII expression are highly correlated at perinatal ages in rat cortical neurons. SynIII acts on downstream Sema3A/Cdk5 signaling to play an important role in neuronal migration and orientation (Perlini et al. 2015; Ferreira et al. 2000). It has been found that phosphorylation of SynIII at Ser9 by PKA and Ser404 by Cdk5 are equally important at the early neuronal development (Piccini et al. 2015). Colectively, the downstream substrates of cAMP/PKA signaling pathwy contain phosphorylation sites of Cdk5 and PKA, summarized as in Table 3.1.

## 3.4 Cdk5 Is Associated with Memory, Learning via the cAMP Signaling Pathway

The hippocampus is considered to be a key region for long-term memory formation in humans and rodents (Morris et al. 1982). Memory formation is modulated by pre- and post-synaptic signaling events in neurons which affect synaptic plasticity. Synaptic plasticity can produce decreases or increases in the amplitude of synaptic responses, called depression or potentiation, respectively.

**Table 3.1** The phosphorylation sites of downstream substrates by Cdk5 or PKA

| Substrates | Cdk5 sites | PKA sites |
|---|---|---|
| PDE4 | Ser 145 | Ser 133 |
| DARPP-32 | Thr 75 | Thr 34 |
| PP1 | Thr 320 | |
| TH | Ser 31 | Ser 40 |
| Cornin1 | Thr418, Thr 424 | Upstream of PKA |
| DSCI1 | Ser 710 | Ser 58 |
| SnyIII | Ser 404 | Ser 9 |

Activation of cAMP/PKA signaling enhances synaptic plasticity through phosphorylation of its downstream target CREB, which activate related genes expression (Bruel-Jungerman et al. 2005). Consistent with this, PDE4 is involved in hippocampal neurogenesis, which is associated with learning and memory (Egawa et al. 1997). Chronic rolipram treatment to specifically inhibit PDE4 increases proliferation and survival of newborn neurons in the hippocampal dentate gyrus (Nakagawa et al. 2002; Sasaki et al. 2007). Inhibition of PDE4 also enhances memory or reverses memory deficits produced by pharmacological, (Egawa et al. 1997; Zhang et al. 2000; Zhang et al. 2004) physical, or genetic approaches (Sierksma et al. 2014; Imanishi et al. 1997; Bourtchouladze et al. 2003). Similar results are observed in PDE4D-deficient mice, which showed increased hippocampal neurogenesis and phosphorylated CREB in the brain. miRNA-mediated PDE4D knockdown in the hippocampus demonstrates that PDE4D, in particular long-form PDE4Ds, plays a critical role in the mediation of memory and hippocampal neurogenesis (Li et al. 2011; Zhang et al. 2014; Wang et al. 2013; Wang et al. 2015). These are consistent with the findings using pharmacological approaches (Sierksma et al. 2014; Bruno et al. 2011).

Cdk5 is also implicated in memory formation by phosphorylating a variety of synaptic substrates. The first hint suggesting a role of Cdk5 in hippocampus-dependent memory formation came from a study with p35 knockout (KO) mice, which displayed normal LTP, but impaired LTD in the CA1 subregions of the hippocampus (Ohshima et al. 2005). Another hint was from p25 transgenic (Tg) mice showing that p25 appeared to have dual effects in synaptic plasticity. Adult CK-p25 Tg mice with p25 overexpressed for 2 weeks, which is driven by the CaMKII promoter and turned on by aTA system, displayed dramatic enhancement of learning and memory in contextual fear conditioning and the Morris water-maze tasks (Fischer et al. 2005). This memory-enhancing effect is consistent with facilitation of LTP and increases in dendritic spines in hippocampal CA1. However, long-term, 6-week induction of p25 resulted in severe neuronal loss, memory impairment, and LTP deficit.

Additional findings associating Cdk5 activity with memory seem controversial. In an inducible Cdk5 conditional knockout (cKO) line, which was derived under a prion promoter (Hawasli et al. 2007), the Cdk5 cKO mice display facilitated LTP and enhanced memory via reduced degradation of the NR2B subunit of NMDA receptors. In contrast, a different line of Cdk5 cKO mice, whose Cdk5 is ablated primarily in CA1 pyramidal neurons of the hippocampus at early age (2.5–3.5 months old), exhibited severe impairment in hippocampus-dependent spatial memory. Memory impairment was also observed in Cdk5 cKO mice with forebrain-targeted Cdk5 deletion in excitatory neurons (Fischer et al. 2005).

In Cdk5f/f/T29 cKO mice in which Cdk5 ablation is restricted mainly to CA1 pyramidal neurons of the hippocampus, it has been demonstrated that Cdk5 mediates synaptic plasticity and hippocampus-dependent memory via modulation of cAMP signaling (Guan et al. 2011). In the Cdk5 KO mice, increased mRNA levels of multiple PDE isoforms, including PDE4B, PDE4D, PDE4D4, PDE1A, and PDE2A, were observed in the hippocampus. Low cAMP causes insufficient CREB

phosphorylation at Ser 133, leading to decreases in synaptic proteins and impairment of learning and memory. Treatment with the PDE4 inhibitor rolipram rescues the behavioral deficits in Cdk5 cKO mice.

The Cdk5 mediation of memory via cAMP/PKA is supported by a recent finding that Cdk5 regulates coronin 1-dependent cAMP/PKA signaling (Liu et al. 2016). Coronin 1, the upstream trigger of cAMP/PKA signaling, has been found to regulate cAMP production and PKA activation (Jayachandran et al. 2014). Coronin 1 deficiency results in severe functional defects at excitatory synapses. Furthermore, in both mice and humans, deletion or mutation of coronin 1 causes severe neurobehavioral defects, including social deficits, increased aggression, and learning disabilities. Infusions of the cAMP analogue 8-Br-cAMP into the amygdala restore synaptic plasticity and behavioral defects in mice lacking coronin 1. It is interesting to note that Cdk5 is able to phosphorylate coronin 1 on Thr 418 and 424 in cultured neurons. This provides evidence that Cdk5 regulates the coronin 1-dependent cAMP/PKA signaling pathway, even if Cdk5-dependent phosphorylation of coronin 1 is not sufficient for Gαs-mediated cAMP production (Liu et al. 2016). It will be important to check this pathway in extended studies with animal models.

Together, both cAMP/PKA signaling and Cdk5 are involved in the mediation of learning and memory. Cdk5 regulates cAMP/PKA signaling via phosphorylation of the elements upstream and downstream of the pathway.

## 3.5 Cdk5 Regulates PDE4 Signaling on Stress Exposure and Its Association with Anxiety and Depression

Under acute and chronic stress procedures, several brain areas are important for neurobiological responses to stress exposure, including the amygdala and the ventral tegmental area (VTA). The limbic system controls emotional behavior and motivational drives. The amygdala, in particular the basolateral amygdala (BLA), modulates negative emotional reactions to threatening environment. Dopamine neurons in the VTA govern reward and motivation and mediate stress-induced behaviors (Chaudhury et al. 2013; Tye et al. 2013).

The activity of both Cdk5 and p35 is increased in various brain areas of the limbic system in response to stress stimulation. Stress exposure increases p35 levels particularly in the BLA, which is correlated with the occurrence of exaggerated anxiety. This is selectively reversed by infusions of olomoucine, a Cdk5 inhibitor, into the BLA, but not the adjacent CeA, prior to the restraint session, suggesting a role of Cdk5 (Bignante et al. 2010; Bignante et al. 2008). In a p25 transgenic (p25-Tg) mouse model created using the neuron-specific enolase promoter that expresses human p25 cDNA, (Ahlijanian et al. 2000) it has been demonstrated that upregulation of p25 increases locomotor activity and decreases anxiety-like behavior. These results suggest a pivotal role of the Cdk5/p35 complex in excessive anxiety induced by a previously stressful experience.

Early studies suggest a reciprocal, regulatory relationship between PKA and Cdk5 activity (Bibb et al. 1999). Infusions of a Cdk5 inhibitor into the hippocampal dentate gyrus (DG), but not CA1 or CA3, increase sucrose preference and prevent locomotor impairment in response to chronic mild stress, supporting antidepressant activity (Zhu et al. 2012). Since selective increases in cAMP levels in VTA dopamine neurons reverse behavioral deficits induced by Cdk5 deletion, the results imply that Cdk5 may regulate cAMP/PKA signaling upstream. This hypothesis has been demonstrated by a recent study showing that Cdk5 directly potentiates PDE4B1 activity via phosphorylation, causing downregulation of cAMP levels in striatal slices (Plattner et al. 2015). Inhibition of Cdk5 by roscovitine increases phosphorylation of cAMP/PKA downstream substrates in striatal slices, including CREB (Ser133) and DARPP-32 (Thr34). This observation was further confirmed in an AAV2-mediated mouse model, in which medium spiny neurons in the ventral striatum and D1 dopamine receptor positive neurons were specifically targeted. Consistent with these results, virus-mediated Cdk5 KO in the ventral striatum and D1R-Cdk5-KO mice all showed consistent biochemical and behavioral effects suggesting antidepressant-like effects (e.g. reduced immobility time in Porsolt forced-swim test, increased time struggling in tail suspension test and social interaction ratio in social defeat stress, and elevated sucrose preference). In addition, specific disruption of Cdk5 in the VTA or dopamine neurons by VTA infusions of adeno-associated viral-Cre in Cdk5loxP/loxP mice or breading dopamine transporter (DAT)-Cre mice with Cdk5loxP/loxP mice decreases dopamine-release in the ventral striatum, reduces motor activity in response to acute stress, prolongs novel environment-related feeding delay, and reduces sucrose preference, which paradoxically suggest anxiety- and depressive-like behaviors (Zhong et al. 2014). These mice also show decreases in TH phosphorylation at Ser31 (Cdk5 site) and Ser40 (PKA site), cAMP, and phosphorylated CREB (ser133) in the VTA. The reason for the contradictory observations remains to be clarified, while brain region-specific responses to Cdk5 disruption cannot be excluded.

Overall, in the VTA of the striatum, Cdk5 provides a negative feedback on cAMP/PKA signaling by potentiating PDE4 activity via phosphorylation. Deletion of Cdk5 in the VTA increases cAMP levels and PKA activity, thereby affecting behavioral responses induced by acute and chronic stress, as indicated in Fig. 3.1. Nevertheless, it remains to be resolved how biological responses cause the behavioral changes.

## 3.6 Reciprocal Regulation of Cdk5 and cAMP/PKA Signaling on Dopaminergic Signaling and Its Association with Parkinson's Disease

Striatal functions depend on an activity balance between dopamine and glutamate transmissions that produce opposing physiological effects (Greengard 2001; Chergui et al. 2004). Dopamine inputs activate PKA, thus phosphorylating

DARPP-32 at Thr 34, which inhibits PP1, the enzyme responsible for dephosphorylation of Ser-133 of CREB (Hemmings et al. 1984). Glutamate inputs activate Cdk5, thus phosphorylating DARPP-32 at Thr75, which functions as an inhibitor of PKA. Therefore, DARPP-32 plays as an integrator to balance dopamine and glutamate transmissions (Svenningsson et al. 2004; Fernandez et al. 2006; Bonito-Oliva et al. 2011). It is noted that under resting conditions, DARPP-32 is highly phosphorylated at Thr 75 and slightly phosphorylated at Thr34 (Greengard 2001; Sako et al. 2010). Upon stimulation or under disease conditions, the homeostasis of this balance is disrupted. Dysregulation of Cdk5 activity has been implicated in striatal dopamine-related disorders such as Parkinson's disease (PD) (Chergui et al. 2004; Smith et al. 2003; Qu et al. 2007) and drug addiction (Takahashi et al. 2005; Bibb et al. 2001).

In a rodent model of PD, striatal dopamine deficiency had no effect on phosphorylation of Thr34-DARPP-32, but significantly increased that of Thr75-DARPP-32 (Brown et al. 2005). In MPTP mice, dopamine deficiency increased Cdk5-pTyr15 and Thr75-DARPP-32 via the D2R pathway. In addition, calpain caused aberrant formation of p25 and accompanied Cdk5 hyperactivity in MPTP mice (Qu et al. 2007; Huang et al. 2010; Smith et al. 2006). Since activation of Cdk5 also phosphorylates PDE4 as it does in VTA, it is possible that aberrant Cdk5 activity may increase PDE4 phosphorylation and inhibit PKA activity, and thus worsen DA neuron loss.

## 3.7 Interaction of Cdk5 and cAMP/PKA Pathway in Dopamine Signaling and Its Association with Drug Addiction

Cocaine, a drug of abuse, increases synaptic dopamine levels in the striatum by blocking dopamine reuptake at axon terminals. Acute cocaine inhibits dopamine synthesis in a dose-dependent manner via a putative negative feedback mechanism.

Chronic cocaine exposure increases ΔFosB, a Fos family transcriptional factor in the striatum, resulting in the elevation of Cdk5 and p35 in medium spiny striatal neurons (Bibb et al. 2001). Cdk5 activation increases phosphorylation of DARPP-32 at Thr75 and subsequently attenuates D1R/PKA signaling. This is supported by the observation in DARPP-32 mutant mice (Hiroi et al. 1999). Cdk5 activation also phosphorylates TH at Ser31 in dopaminergic neurons of rats trained to chronically self-administer cocaine (Lu et al. 2003). Inhibition of Cdk5 in the striatum has been shown to potentiate behavioral effects of chronic cocaine treatment in animals (Taylor et al. 2007). In a p35 transgenic mouse line, overexpression of p35 decreases cocaine-induced phosphorylation of CREB (at Ser133) and that of MEK1/2 (at Ser217/Ser221 or Thr202/Tyr204), and DARPP-32 (at Thr34), but increases cocaine-induced phosphorylation of DARPP-32 (at Thr75) and MEK1/2 (at Thr286) (Ohshima et al. 1996). The results provide further evidence that Cdk5 mediates

cocaine-induced dopamine signaling through inhibition of the PKA and ERK cascades, leading to less induction of CREB phosphorylation and c-fos in the striatum.

Methamphetamine (METH), another illicit substance of abuse, acts as a substrate for the dopamine transporter and the vesicular monoamine transporter and causes intense psychomotor activating and motivational properties (Bosse et al. 2015). Repeated use of METH leads to behavioral sensitization and addition. The cAMP/PKA pathway implicates in conferring METH-induced synaptic modifications in striatal reward neurocircuits (Bosse et al. 2015; Moriguchi et al. 2002; Miyazaki et al. 2013). For example, a recent study demonstrates a blunted, acute and sensitized locomotor response to METH in mice with AC1 and AC8 double knockout (DKO) (Bosse et al. 2015). Compared to WT controls, DKO mice displayed significantly low levels of dopamine and decreases in the ratio of phosphorylation of DARPP-32 at Thr-34 (the PKA site) relative to Thr-75 (the Cdk5 site) after repeated exposure to METH. This study suggest that AC modulates interaction between Cdk5 and cAMP/PKA pathway in drug addiction.

## 3.8 Role of Cdk5 and cAMP/PKA Signaling in Mediating Neuropsychiatric Disorders

The DISC1 gene is a generalized risk factor in major mental illnesses, including bipolar disorder, major depression, and schizophrenia (Blackwood et al. 2001; Millar et al. 2000; Hennah et al. 2007; Porteous and Millar 2006). Disruption of PDE4B due to a balanced translocation is also identified as a genetic risk factor for psychiatric illnesses such as schizophrenia, (Hansen et al. 2014) which is supported by the association of PDE4B polymorphisms with schizophrenia (Guan et al. 2012). The interaction between DISC1 and several other proteins, including PDE4B, NDEL1, FEZ1, and GSK3β, is involved in the molecular mechanism of schizophrenia. In addition, phosphorylation of DISC1 at Ser710 by Cdk5 triggers the recruitment of Bardet-Biedl-Syndrome (BBS) proteins to the centrosome, which underlie neuronal migration (Kamiya et al. 2008). It is speculated that disturbance of this switch mechanism may contribute to hypertrophic and disturbed corticogenesis observed in brains of patients with autism.

Several reports suggest an association of SynIII with neurodevelopmental disorders such as schizophrenia by analysis of postmortem samples (Porton and Wetsel 2007) or genetic studies (Porton et al. 2004; Chen et al. 2009). Synapsins play a primary role in synaptic transmission and plasticity (Valtorta et al. 1992; Cesca et al. 2010; Fornasiero et al. 2012). In addition, Syns also play a critical role in neuronal development by regulating neurite outgrowth and synapse formation (Fornasiero et al. 2010; Perlini et al. 2011). SynIII is the isoform expressed earliest in neurons compared to Syn I and II. Structurally, SynIII contains a major Cdk5 phosphorylation site (Ser404) in the unique domain J, while all three Syns share a

highly conserved PKA phosphorylation site (Kao et al. 1999). Phosphorylation of SynI by PKA modulates synapse formation in vitro (Perlini et al. 2011), and phosphorylation of SynII by PKA plays a crucial role in Xenopus spinal neurons. Phosphorylation of SynIII by Cdk5 in vivo regulates the radial migration of pyramidal neurons in cortical development (Perlini et al. 2015). SynIII KO in embryonic neurons impairs inhibitory transmission, but the phenotype is mild (Feng et al. 2002). Furthermore, phosphorylation of SynIII by PKA and Cdk5 are both required at the early neuronal development, because the Cdk5 or PKA phospho-mimetic mutation of SynIII only partially rescues the developmental phenotype of SynIII KO (Piccini et al. 2015).

Taken together, both Cdk5 and cAMP/PKA signaling pathways are involved in DISC1- and SnyIII-mediated mental illnesses including schizophrenia. Determination of whether these two pathways are independent or in crosstalk will help us better understand the mechanisms underlying the development of the psychiatric diseases, which could lead to novel therapeutic strategies.

## 3.9 Conclusions

Accumulating evidences support significant interactions between Cdk5 and cAMP/PKA signaling, which play an important role in multiple important functions of the CNS, including cognition, drug addiction, and mental behaviors. Cdk5 regulates cAMP signaling via phosphorylation of its upstream and/or downstream components, including PDE4B, DARPP-32, ERK, and CREB. PKA in turn also regulates Cdk5 activity via phosphorylation of DARPP-32 at a different phosphorylation site. It should be noted that other critical players not summarized here such as anchoring proteins also serve as mediators to integrate the activity of Cdk5 and PKA in neuronal environment. Primarily through these mechanisms, Cdk5 is involved in the mediation of a variety of CNS disorders, including AD, PD, depression, anxiety, schizophrenia, and drug addiction, in which Cdk5 is in hyperphosphorylation and/or dysfunction. More studies are needed to understand the related cellular and molecular mechanisms underlying neuropsychiatric and neurodegenerative diseases, which could aid in the development of novel treatments of these diseases.

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

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# Chapter 4
# The PDE4 cAMP-Specific Phosphodiesterases: Targets for Drugs with Antidepressant and Memory-Enhancing Action

**Graeme B. Bolger**

**Abstract** The PDE4 cyclic nucleotide phosphodiesterases are essential regulators of cAMP abundance in the CNS through their ability to regulate PKA activity, the phosphorylation of CREB, and other important elements of signal transduction. In pre-clinical models and in early-stage clinical trials, PDE4 inhibitors have been shown to have antidepressant and memory-enhancing activity. However, the development of clinically-useful PDE4 inhibitors for CNS disorders has been limited by variable efficacy and significant side effects. Recent structural studies have greatly enhanced our understanding of the molecular configuration of PDE4 enzymes, especially the "long" PDE4 isoforms that are abundant in the CNS. The new structural data provide a rationale for the development of a new generation of PDE4 inhibitors that specifically act on long PDE4 isoforms. These next generation PDE4 inhibitors may also be capable of targeting the interactions of select long forms with their "partner" proteins, such as RACK1, β-arrestin, and DISC1. They would therefore have the ability to affect cAMP levels in specific cellular compartments and target localized cellular functions, such as synaptic plasticity. These new agents might also be able to target PDE4 populations in select regions of the CNS that are implicated in learning and memory, affect, and cognition. Potential therapeutic uses of these agents could include affective disorders, memory enhancement, and neurogenesis.

**Keywords** cAMP • Phosphodiesterase • PDE4 • Beta-arrestin • RACK1 • PKA • ERK1/2 • Learning • Memory • Depression

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## 4.1 Introduction

The cAMP-specific phosphodiesterases (PDE4) enzymes) hydrolyze the ubiquitous "second messenger" cAMP and thereby serve to regulate its abundance in specific sub-cellular compartments (Francis et al. 2011; Conti and Beavo 2007; Houslay 2010; Maurice et al. 2014; Baillie 2009; Menniti et al. 2006). They are an essential component of the cAMP signal transduction system, which also includes adenylyl cyclase, specific G-proteins, G-protein coupled receptors (GPCRs), the cAMP-dependent protein kinase (PKA) and the cAMP target, Epac (Beavo and Brunton 2002). The PDE4 family is a member of the cyclic nucleotide PDE super-family, which consists of 11 distinct families (PDE1 through PDE11, respectively) that can be distinguished by their substrate specificity (cGMP and/or cAMP), molecular structure, and their ability to be inhibited by family-selective inhibitors (Bolger 2007). Like all members of the PDE super-family, the PDE4s are important targets for drug discovery. Currently, three PDE4-selective inhibitors, roflumilast, apremilast and crisaborole, have been developed for clinical use, in COPD and inflammatory disorders (Fabbri et al. 2009; Calverley et al. 2009; Hatzelmann et al. 2010; Page and Spina 2012; Schafer et al. 2014; Kavanaugh et al. 2015; Papp et al. 2015; Murrell et al. 2015), and additional PDE4 inhibitors are being tested in a wide variety of pre-clinical models and in clinical trials (Page and Spina 2012; Zhang et al. 2005a; Bruno et al. 2011; Giembycz and Maurice 2014; Richter et al. 2013). PDE4s are expressed in many areas of the CNS and PDE4 inhibitors have been shown to have antidepressant, anti-psychotic, and memory-enhancing actions in both rodent models and in humans (Fleischhacker et al. 1992; Scott et al. 1991; Hebenstreit et al. 1989; Eckmann et al. 1988; Zeller et al. 1984; Bobon et al. 1988; Barad et al. 1998; Bach et al. 1999; Titus et al. 2013; Mueller et al. 2010; Nibuya et al. 1996; O'Donnell and Zhang 2004; Kanes et al. 2007; Halene and Siegel 2008). However, the development of clinically-effective PDE4 inhibitors in CNS disorders has been hampered by lack of effectiveness and significant side effects, such as nausea (Higgs 2010; Gavalda and Roberts 2013).

This review discusses recent advances in the PDE4 field that promise to greatly enhance our understanding of the biology of PDE4 isoforms and also to accelerate the development of PDE4-selective inhibitors with greater activity and selectivity in the CNS. It will first review the structure of PDE4 genes and their transcripts. It will then discuss recent advances in the structure and function of PDE4 proteins, with emphasis on dimerization of PDE4 isoforms, the role of phosphorylation, and the interactions of PDE4s with their "partner" proteins, such as DISC1, RACK1 and β-arrestin2. The focus will then change to the cellular functions of the PDE4s, with special emphasis on their differential effects on important PKA substrates in the CNS. It will then review briefly the functional roles of the PDE4s in the intact brain, with emphasis on both the CNS effects of PDE4-selective inhibitors and on the CNS phenotypes of PDE4-mutant mice, especially those of newer dominant-negative models. Finally, it will discuss the implications of all these developments for drug discovery, with special emphasis on the potential of PDE4-selective inhibitors for CNS disorders.

## 4.2 The Structure of the PDE4 Genes and Their Transcripts

One of the most important aspects of PDE4 biology is the marked diversity of PDE4 isoforms, with over 20 isoforms having been identified to date (Conti and Beavo 2007; Houslay 2010; Maurice et al. 2014; Bolger 2007; Bolger et al. 1993; Swinnen et al. 1989). The PDE4s are encoded by four different genes in mammals (called *PDE4A, PDE4B, PDE4C* and *PDE4D* in humans), with additional diversity being produced by alternative mRNA splicing and the use of several isoform-specific promoters within each gene (Conti and Beavo 2007; Houslay 2010; Maurice et al. 2014; Bolger 2007; Bolger et al. 1993; Swinnen et al. 1989). Each of the PDE4 isoforms has a distinct pattern of expression in cells and tissues and the vast majority of them has been demonstrated to have an isoform-specific pattern of expression in the CNS (Bolger et al. 1994; Cherry and Davis 1999; Miro et al. 2002; D'Sa et al. 2005; D'Sa et al. 2002; Reyes-Irisarri et al. 2008; Nishi et al. 2008; Mori et al. 2010; Kuroiwa et al. 2012; Ahmed and Frey 2003). These pronounced differences in regional expression in the CNS suggest that each isoform has a distinct function; a concept that will be discussed in more detail, below.

The PDE4 isoforms can be categorized into "long" forms, which possess both UCR1 and UCR2 regulatory domains, "short" forms that lack UCR1, and "super-short" forms that lack UCR1 and have a truncated UCR2 (Conti and Beavo 2007; Bolger 2007; Bolger et al. 1993). In addition, each isoform has a unique amino-terminal region, encoded by one or more exons specific to that isoform, that frequently has unique properties. For example, the unique amino-terminus of the widely-found PDE4D5 isoform (Fig. 4.1) is essential for its interaction with its "partner" proteins (Bolger et al. 1997; Perry et al. 2002; Bolger et al. 2003; Baillie et al. 2003; Shukla et al. 2014; Yarwood et al. 1999; Bolger et al. 2002; Steele et al. 2001; Li et al. 2009a; Bolger et al. 2006; Baillie et al. 2007; Smith et al. 2007). PDE4D5 interacts selectively with β-arrestin2, implicated in the regulation of GPCRs and other cell signaling components (Perry et al. 2002; Bolger et al. 2003; Baillie et al. 2003; Li et al. 2009a; Bolger et al. 2006; Baillie et al. 2007; Smith et al. 2007; Bradaia et al. 2005; Lynch et al. 2005), and also with the β-propeller protein RACK1 (Yarwood et al. 1999; Bolger et al. 2002; Steele et al. 2001; Bolger et al. 2006; Smith et al. 2007; Bird et al. 2010). In contrast, the PDE4B1 isoform, which has an amino-terminal region completely different from that of PDE4D5, interacts selectively with the DISC1 protein, implicated in affective disorders and schizophrenia (Millar et al. 2005; Murdoch et al. 2007; Bradshaw et al. 2011; Hayashi-Takagi et al. 2010).

The catalytic regions of all PDE4 isoforms encoded by any individual PDE4 gene are identical in amino acid sequence and, in general, the biochemical and pharmacologic properties of each of the isoforms encoded by any individual PDE4 gene differ only modestly. For example, five different isoforms encoded by the *PDE4D* gene have differ less than fivefold in their $K_m$ for cAMP and in their $IC_{50}$ for the prototypical PDE4-selective inhibitor rolipram (Bolger et al. 1997). The catalytic regions of the proteins encoded by the four different PDE4 genes are extremely

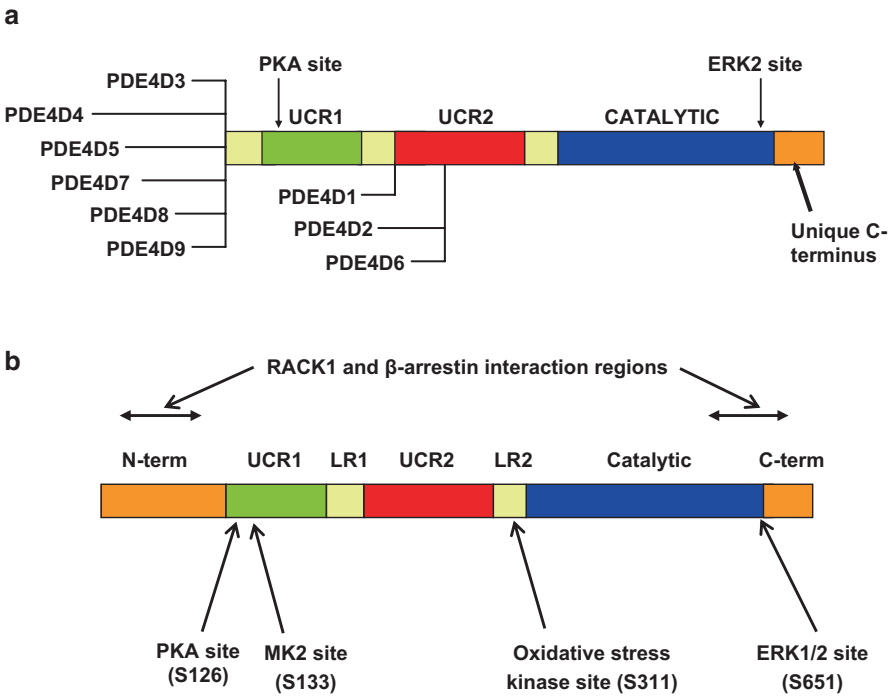


**Fig. 4.1** Primary structures of PDE4D isoforms. (**a**) Schematic representation of the nine different isoforms encoded by the human *PDE4D* gene. The isoforms are divided into long isoforms, such as PDE4D5, that contain both UCR1 and UCR2, short isoforms, such as PDE4D1, that contain only UCR2, and super-short isoforms, such as PDE4D2, that contain only a truncated UCR2. Also shown is the C-terminal region, present in all PDE4D isoforms, but differing from the C-terminal regions of isoforms encoded by other PDE4 genes. (**b**) Schematic representation of human PDE4D5. PDE4D5 contains UCR1, UCR2, and catalytic domains, which are separated by the unstructured LR1 and LR2 regions. Also shown are the 88 amino acid unique N-terminal region (N-term), the C-terminus (C-term), and regions required for the interaction of PDE4D5 with RACK1 and β-arrestin2. The locations of PKA, ERK1/2, MK2, and oxidative stress kinase sites are also shown

similar (approximately 90% sequence identity). As all PDE4-selective inhibitors act, at least in part, at the catalytic sites of the PDE4 enzymes (Lee et al. 2002; Zhang et al. 2004a; Card et al. 2004; Huai et al. 2004; Burgin et al. 2010; Wang et al. 2007a; Kranz et al. 2009; Fox et al. 2014; Gurney et al. 2011), and therefore act, at least in part, as competitive inhibitors of cAMP hydrolysis, the similarity among the catalytic sites of the isoforms has greatly complicated the development of inhibitors selective for any individual isoform, or even for all the isoforms encoded by one PDE4 gene. Although some newer compounds may be more selective (Bruno et al. 2011), most PDE4-selective inhibitors have less than a tenfold difference in potency (i.e., $IC_{50}$) for isoforms encoded by different PDE4 genes (Hatzelmann et al. 2010; Burgin et al. 2010; Wang et al. 2007a).

## 4.3 Dimerization of PDE4 Isoforms and Its Implication for Drug Discovery

Long PDE4 isoforms, such as PDE4B1 and PDE4D5, have been demonstrated by a variety of assays to form homodimers (Richter and Conti 2002; Richter and Conti 2004; Xie et al. 2014; Bolger et al. 2015). Recently, the dimerization of long PDE4 isoforms has been greatly illuminated by structural and enzymatic studies (Cedervall et al. 2015). The structural data built on prior interaction studies, including yeast 2-hybrid and co-immunoprecipitation, and extensive mutagenesis studies, that suggested an interaction between specific regions of UCR1 and UCR2, which appeared to form a module that in turn interacted with the catalytic domain (Bolger et al. 1993; Richter and Conti 2002; Richter and Conti 2004; Xie et al. 2014; Bolger et al. 2015; Lim et al. 1999; Beard et al. 2000). They have also demonstrated conclusively, consistent with previous data (Richter and Conti 2002; Richter and Conti 2004; Xie et al. 2014; Bolger et al. 2015), that long PDE4 isoforms can form dimers, with UCR1 and UCR2 being essential components of the dimeric structure (Cedervall et al. 2015). Collectively, these approaches have shown that dimerization is mediated by an interaction of α-helical regions in the C-terminus of UCR1 with the N-terminus of UCR2, forming a tight 4-helix bundle (Richter and Conti 2002; Cedervall et al. 2015; Beard et al. 2000). Also present in the dimer is an interaction between UCR2 of one member of the dimer and the catalytic region of the other, providing a mechanism by which UCR2 serves as an auto-inhibitory domain (Cedervall et al. 2015). Finally, there is a smaller, but nonetheless biochemically significant, interface between the two catalytic domains, mediated by electrostatic interactions between Asp463 and Arg499 (PDE4D5 co-ordinates; Asp 471 and Arg507 in PDE4B1; refs. (Bolger et al. 2015; Cedervall et al. 2015)).

Dimerization provides many new insights into the enzymology and pharmacology of long PDE4 isoforms. The enzymatic and pharmacologic characteristics of the dimeric form are markedly different from those of the corresponding monomer. The dimeric form appears to exist as a “closed” or less-active conformation of the enzyme, with a specific activity for cAMP hydrolysis of dimeric PDE4B1 being roughly 50-fold lower than the corresponding monomeric form (Cedervall et al. 2015). Dimerization also affects the ability of long PDE4 isoforms to be inhibited by many PDE4-selective inhibitors; the effect of dimerization has been best-studied with the prototypical PDE4 inhibitor rolipram (Cedervall et al. 2015). These differences are mediated by a specific α-helical domain in the C-terminal half of UCR2 that, in the dimer, associates *in trans* with the catalytic domain (Cedervall et al. 2015), to create a high-affinity rolipram binding site (HARBS). In contrast, in the monomer, inhibitor binding is mediated exclusively by the catalytic region, to form a low-affinity rolipram-binding site (LARBS). The presence of a HARBS therefore reflects a conformational state unique to long PDE4 isoforms; short PDE4 isoforms, which lack UCR1 and therefore cannot dimerize, do not have a HARBS (Richter and Conti 2004; Huston et al. 1996; Rocque et al. 1997a; Rocque et al. 1997b; Souness and Rao 1997). These insights expand and modify prior models of PDE4

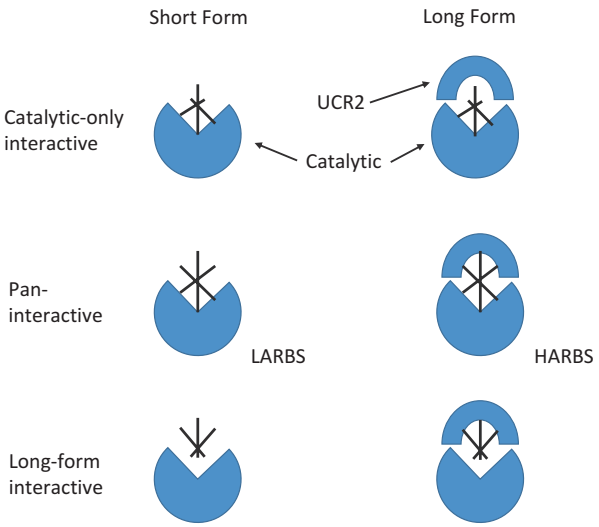


**Fig. 4.2** Structure of the drug-binding site in short and long PDE4 isoforms and the effects of various classes of inhibitors. Schematic representations of the PDE4 short and long isoforms are shown in the *left and right columns*, respectively. Short isoforms form monomers with no UCR2-catalytic interaction; long isoforms form dimers with a specific UCR2-catalytic interaction. PDE4-selective inhibitors are represented by the *intersecting black bars*. Catalytic-only inhibitors (*top row*) interact primarily with the catalytic region and less avidly with UCR2; they would have activity against both long and short isoforms. Pan-interactive inhibitors (*middle row*) interact with both the catalytic regions and UCR2; when UCR2 is not present, the interaction site has the conformation of a LARBS; when UCR2 is present, the interaction site has the conformation of a HARBS. They would have activity against both long and short isoforms, but with different inhibitory characteristics. Long-form inhibitors (*lower row*) interact primarily with UCR2 and less avidly with the catalytic region and therefore would have activity against only long isoforms

active site conformation (Lee et al. 2002; Zhang et al. 2004a; Card et al. 2004; Burgin et al. 2010; Wang et al. 2007a; Kranz et al. 2009; Fox et al. 2014; Gurney et al. 2011; Huai et al. 2006) and are highly likely to stimulate the identification of inhibitors that interact primarily with UCR2, with relatively less interaction with the catalytic domain (Fig. 4.2). These "long-isoform interactive" PDE4 inhibitors might therefore have a safety and/or efficacy profile distinct from the current generation of PDE4 inhibitors (Cedervall et al. 2015; Zhang et al. 2006; Zhao et al. 2003a).

Given these new findings, it is of interest to review the action of currently-approved PDE4 inhibitors. Roflumilast clearly acts similarly (i.e., with an $IC_{50}$ less than fivefold different) on the long and short forms encoded by any individual PDE4 gene (Hatzelmann et al. 2010). Similarly, the data on apremilast suggests that, like cilomilast (Giembycz 2001), it acts roughly equally on both long and short forms (Schafer et al. 2014). Another important characteristic of both roflumilast and apremilast is that their penetration into the CNS may be limited by the blood-brain barrier. There is little published pre-clinical data on crisaborole, which is designed for topical application. These characteristics of the currently-approved PDE4

inhibitors probably account for their improved tolerability in inflammatory and pulmonary disorders, compared to older agents, such as rolipram. However, it is clear that these clinically-useful characteristics of these three drugs actually reduces their potency in the CNS, indicating that further compound development work is essential to optimize the CNS-selectivity and effectiveness of PDE4-selective inhibitors. I present a potential pathway for these developmental activities below.

## 4.4 Dimerization and the Phosphorylation of PDE4s

The functions of PDE4 isoforms are dynamically regulated through phosphorylation by kinases such as PKA, ERK1/2, MK2, and AMPK, as well as modification by ubiquitination and sumoylation (Marchmont and Houslay 1980; Sette et al. 1994a; Sette et al. 1994b; Sette and Conti 1996; Hoffmann et al. 1998; MacKenzie et al. 2002; Collins et al. 2008; Baillie et al. 2001; Hoffmann et al. 1999; Baillie et al. 2000; MacKenzie et al. 2000; Mackenzie et al. 2011; Sheppard et al. 2014; Hill et al. 2006; Li et al. 2010). The activity of all long PDE4 isoforms is increased by two- to sixfold upon PKA phosphorylation, and PKA phosphorylation also changes the ability of the enzyme to be inhibited by PDE4-selective inhibitors, such as rolipram (Sette et al. 1994a; Sette et al. 1994b; Sette and Conti 1996; Hoffmann et al. 1998; MacKenzie et al. 2002). In contrast, ERK1/2 phosphorylation attenuates PDE activity (Hoffmann et al. 1999; Baillie et al. 2000; MacKenzie et al. 2000; Mackenzie et al. 2011). MK2 kinase serves to attenuate the degree of activation conferred by PKA phosphorylation and, in the case of PDE4D5, serves as a site for mono-ubiquitination by the β-arrestin-sequestered E3 ligase, Mdm3, which gates poly-ubiquitination of the PDE4D5 isoform-specific N-terminal region (Sheppard et al. 2014).

Recently, we have assessed the effects of phosphorylation on PDE4 dimerization. PKA phosphorylates a site (S54 in PDE4D3, S126 in PDE4D5 and S133 in PDE4B1; Fig. 4.1) in the motif QRRES located at the N-terminus of UCR2 (Sette et al. 1994a; Sette et al. 1994b; Sette and Conti 1996; Hoffmann et al. 1998; MacKenzie et al. 2002). ERK1/2 phosphorylates a site (S579 in PDE4D3, S651 in PDE4D5 and S659 in PDE4B1) located on the outer surface of the catalytic domain (Hoffmann et al. 1999; MacKenzie et al. 2000). MK2 phosphorylates a serine (S61 in PDE4D3, S133 in PDE4D5 and S140 in PDE4B1) close to the PKA site, within UCR1 (Sheppard et al. 2014).

Although all of these phosphorylation sites are located in highly flexible areas of the protein that are disordered in the crystal structure, suggesting that these regions are not essential for creation or maintenance of the dimer (Cedervall et al. 2015), we have shown recently that mutations of PKA, ERK1/2, MK2 and oxidative stress kinase phosphorylation sites can affect dimerization. Specifically, blocking phosphorylation at both the PKA and ERK1/2 phosphorylation sites diminished dimerization; mutations of each individual site had only modest effect (Bolger 2016). The precise mechanism of how PKA-ERK1/2 phosphorylation might

promote dimerization is uncertain; however, it is likely that phosphorylation at these sites would affect the conformation of the dimer and thereby push the equilibrium towards the dimeric form. In contrast, our analysis of phospho-mimetic mutations at the MK2 and stress oxidation kinase sites suggests that their action would be to promote the monomeric form.

## 4.5 Dimerization and Interaction of PDE Isoforms with Their Protein "Partners"

Given the extensive surfaces on PDE4 long forms that are necessary for dimerization (Cedervall et al. 2015), we felt that it was highly possible that their protein partners would restrict access to these surfaces and thereby inhibit dimerization. Recently, we demonstrated that the dimerization of PDE4D5 was blocked by two well-characterized protein partners, specifically RACK1 and β-arrestin2 (Bolger 2016). Given the high avidity and multiple sites of interaction between PDE4D5 and both of these proteins (Perry et al. 2002; Bolger et al. 2003; Baillie et al. 2003; Yarwood et al. 1999; Bolger et al. 2002; Steele et al. 2001; Li et al. 2009a; Bolger et al. 2006; Baillie et al. 2007; Smith et al. 2007), it is perhaps not surprising that they would have such an effect. However, since our prior studies have shown that both RACK1 and β-arrestin2 largely interact with the unique N-terminal and C-terminal regions of PDE4D5 (Bolger et al. 2003; Yarwood et al. 1999; Bolger et al. 2002; Bolger et al. 2006; Smith et al. 2007), which are unstructured in the dimer (Cedervall et al. 2015), it is unlikely that they act to directly restrict interaction at the UCR1/UCR2/catalytic or catalytic/catalytic interfaces that mediate dimerization. Instead, they presumably have indirect effects, possibly by sequestering the monomeric protein and thereby preventing it from forming a dimer, or by affecting its conformation in other ways. Inhibiting the dimerization of PDE4D5 could have multiple possible functional roles, such as increasing the enzymatic activity of PDE4D5 in certain cellular contexts, or targeting monomeric PDE4D5 to specific subcellular compartments.

RACK1 and β-arrestin2 have very different avidities for the "closed" or obligate-dimer conformation of PDE4D5. RACK1 interacts avidly with the "closed" conformation of PDE4D5, which is not entirely surprising, given its high avidity and selectivity for PDE4D5 and the extensive regions on PDE4D5 that can interact with RACK1 (Bolger 2016). However, in contrast, β-arrestin2 did not detectably interact with the "closed" conformation (Bolger 2016). This observation could provide novel insight into the physiological mechanism of the PDE4D5-β-arrestin2 interaction, in which β-arrestin2 serves to recruit PDE4D5 to the ligand-occupied, GRK2-phosphorylated state of the $\beta_2$-adrenergic receptor and thereby down-regulate cAMP signaling (Perry et al. 2002; Baillie et al. 2003). Since the major function of this recruitment is to move PDE4 enzymatic activity close to the $\beta_2$-adrenergic receptor, it would be logical that β-arrestin2 preferentially recruit the monomeric, or "open," form of PDE4D5, as this has much higher catalytic activity (50-fold greater, as

measured for PDE4B1; Cedervall et al. 2015). Therefore, the preferential interaction of β-arrestin2 with the monomeric form would maximize its physiologic function.

In summary, much has now been learned about the regulation of PDE4 isoforms by protein-protein interactions, including dimerization, and by phosphorylation. Since both of these processes require intimate contact between a PDE4 protein and its "partner" or kinase, these studies have also provided support for the concept that PDE4 regulation is highly spatially-dependent in cells, thereby providing a mechanism for the regulation of cAMP abundance in specific sub-cellular compartments (Francis et al. 2011; Conti and Beavo 2007; Houslay 2010; Bolger et al. 2007). This concept is particularly attractive in neurons, where PDE4 action could be targeted to specific synapses, axons, or dendrites, or other sub-cellular structures, rather than modulating cAMP levels globally throughout the cell. This compartmentalization of cAMP signaling, and PDE4 action in particular, is in turn compatible with PKA having different substrates in specific cellular compartments that are in turn regulated by different PDE4 isoforms. Selective targeting of these PDE4 isoforms could therefore produce highly specific pharmacologic effects, as discussed in the next section.

## 4.6 PKA Substrates as Mediators of PDE4 Action in the CNS

Key to understanding the cellular and organismal functions of the PDE4s is determining their downstream targets of action. Extensive research has demonstrated that cAMP binds to, and regulates the activity of, three effectors: (1) the regulatory subunit of cAMP-dependent protein kinase (kinase A; PKA); (2) the exchange protein directly activated by cAMP (Epac; refs. (de Rooij et al. 1998; Kawasaki et al. 1998; Gloerich and Bos 2010)) and (3) cAMP-gated ion channels. The cAMP-binding domains of each of these targets show significant structural similarity, reflecting their common function in binding cAMP (Rehmann et al. 2003; Kim et al. 2005; Zagotta et al. 2003). Epac acts as a cAMP-regulated guanine nucleotide exchange factor for Rap1 and has a range of physiologic functions (Gloerich and Bos 2010; Munoz-Llancao et al. 2015; Consonni et al. 2012; Gloerich et al. 2011). In contrast to the unique downstream effector of Epac, PKA has numerous substrates, the physiologic significance of which continues to evolve. In this section, we will focus on the following PKA substrates as being especially important in explaining PDE4 functions in the CNS:

### *4.6.1 CREB*

The loop-helix loop transcription factor cAMP-response element binding protein (CREB) is phosphorylated by PKA, ERK1/2 and several other kinases at a single serine (S133). CREB and phospho-CREB are expressed widely in the brain and their abundance changes in response to numerous neurotransmitters, drugs, and

stimuli, including those necessary for learning/memory and other behavioral processes (Silva et al. 1998; Frank and Greenberg 1994). Knock-out and dominant-negative genetic approaches have demonstrated that CREB has an essential role in learning and memory in a wide range of organisms, from *Aplysia californica*, to *Drosophila melanogaster*, rodents, and humans (Bourtchuladze et al. 1994; Yin et al. 1995; Cho et al. 1998; Kida et al. 2002; Ahn et al. 1999; Bartsch et al. 1998; Pittenger et al. 2002; Barco et al. 2002; Pittenger et al. 2006; Han et al. 2009; Lonze et al. 2002). CREB has been implicated in a variety of CNS phenotypes, including those implicated in affect (depression), reward (drug-seeking behavior and addiction) and several others (Newton et al. 2002; Carlezon et al. 1998). Investigators using PDE4 mutant mice have implicated CREB as an important contributor to the phenotypes seen in these mice, as described in more detail below.

A number of gene-expression and proteomic studies have attempted to identify CREB-responsive genes. Whole-genome sequencing has identified cAMP-response elements (CREs) in the promoters of numerous genes, some of which have been determined experimentally to be of functional significance in the transcriptional regulation of those genes (Kim et al. 2010). mRNA expression studies have identified numerous genes that are differentially regulated upon phosphorylation of CREB in cells, many of which contribute to neuronal growth and differentiation and synaptic plasticity (Casadio et al. 1999; Barco et al. 2005; Crino et al. 1998). However, the precise role of CREB phosphorylation in the regulation of many of these genes is not known. Collectively, however, these studies suggest strongly that many of the biochemical and cellular effects of PDE4 modulation in the CNS might be mediated through CREB, a hypothesis that has been tested extensively in the cellular and animal experiments reviewed below.

### *4.6.2 Cytoplasmic PKA Targets: LKB1 and GSK-3β Kinases*

PKA phosphorylates a number of kinases implicated in neuron growth and differentiation, especially in the hippocampus (Seino and Shibasaki 2005). Among the best-studied of these kinases are LKB1 and GSK-3β, both of which are essential for neuronal polarity during development and hippocampal neurogenesis (Song et al. 1997; Shelly et al. 2007; Ming et al. 1997; Huang et al. 2014; Barnes et al. 2007; Jiang et al. 2005; Yoshimura et al. 2005; Shelly et al. 2010). Treatment of cultured cortical neurons with rolipram, or transfection with siRNA directed against PDE4D isoforms, increases phosphorylation of LKB1 by PKA and impairs the development of neural polarity and reduces neural migration (Shelly et al. 2010). A number of extracellular or cell-surface components implicated in neuronal growth and differentiation, such as brain-derived neurotrophic factor (BNDF), NGF, netrin-1, laminin, or Wnt, could modulate cAMP levels in these cells. Although the physiological mechanism of cAMP elevation remains uncertain, these experiments implicate LKB1 and GSK-3β as likely PDE4-regulated PKA substrates in cortical neurons. It is highly possible that additional kinases, some of which may also be PKA substrates, contribute to these effects.

### 4.6.3 Cytoplasmic PKA Targets: DARPP32

The primarily cytoplasmic protein DARPP32 is an important PKA substrate in the CNS (Svenningsson et al. 2004). It is a 32 kDa protein that is phosphorylated at T34 by several kinases, including PKA, and at T75 by Cdk5. Phosphorylation of DARPP32 at T34 in turn depends on the phosphorylation state of S102 and S137, which are phosphorylated by CK2 and CK1, respectively (Svenningsson et al. 2004). Activation of the D1 dopamine receptor, a GPCR, by dopamine activates adenylyl cyclase and thereby PKA, increasing pT34-DARPP32 (Svenningsson et al. 2004; Stipanovich et al. 2008). Dopamine antagonists, such as haloperidol, and many drugs of abuse, such as cocaine, exert many of their effects through T34-DARPP32 phosphorylation (Bateup et al. 2008; Volkow and Morales 2015). As pT34-DARPP32 is in turn a potent inhibitor of PPT1 and pT75-DARPP32 is a potent inhibitor of PKA (Svenningsson et al. 2004), phosphorylation of DARPP32 produces profound changes in many cellular signaling pathways (Nishi et al. 2008; Svenningsson et al. 2004). pT34-DARPP32 can translocate to the nucleus, where it can inhibit nuclear PPT-1, enhance phosphorylation of histone H3, and regulate transcription (Stipanovich et al. 2008). Rolipram has been shown to enhance pT34-DARPP32 phosphorylation in striatopallidal neurons; this effect is accompanied by significant PKA-mediated phosphorylation of tyrosine hydroxylase (TH), essential for dopamine synthesis and turnover (Nishi et al. 2008). In contrast, PDE10 inhibition has no effect on TH phosphorylation, but substantially increases pT34-DARPP32 phosphorylation in striatal neurons (Nishi et al. 2008). The differential effects of these PDE4 inhibitors on dopamine signaling support investigation of PDE4-selective inhibitors as therapy in psychiatric and drug abuse disorders mediated, at least in part, by dopamine neurotransmission.

### 4.6.4 Ion Channels

There are two mechanisms by which cAMP can regulate ion channel activity. In the first mechanism, cAMP binds directly to a conserved intracellular cyclic nucleotide-binding domain (CNBD); this mechanism is important in several classes of cyclic nucleotide-gated ion channels (CNGs and HCNs; refs. (Zagotta et al. 2003; Craven and Zagotta 2006; Puljung et al. 2014)) whose functions in the mammalian CNS are an active area of research (DiFrancesco and DiFrancesco 2015; Nolan et al. 2004; Wang et al. 2007b; Kaupp and Seifert 2002). In the second mechanism, the ion channel is phosphorylated by PKA; a classical example of this mechanism is the cystic fibrosis transmembrane regulator (CFTR), which is a $Cl^-$ ion channel that is mutated in the disease cystic fibrosis and which has multiple PKA phosphorylation sites (Lambert et al. 2014; Baker et al. 2007).

PKA modulates the activity of a number of CNS-expressed ion channels, largely through the property of PKA to be tethered close to these ion channels by its interaction

with specific A-kinase anchoring proteins (AKAPs). For example, the strong inwardly rectifying potassium channel Kir2.1 forms a complex with AKAP79/150 and the related channel Kir6.2 is PKA-phosphorylated in its regulatory region in response to GPCR activation (Dart and Leyland 2001; Light et al. 2002). AKAPs are likely to be involved in the PKA-mediated phosphorylation at S333 of the potassium ion channel TREK-1, which is expressed widely in the CNS (Maingret et al. 2000). PKA-mediated phosphorylation of the A-type potassium channel Kv4.2 subunit occurs at two sites and requires the participation of a multi-protein regulatory complex (Schrader et al. 2002). The role of PDEs in the regulation of these channels remains to be determined.

AKAP79/150 also recruited into complexes at the postsynaptic membrane of excitatory synapses with N-methyl-d-aspartic acid (NMDA) or alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)-subtype glutamate GluA receptors, where it tethers PKA, protein kinase C, and protein phosphatase-2B (PP2B/calcineurin) into a dynamic regulatory complex (Westphal et al. 1999; Greengard et al. 1991; Banke et al. 2000; Tavalin et al. 2002). Recent studies have implicated PDE4, most notably ERK1/2-mediated phosphorylation of PDE4, in the regulation of membrane insertion of GluA1 (Song et al. 2013). GluA1 is also PKA-phosphorylated at a specific site (S845), but this phosphorylation is increased by PDE10, rather than PDE4, inhibition (Nishi et al. 2008; Greengard et al. 1991).

Extensive work in models of cardiac function has demonstrated that PDE4D3, and possibly other PDE4 isoforms, forms a complex with, and regulates PKA phosphorylation of, the cardiac ryanodine receptor (Beca et al. 2011; Lehnart et al. 2005) and other structures involved in the generation of cardiac calcium currents (Kerfant et al. 2007; Weninger et al. 2013; Leroy et al. 2011; Sin et al. 2011). Since calcium currents are also essential for many aspects of neuronal function, it would seem reasonable to search for PDE4-dependent activity of neuronal calcium flux; to date, however, such attempts have been unsuccessful.

### *4.6.5 Synaptic Vesicle Proteins*

The synaptic protein Rim1α is an important PKA target, being phosphorylated at two separate sites (Seino and Shibasaki 2005; Lonart et al. 2003; Park et al. 2014). However, Rim1α has also been shown to interact with Epac2 (Seino and Shibasaki 2005). Mutant RIM1α lacking the N-terminal PKA phosphorylation site was unable to rescue LTP in *RIM1α* knockout neurons but selectively suppressed LTP in wild-type neurons, clearly implicating a role of PKA-mediated phosphorylation Rim1α on presynaptic LTP (Lonart et al. 2003). A number of other synaptic vesicle proteins also appear to be PKA substrates (Seino and Shibasaki 2005; Park et al. 2014), although the exact physiological consequences of their PKA phosphorylation are not clear.

### *4.6.6 Ubiquitin Ligases*

The HECT domain E3 ubiquitin ligase UBE3A targets proteins to proteasome-mediated degradation (Yi et al. 2015). Duplication or truncation mutations in UBE3A have been linked to autism, while numerous different single amino acid mutations in UBE3A have been linked to Angelman syndrome (AS), a multi-component CNS disorder (Kishino et al. 1997; Jiang et al. 1998). Deletion of *Ube3a* in mice impairs synapse development and plasticity and produces a number of neurobiological phenotypes that mimic human AS (Yi et al. 2015). UBE3A is phosphorylated at T485 by PKA, and PKA-mediated phosphorylation of T485 inhibits UBE3A activity. Pharmacologic agents that elevate cAMP in dissociated mouse cortical neurons, including rolipram, augment phosphorylation of UBE3A by PKA. An AS-associated single amino acid mutation, T485A, blocks PKA action (Yi et al. 2015), thereby elevating UBE3A activity in cells, with enhanced substrate turnover and excessive dendritic spine development (Yi et al. 2015). These findings implicate a role for PDE4-mediated regulation of PKA activity in CNS development, with potential implications in several genetic disorders, including acrodysostosis, as discussed in greater detail in a section below.

## 4.7 Cellular Functions of PDE4 Action in the CNS

Given the diversity of PDE4 isoforms, and the large number of PKA substrates both in and outside of the CNS, it should not be surprising that numerous cellular functions are influenced in some way by the actions of PDE4 isoforms (Conti and Beavo 2007; Houslay 2010; Maurice et al. 2014; Bolger et al. 2007). Many of these functions are specific to organs or tissues outside the CNS (e.g., cardiac function, refs. (Maurice et al. 2014; Zaccolo 2009; Nikolaev et al. 2010; Richter et al. 2011; Eschenhagen 2013)) and are not discussed here. For this review, we will focus on two CNS-specific cellular functions: neurogenesis and synaptic plasticity.

### *4.7.1 PDE4s and Neurogenesis*

Appropriate levels of hippocampal neurogenesis are essential for normal learning and memory, pattern and spatial recognition, and potentially other functions (Gage 2000; Lie et al. 2004; Sahay et al. 2011; Zhao et al. 2008; Kitamura et al. 2009). Hippocampal neurogenesis occurs throughout human life, with a modest decline accompanying aging (Spalding et al. 2013). Neurogenesis appears to be essential for the anti-depressant effects of fluoxetine, a serotonin-selective re-uptake inhibitor (SSRI), in murine models of depression (Malberg et al. 2000; David et al. 2009; Santarelli et al. 2003). One study has shown that chronic fluoxetine can increased

dendritic arborization of newly-generated immature neurons (Wang et al. 2008). This study also showed that chronic fluoxetine accelerated the maturation of immature neurons. The effects of fluoxetine on neurogenesis are generalizable to other anti-depressants, such as rolipram and other PDE4-selective inhibitors (Li et al. 2009b; Xiao et al. 2011). They are also consistent with the results from the study of genetically-altered PDE4 mice, as described in more detail below.

### *4.7.2 PDE4s, Neuronal Polarity and the Formation of Axons and Dendrites*

As described above, the phosphorylation of LKB1 is dependent on a reciprocal interaction between cAMP and cGMP (Shelly et al. 2010). This reciprocal interaction also has an important role in neuronal development. High local concentrations of cAMP stimulate the differentiation of neurites from embryonic hippocampal neurons into axons, while cGMP stimulates the development of dendrites (Shelly et al. 2010). As predicted, PDE4D siRNA impaired the migration of neural precursor cells to the cortical plate and suppressed neuronal polarity during embryogenesis (Shelly et al. 2010). Although the functional implications of these processes in the intact brain remain uncertain, they may have important implications in a number of neurobiological processes, including cognition, learning and memory, and affect.

### *4.7.3 PDE4s and Synaptic Function*

Modulation of synaptic plasticity underlies, or is influenced by, numerous CNS functions, including learning and memory (Kandel et al. 2014), addiction (Volkow and Morales 2015), and sleep (Yang et al. 2014; Attardo et al. 2015). Emerging evidence from several systems has suggested that select PDE4 isoforms are targeted to synapses, where they can regulate cAMP levels in the local synaptic environment, affect PKA activity, and modulate plasticity. Among the best-understood of these mechanisms is the interaction of PDE4B1 with DISC1 (Millar et al. 2005; Murdoch et al. 2007; Bradshaw et al. 2011; Hayashi-Takagi et al. 2010; Bradshaw and Porteous 2012; Brandon and Sawa 2011), in which PDE4B1 and DISC1 form a complex with several other proteins, including dynein, LIS1, NDE1, and NDEL1 (Collins et al. 2008; Bradshaw et al. 2008). According to some models, DISC1 is felt to act as a scaffold for this complex and to recruit PDE4B1 to the synapse (Hayashi-Takagi et al. 2010; Bradshaw et al. 2008; Wang et al. 2011); other models have suggested that a major location of this complex is in the centrosome or nucleus, where it regulates gene expression (Bradshaw et al. 2011; Sheppard et al. 2014; Bradshaw and Porteous 2012; Soda et al. 2013; Ishizuka et al. 2011) and is active in early brain development (Greenhill et al. 2015; Mao et al. 2009; Niwa et al. 2010).

In both of these models, PDE4B1 is felt to regulate PKA's ability to phosphorylate T131 of NDE1 (Bradshaw et al. 2011) and S58 of DISC1 (Soda et al. 2013). DISC1 has been shown to interact with numerous proteins (Bradshaw and Porteous 2012), not all of which appear to be present in the complex under all physiologic circumstances, and the precise protein components of the PDE4B1-DISC1 complex, and its precise physiologic function(s), remain objects of intense investigation.

A number of PDE4 isoforms other than PDE4B1 have been implicated in synaptic function and, specifically, in hippocampal functions essential to learning and memory (see Sanderson and Sher 2013 for a review). For example, the PDE4B3 isoform has been implicated in LTP, especially late-phase LTP, in rat hippocampal neurons, where it has been localized to cell bodies and dendrites of neurons in hippocampal CA1 (Ahmed and Frey 2003). Another group has demonstrated that the anchoring protein gravin recruits a signaling complex containing PKA, PKC, calmodulin, and PDE4D isoforms to the $\beta_2$-adrenergic receptor (Havekes et al. 2012). Mice lacking the alpha-isoform of gravin have deficits in PKA-dependent long-lasting forms of hippocampal synaptic plasticity, including $\beta_2$-adrenergic receptor-mediated plasticity, and selective impairments of long-term memory storage (Havekes et al. 2012). These studies have collectively implicated a number of different PDE4 isoforms in synaptic plasticity, and particularly in learning and memory, and provide an essential background to interpretation of studies on genetically-modified PDE4 mice, which will be described in detail below.

## 4.8 Regional Expression of PDE4 Isoforms in the CNS and Potential Functional Implications

Each of the PDE4 isoforms has a distinct pattern of expression in cells and tissues and the vast majority of them has been demonstrated to have an isoform-specific pattern of expression in the CNS (Bolger et al. 1994; Cherry and Davis 1999; Miro et al. 2002; D'Sa et al. 2005; D'Sa et al. 2002; Reyes-Irisarri et al. 2008; Nishi et al. 2008; Mori et al. 2010; Kuroiwa et al. 2012; Ahmed and Frey 2003; Shakur et al. 1995; Suda et al. 1998; Farooqui et al. 2000; Zhang et al. 1999a; McPhee et al. 2001; Mackenzie et al. 2008; Perez-Torres et al. 2000; Johansson et al. 2012; Johansson et al. 2011; Braun et al. 2007). The regional expression of many isoforms, especially those identified recently, has yet to be determined. Unfortunately, there is little or no isoform-specific data in commonly-used CNS gene expression databases, such as the Allen Brain Atlas. Some isoforms, such as PDE4D5, are broadly-expressed in multiple CNS and non-CNS tissues (Miro et al. 2002; Bolger et al. 1997), while others, such as PDE4A1, are expressed strongly in a few tissues (e.g., cerebellum for PDE4A1) and expressed at much lower levels elsewhere (Shakur et al. 1995). These pronounced differences in regional expression in the CNS suggest strongly that each isoform has a distinct function; however, in most cases, the precise neurobiological function(s) of each isoform have only begun to be

appreciated. Better knowledge of the regional expression of PDE4 isoforms would in turn provide improved understanding of the phenotypes of genetically-altered PDE4 mice, as described in detail in a subsequent section.

### *4.8.1 Regional Distribution of PDE4 Isoforms in Brain Regions Involved in Dopaminergic Signaling: Addictive Behaviors, Depression and Schizophrenia*

A major objective of PDE4 CNS research has been to identify the functional role(s) of PDE4s in additive behavior. Experimental studies of addiction in a variety of model systems have identified many of the neuronal circuit, behavioral, and synaptic mechanisms involving this process (Volkow and Morales 2015). These studies have identified and characterized a drug-reward neuronal pathway in the CNS, extending from dopaminergic neurons in the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Many drugs of abuse, including opioids and cocaine, increase dopamine release in the shell subregion of the NAc (Di 2002) and elsewhere. Dopaminergic D1 and D2 receptors increase cAMP levels and the phosphorylation of CREB (Bibb 2005; Dudman et al. 2003; Antoine et al. 2013). Rolipram administration given prior to drug administration substantially reduced morphine-, cocaine- and cannabinoid-induced conditioned place preference in mice (Thompson et al. 2004; Zhong et al. 2012; Janes et al. 2009). Additionally, rolipram and other PDE4-selective inhibitors blocked inhibitory LTD and acute depression of inhibitory postsynaptic currents induced by D2 receptor and cannabinoid receptor agonists in VTA dopamine neurons (Zhong et al. 2012).

A number of studies have also implicated PDE4 isoforms in the NAc shell in the pathogenesis of depression. PDE4B and PDE4D isoforms are present in the NAc shell and that their expression is increased upon chronic administration of antidepressants (Cherry and Davis 1999; Takahashi et al. 1999). These effects are likely to be mediated by CREB, as over-expression of dominant-negative CREB in the NAc had an antidepressant effect in the learned-helpless model, while over-expression of wild-type CREB had an opposite effect (Newton et al. 2002). The specificity of these studies to depression is not clear, especially as chronic treatment with a number of antidepressants having different mechanisms of action (including tricyclics, SSRIs and PDE4 inhibitors) all increase levels of various PDE4 isoforms in a number of different areas of the brain (D'Sa et al. 2005; D'Sa et al. 2002; Ye et al. 1997; Ye et al. 2000; Zhao et al. 2003b; Dlaboga et al. 2006). In contrast, diminished stimulation of beta-adrenergic receptors, either by loss of noradrenergic innervation or by receptor blockade, reduces PDE4 activity (Farooqui et al. 2000; Ye and O'Donnell 1996; Zhang et al. 1999b).

Finally, immunohistochemical studies have demonstrated expression of PDE4A, PDE4B and PDE4D isoforms in frontal cortex, probably in D1-receptor-positive

neurons (Kuroiwa et al. 2012). Its location in these areas may contribute to the anti-schizophrenic effect of D1-receptor agonists.

Related to the role of PDE4 isoforms in depression and learning is the important influence of sleep and sleep disorders in these processes (Yang et al. 2014; Vecsey et al. 2009; Havekes et al. 2014); see refs. (Havekes et al. 2015; Meerlo et al. 2015) for a review. Normally, sleep promotes the development of dendritic spines after learning, implicating a beneficial role of sleep in memory consolidation (Yang et al. 2014). In contrast, sleep deprivation has been shown to produce memory loss in a number of rodent models of learning and memory, which is associated with impairment of cAMP- and PKA-dependent forms of hippocampal synaptic plasticity (Vecsey et al. 2009). Sleep deprivation increases PDE4 activity, possibly as a compensatory process (Vecsey et al. 2009). Transiently elevating cAMP levels in hippocampal excitatory neurons during sleep deprivation prevents memory consolidation deficits associated with sleep loss. These observations provide further evidence for the benefit of PDE4 inhibition on cognition and memory. The specificity of the benefit of PDE4 inhibition to sleep-disordered memory loss is uncertain, however, as rolipram and other PDE4 inhibitors improve cognitive function generally in mice, as described in greater detail in the next section.

Chronic stress (modeled in mice by an acute and unpredictable tail-shock), like sleep deprivation, increases PDE4 activity in hippocampal CA3 neurons and is associated with a marked impairment of hippocampal LTP (Chen et al. 2010).

## 4.9 CNS Effects of PDE4 Inhibitors

The molecular, cellular and regional studies described in the preceding sections provide a perspective essential to studying the phenotypes of PDE4 inhibition or ablation in the intact organism. Therefore, we will now discuss the whole-organism pharmacology of PDE4 inhibitors and then move to genetic models.

The prototypical PDE4 inhibitor rolipram was first identified by virtue of its antidepressant-like activity in humans and rodents (Fleischhacker et al. 1992; Scott et al. 1991; Hebenstreit et al. 1989; Eckmann et al. 1988; Zeller et al. 1984; Bobon et al. 1988; Kehr et al. 1985; Wachtel 1983). Its activity as a highly-selective PDE4 inhibitor was determined only after the publication of these early behavioral studies (Nemoz et al. 1985). Extensive testing of rolipram and numerous other PDE4-selective inhibitors in behavioral assays in rodents has demonstrated that they have activity that is broadly similar to other antidepressant agents, such as tricyclic antidepressants, SSRIs and SNRIs. Specifically, PDE4-selective inhibitors have antidepressant-like activity in hypothermia assays and in the forced-swim and tail-suspension tests (Barad et al. 1998; Bach et al. 1999; Titus et al. 2013; Mueller et al. 2010; Nibuya et al. 1996; O'Donnell and Zhang 2004; Zhang et al. 2006; Xiao et al. 2011; Jindal et al. 2012) and other assays (Wachtel 1983; O'Donnell 1993; O'Donnell and Frith 1999; Wachtel and Schneider 1986) used in the pre-clinical

testing of antidepressants. Numerous studies have also demonstrated that most classes of antidepressant drugs, although having disparate immediate targets, ultimately have overlapping effects on cAMP signaling pathways (Zhang et al. 2005b). For example, in rodents, several different classes of antidepressants elevate PDE4 levels, especially levels of PDE4D (Takahashi et al. 1999; Ye et al. 1997; Ye et al. 2000; Zhao et al. 2003b; Dlaboga et al. 2006) and increases levels of CREB (Nibuya et al. 1996) and phospho-CREB (Li et al. 2009b).

In addition to their antidepressant effects, PDE4 inhibitors have cognitive and memory-enhancing effects in rodents and possibly in humans. The potential memory-enhancing effects of PDE inhibition have been investigated for decades (Villiger and Dunn 1981) and the effects of rolipram studied soon after it was first synthesized (Randt et al. 1982) and subsequently (Egawa et al. 1997; Imanishi et al. 1997). The potential value of PDE4 inhibition in disorders of cognition and memory received support from two studies from the Kandel laboratory in 1999 that suggested that PDE4-selective inhibitors have cognitive- and memory-enhancing activity in mice (Barad et al. 1998; Bach et al. 1999). These results have been confirmed by other groups, using a range of experimental conditions (Zhang et al. 2005a; Titus et al. 2013; Mueller et al. 2010; Kuroiwa et al. 2012; Ahmed and Frey 2003; Xiao et al. 2011; Zhang et al. 2000; Zhang et al. 2004b; Hajjhussein et al. 2007; Rutten et al. 2009; Rutten et al. 2007a; Rutten et al. 2007b; Cheng et al. 2010; Li et al. 2011a; Rutten et al. 2008a; Rutten et al. 2006; Navakkode et al. 2005; Wang et al. 2012; Wang et al. 2013; Guan et al. 2011; Werenicz et al. 2012; Hotte et al. 2012; Giralt et al. 2011; Li et al. 2011b). One distinct experimental approach has been the use of NMDA inhibitors as pre-treatment prior to PDE4 inhibition; PDE4 inhibition clearly can reverse, at least in part, memory loss produced by these inhibitors (Zhang et al. 2005a; Zhang et al. 2000; Hajjhussein et al. 2007; Suvarna and O'Donnell 2002; Kato et al. 1997; Wieschollеck and Manahan-Vaughan 2012). These cognition/memory-enhancing effects have also been demonstrated in other rodent models, including the rat (Rutten et al. 2007a; Wieschollеck and Manahan-Vaughan 2012; Schaefer et al. 2012; Zhang and O'Donnell 2000). The effects of rolipram and other PDE4-selective inhibitors on cognition, learning and memory appear to be distinct from their antidepressant effects, as antidepressants of other classes do not seem to have these effects (Makhay et al. 2001). The results of all these studies have stimulated the development of PDE4 inhibitors specifically targeted at cognition and memory enhancement (Zhang et al. 2005a; Zhang et al. 2006); however, clinical trials of these compounds to date have proved to be disappointing.

Pre-clinical testing of PDE4-selective inhibitors in rodent models of emesis, such as in the ferret, have shown consistently that they have pro-emetic properties; this effect is mediated, at least in part, by central mechanisms (i.e., via the area postrema; refs. (Mori et al. 2010; Robichaud et al. 1999; Robichaud et al. 2002; Duplantier et al. 1996)). PDE4-selective inhibitors also have significant class-specific effects on the GI tract, in that they increase gastric production and bowel chloride secretion, leading to emesis and diarrhea (Fabbri et al. 2009; Calverley

et al. 2009; Schafer et al. 2014; Kavanaugh et al. 2015; Papp et al. 2015). These side effects of PDE4-selective inhibitors appear to be related to their pharmacologic mechanism of action, in that gastric acid production and secretory diarrhea are both caused by elevation of cAMP levels in GI epithelium (Hatzelmann et al. 2010; Lambert et al. 2014; Barnette et al. 1995; Okuda et al. 2009). Studies of both the CNS and non-CNS side effects of PDE4 inhibitors have been complicated by the lack of selectivity of PDE4 inhibitors for any individual PDE4 isoform, or subset of PDE4 isoforms, thereby rendering it uncertain which PDE4 isoform(s) are responsible for any specific side effect. However, experimental studies of emesis in *Pde4d* knockout mice have implicated the isoforms encoded by this gene as being most likely to be contributing to this effect (Robichaud et al. 2002).

## 4.10 Studies of PDE4 Function in the CNS Using Genetically-Modified Mice

Essential to the understanding of the functions of PDE4 isoforms in the CNS has been the development of mice with mutations or knockdowns in specific PDE4 isoform(s). Three approaches have been employed: gene knockouts, lentiviral siRNA, and dominant-negative approaches, respectively.

### *4.10.1 PDE4 Gene Knockouts*

The phenotypes of mice with knockouts in each of the *Pde4a, Pde4b* and *Pde4d* genes have been generated and studied extensively.

*Pde4a−/−* mice have been studied to date by a single group (Hansen et al. 2014). The knockout seems to have a beneficial effect on cognition and/or memory, based on one assay (the step-through-passive-avoidance test), but not in other assays, such as the Morris water maze. The mice also seem to have increased anxiety-like behavior, based on the elevated-plus maze, holeboard, light-dark transition, and novelty suppressed feeding tests. Consistent with the anxiety profile, *Pde4a−/−* mice had elevated corticosterone levels. The knockout did not seem to produce any change on tests of depression, such as the forced swim or tail suspension tests. Therefore, *Pde4a* may be important in the regulation of emotional memory and anxiety-like behavior.

*Pde4b−/−* mice have been studied by a number of groups, with disparate results (Zhang et al. 2008; Siuciak et al. 2008; Siuciak et al. 2007; Rutten et al. 2011). Some studies of *Pde4b−/−* mice have shown them to have behavioral characteristics that mimic the actions of antidepressants (Zhang et al. 2008; Siuciak et al. 2008; Zhang et al. 2002); for example, decreased immobility in tail-suspension and

forced-swim tests. However, other studies of the same genotype show only weak or modest effects in what appear to be similar assays (Siuciak et al. 2007; Rutten et al. 2011). Increased activity was also noted by some groups. There was no consistent effect on cognition or memory among the studies. These disparate findings are difficult to reconcile, although differences in genetic background, age at the time of study, or assay conditions could be responsible.

*Pde4d−/−* mice have also been studied by several groups. Some studies of *Pde4d−/−* mice have shown them to have augmented activity in tests of learning and memory (Li et al. 2011a; Zhang et al. 2002), while studies of the identical genotype by other groups do not show this effect (Rutten et al. 2008b). Almost all studies have shown increased levels of pCREB and increased hippocampal neurogenesis in these mice. Some groups also have shown that this knockout has an anti-depressant phenotype, consistent with the concept that PDE4D mediates antidepressant effects (Zhang 2009).

PDE4D−/− rats have also been generated recently (Kaname et al. 2014), although detailed characterization of their CNS phenotype awaits further publication. Of interest, however, is that they have skeletal abnormalities reminiscent of those seen in the human *PDE4D*-mutant disorder, acrodysostosis (see below).

Study of all PDE4 mouse knockouts have been complicated by non-CNS effects (Jin et al. 1999; Jin and Conti 2002), such as slow growth, small adult size and impaired fertility. In addition, assessment of the CNS phenotype of these knockouts has also been complicated by the fact that all of them have knocked out their respective gene in the entire organism, which, given the given the expression of isoforms from their respective genes in a number of brain areas (see section above), complicates assessment of their phenotype in any one area of the brain, such as the striatum or forebrain/hippocampus. Region-specific knockouts would allow exploration of these phenotypes.

### *4.10.2 Lentiviral siRNA*

Several groups have employed lentiviruses expressing siRNA to knock down a specific PDE4 isoform in the murine or rat CNS (Li et al. 2011a; Wang et al. 2013; Schaefer et al. 2012; Wang et al. 2015). The lentiviruses were injected into specific areas of the brain, typically the hippocampus, of wild-type or knockout mice. These experiments have the advantage of targeting both a specific PDE4 isoform and a specific region of the CNS. However, potential off-target effects of the siRNA and trauma related to the injection process remain legitimate concerns. These studies have confirmed and expanded the concept the *Pde4d* is essential to memory, hippocampal neurogenesis and the regulation of pCREB. *Pde4d* siRNA also has a profound effect on neuronal polarization, with potential implications for neural development and learning (Shelly et al. 2010).

### *4.10.3 Dominant-Negative PDE4 Mutants*

Two groups have now reported studies in which they used the over-expression of a dominant-negative PDE4B1 mutant as a transgene in the murine CNS (McGirr et al. 2016). As a precedent for this approach, we and our collaborators have used dominant-negative PDE4 mutants successfully in cell-based studies (Perry et al. 2002; Baillie et al. 2003; Bolger et al. 2006). In these cell-based studies, the dominant-negative mutant protein has been shown to displace the corresponding endogenous PDE4 isoform from its protein partner(s) and therefore disrupt its cellular function(s). The use of a dominant-negative mutant has the potential to be more isoform-selective than a gene knockout: The murine *Pde4b* and human *PDE4B* gene both encode five isoforms (Bolger et al. 1993; Bolger et al. 1994; Swinnen et al. 1991; Huston et al. 1997; Shepherd et al. 2003; Cheung et al. 2007; Johnson et al. 2010), each with a distinct protein structure and pattern of expression in tissues. Therefore, the *Pde4b−/−* mice described above have a phenotype that reflects the combined deficiency of all five PDE4B isoforms, which greatly complicates analysis of the effect(s) of any individual isoform, such as PDE4B1. The generation of dominant-negative mutants as transgenes also follows a strategy used by other groups who have expressed a dominant-negative PKA RIα subunit (Abel et al. 1997), or a dominant-negative CREB mutant (Silva et al. 1998; Kida et al. 2002; Ahn et al. 1999; Pittenger et al. 2002; Barco et al. 2002; Pittenger et al. 2006; Lonze et al. 2002; Vecsey et al. 2009) in the CNS. In the vast majority of these studies, the dominant-negative transgene was expressed off the CaMKIIα promoter (Mayford et al. 1996a; Mayford et al. 1996b; Tsien et al. 1996). This promoter is active preferentially in excitatory neurons of forebrain areas, including the hippocampus, amygdala, cortex and striatum (Mayford et al. 1996a; Mayford et al. 1996b). It is also silent until several days after birth (Burgin et al. 1990), when most neural circuits are already formed, thereby possibly minimizing any adverse effects of the transgene on the normal development of the brain (Tsien et al. 1996). The PDE4B1 dominant-negative approach is designed to target just the PDE4B1 isoform and therefore has greater specificity than a *Pde4b* knockout. This specificity is the likely explanation for the differences in phenotype in PDE4B1 dominant-negative mice, compared to *Pde4b−/−* mice. The PDE4B1 dominant-negative transgene clearly produces increased activity, levels of pCREB and neurogenesis, and may produce antidepressant effects in several assays (McGirr et al. 2016). One potential drawback of this approach is that the PDE4B1 dominant-negative transgene might not fully block PDE4B1 function, or, alternatively, might have some action against other PDE4 isoforms, including those encoded by the *Pde4a* and *Pde4d* genes. Despite these potential issues, the dominant-negative approach has merit and indeed appears to be best available way to study the relationship of a PDE4 isoform with its specific interacting partners, such as the interaction of PDE4B1 and DISC1.

### *4.10.4 What Have We Learned from the Mouse Models?*

The mouse genetic models collectively appear to have phenotypes that are broadly similar to those that would be predicted on the basis of the known CNS actions of PDE4-selective inhibitors: there is activation of PKA and phosphorylation of CREB, with antidepressant-like activity being detected in most although certainly not all, of the models. There also appears to be some effect on learning and memory in many of the models. Augmented neurogenesis has been detected in almost all the models that have been assayed and provides a likely cellular mechanism for both the antidepressant and memory-augmentation phenotypes that have been observed. The antidepressant effects seem to be mediated more by *pde4b* isoforms, whereas the memory effects are mediated more by *pde4d* isoforms, although the relative contributions of these two genes are likely to overlap substantially. These results are generally reassuring for drug development: they provide essential confirmation that the CNS effects of PDE4-selective inhibitors are indeed produced by their ability to inhibit PDE4 enzymatic activity, and not by some as-yet-unappreciated off-target effect. They are also compatible with generally-accepted theories of learning and memory (Silva et al. 1998; Volkow and Morales 2015; Kandel et al. 2014), and depression (Gage 2000; Lie et al. 2004; Zhao et al. 2008; Spalding et al. 2013; Nestler and Hyman 2010), and thereby provide continued impetus for the development of PDE4-selective inhibitors that can produce such effects therapeutically in humans.

A number of questions remain. One important question is determining the specific region(s) of the brain that are essential for PDE4-mediated phenotypes. The dominant-negative models that use the CamIIα promoter tend to confirm numerous prior observations that the hippocampus and forebrain are essential for the PDE4-related learning and memory phenotype; however, this conclusion is obviously dependent on the accuracy of prior observations on the tissue specificity of this widely-used promoter (Mayford et al. 1996a; Mayford et al. 1996b; Tsien et al. 1996; Mayford et al. 1995); see also (Hitti and Siegelbaum 2014). The models provide fewer insights into the regions essential for the antidepressant actions of PDE4-selective inhibitors. Further studies that employ tissue-specific or region-specific methods, such as cre/lox knock-out/knock-in methods, or optogenetic approaches, should provide additional insights.

## 4.11 Human PDE4D Mutations: Acrodysostosis Syndromes

The phenotypes of mice with PDE4 mutations contrast sharply with those identified to date in humans. Mutations in the gene encoding the PKA regulatory subunit Type 1A (PRKAR1A) have been identified as the cause of Carney Complex, a multi-spectrum disorder with cutaneous, cardiac and endocrine features and a

predisposition to several cancers (Carney et al. 1985; Kirschner et al. 2000; Salpea and Stratakis 2014). Intriguingly, a different set of PRKAR1A mutations have been detected in patients with acrodysostosis, a complex disorder affecting bone formation, growth and the CNS (Linglart et al. 2011; Lee et al. 2012; Linglart et al. 2012; Michot et al. 2012; Nagasaki et al. 2012; Muhn et al. 2013; Lindstrand et al. 2014). More recently, PDE4D mutations have been identified in patients with acrodysostosis that lack PRKAR1A mutations (Lee et al. 2012; Linglart et al. 2012; Michot et al. 2012; Lindstrand et al. 2014; Lynch et al. 2013). The skeletal dysplasia in patients with acrodysostosis with PRKAR1A mutations resembles the osteodystrophy seen in patients with pseudohypoparathyroidism Type 1a, in that they are resistant to the action of the hormones PTH and TSH (Linglart et al. 2011; Linglart et al. 2012), two hormones that activate adenylyl cyclase through GPCRs. However, patients with PDE4D mutations do not demonstrate resistance to these hormones (Linglart et al. 2011; Linglart et al. 2012), consistent with the gene defect being in a different portion (PDE4D v PKA) of the cAMP signaling pathway.

Of considerable interest to neurobiologists is that most patients with acrodysostosis and PDE4D mutations have significant mental retardation (Lee et al. 2012; Linglart et al. 2012; Michot et al. 2012; Lindstrand et al. 2014; Lynch et al. 2013); this is not typically seen in patients with acrodysostosis and PRKAR1A mutations, although some of those individuals have behavioral disorders. The presence of intellectual disorders in PDE4D acrodysostosis patients has led a number of investigators to test for PDE4D mutations in a broader population of patients with mental retardation and skeletal abnormalities. These efforts have led to the recent study of a mirror phenotype, involving intellectual disability and skeletal abnormalities different from acrodysostosis; genetic testing revealed PDE4D haploinsufficiency in these patients (Lindstrand et al. 2014). It seems quite likely that additional PDE4D-mutant syndromes affecting the CNS will be identified in the near future.

It is of considerable interest to compare the CNS phenotypes in the acrodysostosis patients to those seen in the PDE4D knockout mice. It is clear that the human phenotype is considerably more severe and affects multiple aspects of cognition and memory. Whether this reflects a purely species difference, or a different mutation mechanism (the acrodysostosis mutants may have a dominant-negative effect, as discussed below) is uncertain. There are no murine models of the acrodysostosis mutations; the CNS phenotype of the rat PDE4D−/− rat, which has skeletal abnormalities reminiscent of acrodysostosis, has yet to be published (Kaname et al. 2014).

## 4.12 Dimerization and the PDE4D Acrodysostosis Mutations

The structural data on the PDE4 dimer also provide great insight into the possible functional effects of PDE4D mutations that have been implicated in acrodysostosis. Of the 16 different single amino acid acrodysostosis mutations that have been identified to date, 15 map to the interface between UCR1/2 and the catalytic domain, or

to the "hinge" region connecting the dimerization domain to UCR1/2 and the catalytic domains (Cedervall et al. 2015). The 16th acrodysostosis mutation is at S133, the PKA catalytic site (Lindstrand et al. 2014); note that this and other genetic references use GenBank NM_001104631.1 for the mutation co-ordinates, with S133 in PDE4D5 being S190 in the GenBank entry.

The structural model may provide insight into the profound disability seen in patients with acrodysostosis mutations. Given that one of the acrodysostosis mutations is at the PKA phosphorylation site and completely blocks PKA phosphorylation of long PDE4D isoforms, and that all the PDE4D acrodysostosis mutations have a similar phenotype, it is quite possible that all PDE4D acrodysostosis mutations serve to inhibit PKA-mediated activation of PDE4D enzymatic activity, or lower PDE4D enzymatic activity in other ways (Kaname et al. 2014). Therefore, cAMP levels would be elevated in PDE4D acrodysostosis cells, activating PKA activity at its substrates and producing a potentially broad range of phenotypes. Consistent with this model is the observation of compensatory activation of other PDE4 isoforms (e.g., PDE4A and PDE4B) in acrodysostosis cells (Kaname et al. 2014).

Since acrodysostosis mutations lower PDE4D enzymatic activity, which is also the pharmacologic effect of rolipram and other PDE4-selective inhibitors, the severe bone and CNS manifestations of acrodysostosis provide a rationale for caution in the human use of PDE4-selective inhibitors. It is possible that disorders of bone (or of the CNS) might be unanticipated side effects of PDE4 inhibition. To date, bone abnormalities (e.g., osteoporosis) have not been reported as a side effect of adult use of PDE4 inhibitors, however, exposure earlier in development (e.g., in utero) might produce profound skeletal development effects and remains a legitimate concern.

## 4.13 Conclusion: Implications for Future PDE4 CNS Drug Development

There are now grounds for reasonable optimism for the development of PDE4-selective inhibitors that would target the CNS and thereby be potentially useful in the treatment of depression and other affective disorders, psychosis, cognitive and memory dysfunction, addictive disorders, and possibly other conditions. I propose that such agents should target the long PDE4 isoforms preferentially. Expression studies show that long forms are preferentially expressed in the CNS. Furthermore, only the long isoforms are capable of dimerization, with the corresponding change in enzymatic properties and the formation of a HARBS. Indeed, it is generally agreed that CNS tissues are enriched in HARBS (Rocque et al. 1997a; Rocque et al. 1997b; Souness and Rao 1997; Zhang et al. 2006; Zhao et al. 2003a; Zhao et al. 2003b). To be effective therapeutically, these new inhibitors would also need to have low emetic potential; given our lack of knowledge of the exact targets for PDE4 inhibitors in the area postrema, this could remain a significant problem.

Finally, these PDE4 inhibitors would need to permeate the blood-brain barrier and have appropriate bioavailability. Despite these obstacles, there are grounds for optimism.

**Conflict of Interest** The author declares that he has no conflicts of interest.

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# Chapter 5
# Phosphodiesterase-4B as a Therapeutic Target for Cognitive Impairment and Obesity-Related Metabolic Diseases

**Steven J. Clapcote**

**Abstract** People in modern, affluent societies are living longer but also becoming increasingly overweight. With increased life expectancy comes increased risk of developing age-related cognitive decline and neurodegenerative diseases, such that an increasing proportion of life may be lived with cognitive impairment as age increases. Obesity is associated with poorer cognitive function in elderly subjects, and often leads to ill-health arising from various complications such as metabolic syndrome and type-2 diabetes mellitus. This chapter provides an overview of the effects of administering pan-phosphodiesterase-4 (PDE4) inhibitors to animal models of cognitive ageing, Alzheimer's disease, frontotemporal dementia, fragile X syndrome, obesity and diabetes. Inhibition of the PDE4B subtype specifically is discussed as an approach to avoid the emetic side effects of pan-PDE4 inhibitors, whilst retaining their therapeutic effects. Finally, the findings of rodent studies that employ genetic and pharmacological approaches to specifically target PDE4B are discussed in relation to the potential utility of PDE4B-selective inhibitors for the treatment of cognitive impairment and obesity-related metabolic diseases.

**Keywords** Phosphodiesterase-4 • PDE4B • cAMP signalling • Rolipram • Cognitive enhancement • Alzheimer's disease • Frontotemporal dementia • Fragile X • Obesity • Diabetes • Traumatic brain injury

## 5.1 Introduction

Cyclic adenosine monophosphate (cAMP) is a second messenger used for intracellular signal transduction to regulate a wide range of biological processes, such as the cellular response to neurotransmitters in the central nervous system (CNS) (Houslay et al. 2007). Intracellular concentrations of cAMP are regulated by its synthetase,

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adenylyl cyclase, and its hydrolase, the cAMP-specific phosphodiesterases. Phosphodiesterase-4 (PDE4) is the predominant enzyme that selectively hydrolyses and inactivates cAMP. Thus, inhibitors of PDE4 provide a pharmacological strategy to increase prevailing cAMP levels in relevant target tissues. The PDE4 family comprises four subtypes (A–D), each encoded by a separate gene, which have clearly distinct expression patterns at the regional and cellular levels (Richter et al. 2013). These enzymes are expressed throughout the body, with the highest levels of activity and protein found in the brain (Richter et al. 2013).

PDE4B is expressed in various isoforms across a wide range of human tissues, including adipose tissue, whole blood, liver and brain (Bunnage et al. 2015). All five PDE4B isoforms identified in mammals contain the catalytic domain (Zhang 2009). The long isoforms PDE4B1 (736 a.a.), PDE4B3 (721 a.a.) and PDE4B4 (659 a.a.) contain both Upstream Conserved Region 1 (UCR1) and Upstream Conserved Region 2 (UCR2); the short isoform PDE4B2 (564 a.a.) lacks UCR1; and the super-short isoform PDE4B5 (484 a.a.) lacks UCR1 and has a truncated UCR2 (Shepherd et al. 2003; Cheung et al. 2007; Fatemi et al. 2008). Long isoforms are activated by protein kinase A (PKA)-mediated phosphorylation of a single serine residue in UCR1 (MacKenzie et al. 2002), and are inhibited by extracellular signal-related kinase-2 (ERK2)-mediated phosphorylation of a single serine residue located within the C-terminal portion of the catalytic domain (Baillie et al. 2000). Conversely, the short isoform PDE4B2 is activated by ERK2 phosphorylation (Baillie et al. 2000). PDE4B2 is the predominant and often only isoform present in tissues outside the CNS, while the CNS contains the long (PDE4B1, 4B3, 4B4), short (PDE4B2) and super-short (PDE4B5) isoforms (Bunnage et al. 2015). This chapter discusses the potential utility of PDE4 inhibitors, especially those selective for PDE4B, in the treatment of cognitive impairment and obesity-related metabolic diseases.

## 5.2 Effects of Pan-PDE4 Inhibitors on Cognitive Impairment

Long-term memory formation following new protein synthesis relies on the expression of genes upregulated by cAMP response element binding protein (CREB) (Guzowski and McGaugh 1997). A transcription factor, CREB is phosphorylated and activated by PKA via an increase in cAMP, and regulates gene transcription by binding to the cAMP response element on target genes (Habener 1990). As a consequence, PDE4 enzymes, as regulators of cAMP gradients and ultimately CREB, are promising targets for the development of cognition-enhancing agents (Randt et al. 1982; Ghavami et al. 2006; Richter et al. 2013). PDE4 inhibitors are drugs used to block the degradative action of PDE4 on cAMP. The prototypical PDE4 inhibitor is rolipram (CAS: 61413-54-5), a non-subtype-selective or pan-PDE4 inhibitor (targeting all of four subtypes) with a half-life of 3 h that readily passes through the blood-brain barrier, is rapidly cleared by the kidneys, and does not accumulate in the tissues (Schneider 1984; Krause and Kuhne 1988). Rolipram engages the N-terminal UCR2 domain (truncated in super-short isoform PDE4B5), thereby closing UCR2 across the PDE4

active site and preventing access of cAMP (Burgin et al. 2010). It has an inhibitory concentration 50% ($IC_{50}$) value of 225 nM against PDE4B and 228 nM against PDE4D (Burgin et al. 2010; Fox et al. 2014). PDE4B knockout (KO; *Pde4b*$^{tm1Mct}$) mice (see Sects. 5.3.1 and 5.5.1) show a partial (~50%) reduction in sensitivity to rolipram (3.2 mg/kg) in a conditioned avoidance response task (Siuciak et al. 2007), suggesting that the effect of rolipram in this test is mediated, in part, through PDE4B.

The primary effect of inhibition of PDE4 is the elevation in intracellular levels of cAMP (Scheider 1984), which is assumed to regulate transcription of various target genes by increasing cAMP-dependent PKA activity. Pan-PDE4 inhibitors, such as rolipram, have shown therapeutic benefit in preclinical models of psychiatric and neurological conditions including memory and cognition impairments (Ghavami et al. 2006; Richter et al. 2013). It has thus been postulated that PDE4 inhibition may promote improved synaptic function or 'synaptic resilience' (Bales et al. 2010). This section gives an overview of inhibition of PDE4 as a potential therapeutic strategy for cognitive impairment, focussing on evidence from animal model studies of cognitive ageing, Alzheimer's disease, frontotemporal dementia, and fragile X syndrome (Table 5.1).

### *5.2.1 Age-Associated Cognitive Decline*

Cognitive ageing is a lifelong process of gradual, ongoing, yet highly variable changes in cognitive function that occurs as people get older. Mental capabilities that decline from middle age onwards include aspects of memory, executive functions, processing speed and reasoning. All of these so-called 'fluid' mental abilities are important for carrying out everyday activities, living independently and leading a fulfilling life (Deary et al. 2000). Individuals may, for example, have difficulty driving a car, making financial decisions, or following directions for prescription medicines (Blazer et al. 2015). Life expectancy in developed countries has increased substantially (Hicks and Allen 1999), such that an increasing proportion of life may be lived with cognitive impairment as age increases.

In a Barnes maze, aged (18-month-old) C57BL/6 mice given rolipram (0.05 μM/day, i.p.) on days 15–40 of training showed a reduced number of errors, and an increased percentage reached a learning criterion, compared with controls (Bach et al. 1999). In contextual fear conditioning, rolipram (0.1 μmol/kg, s.c.) given 30 min before training increased contextual freezing after 24 h in both young (12–16-week-old) and aged (18 month-old) C57BL/6 mice compared with controls (Barad et al. 1998). The effective dose increased cAMP signalling without affecting basal levels of cAMP (Barad et al. 1998). In a novel object recognition test, aged (23-month-old) rats given rolipram (0.1 mg/kg) immediately after training showed enhanced memory after 24 h, compared with controls (de Lima et al. 2008). The efficacy of this post-training treatment ruled out the possibility that the rolipram effects were due to drug-induced behavioural or sensory alterations at training. In a Morris water maze, aged (20-month-old) C57BL/6NTac mice given another pan-PDE4 inhibitor, HT-0712 (0.15 mg/kg/day, i.p.), 20 min before training over 15

**Table 5.1** Cognitive effects of pan-PDE4 inhibitors in animal models of cognitive impairment

| Animal model | Behavioural test | Drug treatment | Results | References |
|---|---|---|---|---|
| *Age-associated cognitive decline* | | | | |
| Aged mice (18-months-old, C57BL/B6, ♂) | Barnes maze | Rolipram, chronic (0.05 μM/day, i.p., up to 40 day) | Fewer errors, and an increased % reaching a learning criterion | Bach et al. (1999) |
| Aged mice (18-months-old, C57BL/B6, ♂ + ♀) | Contextual fear conditioning | Rolipram, acute (0.1 μmol/kg, s.c., 30 min before training) | Enhanced contextual memory at 24 h | Barad et al. (1998) |
| Aged rats (23-months-old, Wistar, ♂) | Novel object recognition | Rolipram, acute (0.1 mg/kg, i.p., immediately after training) | Enhanced object recognition memory at 24 h | de Lima et al. (2008) |
| Aged mice (20-months-old, C57BL/6NTac, ♂) | Morris water maze | HT-0712, chronic (0.15 mg/kg/day, i.p., 20 min before training, 15 day) | Enhanced spatial reference memory after 10 and 15 d of training | Peters et al. (2014) |
| | Trace fear conditioning | HT-0712, acute (0.15 mg/kg/day, i.p., 20 min before training) | Enhanced trace fear memory at 24 h | Peters et al. (2014) |
| | Contextual fear conditioning | HT-0712, acute (0.15 mg/kg/day, i.p., 20 min before training) | Enhanced trace fear memory at 24 h | Peters et al. (2014) |
| Aged macaque monkeys (18–30 years, ♀) | Spatial delayed response task | Rolipram, acute (0.01–1 μg/kg or 0.01 mg/kg, i.m., 1 h before training) | No effect or fewer trials correct | Ramos et al. (2003) |

| | | | | |
|---|---|---|---|---|
| *Alzheimer's disease* | | | | |
| Aβ25–35-infused rats (Sprague-Dawley, ♂) | Morris water maze | Rolipram, chronic (0.1–0.5 mg/kg/day, i.p., 19–20 day, 1 h before training or testing) | Enhanced spatial reference memory at 24 h | Cheng et al. (2010), Wang et al. (2012) |
| | Step-through passive avoidance | Rolipram, chronic (0.5 mg/kg/day, i.p., 22–23 day, 1 h before training or testing) | Enhanced passive avoidance memory at 24 h | Cheng et al. (2010) Wang et al. (2012) |
| Aβ40-infused rats (Sprague-Dawley, ♂) | Step-through passive avoidance | Rolipram, chronic (0.5 mg/kg/day, i.p., 8–32 day, 1 h before testing) | Enhanced passive avoidance memory at 8–32 d | Cheng et al. (2010) |
| $APP^{K670N,M671L}/PSEN1^{M146L}$ double-transgenic mice (♂ + ♀) | Contextual fear conditioning | Rolipram, chronic (0.03 mg/kg/day, s.c., 3 weeks, 3 months before testing) | Enhanced contextual memory at 24 h | Gong et al. (2004) |
| | Morris water maze | Rolipram, chronic (0.03 mg/kg/day, s.c., 3 weeks, 3 months before testing) | Enhanced spatial reference memory | Gong et al. (2004) |
| | Radial arm water maze | Rolipram, chronic (0.03 mg/kg/day, s.c., 3 weeks, 3 months before testing) | Fewer arm entry errors | Gong et al. (2004) |
| $APP^{K670N,M671L}$ transgenic mice (9-months-old, ♂) | Contextual fear conditioning | Rolipram, acute (0.1 mg/kg, i.p., 30 min before training) | Enhanced contextual memory at 20 h | Comery et al. (2005) |

(continued)

**Table 5.1** (continued)

| Animal model | Behavioural test | Drug treatment | Results | References |
|---|---|---|---|---|
| *Frontotemporal dementia* | | | | |
| rTg(tauP301L)4510 mice (3–4 months-old, ♂) | Morris water maze | Rolipram, chronic (0.03 mg/kg, i.p., twice daily, 21 day, 6 h before training) | Enhanced spatial learning | Myeku et al. (2016) |
| rTg(tauP301L)4510 mice (8–10 months-old, ♂) | Morris water maze | Rolipram, chronic (0.03 mg/kg, i.p., twice daily, 21 day, 6 h before training) | No effect | Myeku et al. (2016) |
| *Fragile X syndrome* | | | | |
| Heterozygous *fmr1* null *Drosophila* | Negatively reinforced olfactory learning | Rolipram, acute (200 μM, diet, 5–6 h, before and after training) | Enhanced olfactory memory at 24 h | Kanellopoulos et al. (2012) |
| Heterozygous *fmr1* null *Drosophila* (♂) | Conditioned courtship-associative memory | Rolipram, chronic (50 μM, diet, 9 day) | Enhanced immediate-recall (<2 min) and short-term (1 h) courtship memory | Choi et al. (2015) |
| | Conditioned courtship-associative memory | Rolipram, chronic (500 μM, diet, 9 day) | Enhanced immediate-recall (<2 min) and short-term (1 h) courtship memory | Choi et al. (2015) |
| Heterozygous *fmr1* null *Drosophila* | Olfactory conditioning | Rolipram, acute (50 μM, diet, 12 h) | Enhanced immediate-recall (<2 min) | Choi et al. (2015) |

♂, male; ♀, female; i.p., intraperitoneal; s.c., subcutaneous; i.m. intramuscular; h, hours; d, days

days demonstrated enhanced memory of the location of an escape platform after 10 and 15 days of training compared with controls (Peters et al. 2014). In both contextual and trace fear conditioning, aged (20–24-month-old) C57BL/6NTac mice given HT-0712 (0.1 mg/kg, i.p.) 20 min before training showed increased freezing after 24 h compared with controls (Peters et al. 2014).

Adult rats of an unspecified age were given four different doses of rolipram (0.01, 0.03, 0.1, and 0.3 mg/kg) 3 h after the last training trial on each of 4 days in the Morris water maze (Hosseini-Sharifabad et al. 2012). Rats given 0.03 mg/kg rolipram showed a shorter escape latency and distance swum on the second day of training. In a probe trial at the end of training, with the escape platform removed, rats treated with 0.03 mg/kg rolipram spent more time at the target location, indicating enhanced spatial reference memory. Other doses of rolipram did not show any differences compared with controls. Thus, rolipram enhanced spatial memory consolidation in an inverted U-shaped dose-response curve, such that higher doses of rolipram were not as efficacious as lower doses in improving hippocampus-dependent learning (Hosseini-Sharifabad et al. 2012). This might be explained by sedative effects, which have been observed in mice following the administration of rolipram at higher doses (≥0.12 mg/kg), demonstrated by dose-dependent hypoactivity in the open field task (Griebel et al. 1991; Barad et al. 1998; Hu et al. 2011).

Presynaptic ChAT activity was found to be reduced in the frontal cortex and the hippocampus of aged (24-month-old) rats, but was restored to young-adult control levels by rolipram treatment (0.1 mg/kg/day) for 14 days (Asanuma et al. 1993). Since the 5′ upstream region of the rat ChAT gene contains a CREB binding site (Bejanin et al. 1992), it is possible that the function of rolipram in improving memory in aged rodents may be partly implemented through the enhancement of ChAT activity via phosphorylation of CREB (pCREB).

By contrast with the rodent findings, aged (18–30 years) macaque monkeys given rolipram 1 h prior to training in a spatial delayed response task showed no difference in working memory (number of trials correct) at lower doses (0.01–1 μg/kg, i.m.), and performed significantly worse than vehicle-treated controls following the highest dose (0.01 mg/kg, i.m.) (Ramos et al. 2003). Another pan-PDE4 inhibitor, etazolate (delivered near the recorded neurones by iontophoresis), was found to reduce the memory-related firing rate of prefrontal cortical neurones in a single aged (17-year-old) male macaque as it performed the oculomotor delayed response spatial working memory task (Wang et al. 2011a). The apparent discrepancy between the aged rodent and monkey findings may exist because only hippocampus-dependent reference memory was assessed in the rodent studies, whereas only prefrontal cortex-dependent working memory was assessed in the monkey studies. The impairment of prefrontal cortical function by PDE4 inhibition in aged monkeys may be related, in part, to possible decreases in cortical PDE4 subtype expression that occur with age. In aged (25-month-old) rats, PDE4D mRNA expression is reduced in the cortex and cerebellum, and PDE4A mRNA expression is reduced in the striatum, whereas PDE4B mRNA expression in the hippocampus, cortex, striatum and cerebellum shows no change compared with young (5-month-old) rats (Kelly et al. 2014).

### 5.2.2 Alzheimer's Disease

Alzheimer's disease (AD) is a chronic neurodegenerative condition characterised by progressive cognitive decline, memory loss and personality changes, eventually leading to death. The prevalence of AD increases with life expectancy, being rare before the age of 60 but affecting more than one-third of people over the age of 90 (Querfurth and LaFerla 2010). Absolute confirmation of diagnosis can only be made by a post-mortem examination of the brain to demonstrate the neuropathological changes first described by Alois Alzheimer, namely plaques and tangles (Alzheimer 1907). The extracellular plaques, formed by fibrillary aggregates of amyloid-β (Aβ) peptides, together with the intraneuronal neurofibrillary tangles formed by hyperphosphorylated tau protein, are thought to be the causative agents in AD (Hardy 2009).

There is a growing body of evidence suggesting that neuroinflammation can contribute to AD pathogenesis (Heppner et al. 2015). High levels of the pro-inflammatory cytokine tumour necrosis factor-alpha (TNFα) have been detected in AD patients (Fillit et al. 1991; Tarkowski et al. 1999). A recent study used PET imaging to measure TSPO, a biomarker for inflammation, in amyloid-positive AD patients and amyloid-negative controls (Kreisl et al. 2013). AD was found to be associated with increased inflammation in the brain, mostly in temporal and parietal regions known to be affected by amyloid plaque pathology. In these same regions, increased inflammation correlated with amyloid burden and with the degree of cognitive impairment. Early-onset AD patients showed greater inflammation than late-onset patients (Kreisl et al. 2013), which might explain the more precipitous disease course typically seen in early-onset patients. The immune system may thus provide novel routes for the treatment of AD.

Cyclic AMP signalling is involved in the regulation of postsynaptic, protein synthesis-dependent long-term potentiation (LTP) in the hippocampus (Kandel 2001), a form of synaptic strengthening thought to underlie the formation and persistence of memory (Malenka and Bear 2004). Rolipram has been shown to potentiate LTP in hippocampal slice preparations (Barad et al. 1998). Aβ peptides strongly inhibit LTP in hippocampal slices (Cullen et al. 1997; Itoh et al. 1999; Vitolo et al. 2002), but Aβ-induced inhibition of LTP is reversed by rolipram (Vitolo et al. 2002), suggesting a direct effect of Aβ on the cAMP signalling pathway. Similarly, Aβ-induced reduction in the spine density of pyramidal hippocampal neurones is attenuated by rolipram (Smith et al. 2009). Treatment with rolipram (0.5 mg/kg/day, i.p.) beginning 12–13 prior to the onset of training reversed the impairments in Morris water maze and passive avoidance tests of rats infused with aggregated Aβ peptides into the hippocampal CA1 (Cheng et al. 2010; Wang et al. 2012). Rolipram also reversed the LTP deficit of hippocampal slices from 3-month-old APP/PSEN1 double-transgenic mice, which overexpress both mutant APP-K670N,M671L under the control of the hamster PRNP promoter (Tg(APPSWE)2576Kha) and PSEN1-M146L under the control of the human PDGFB promoter (Tg(PDGFB-PSEN1M146V)3Jhd) (Gong et al. 2004). Acute rolipram treatment (0.03 mg/kg, s.c.) 30 min before training reversed the deficit of APP/PSEN1 mice in contextual

fear conditioning but not in a radial-arm water maze. Chronic rolipram treatment (0.03 mg/kg/day, s.c.) for 3 weeks, followed by a 3-month hiatus, showed persistent effects by reversing the deficits of 6-month-old APP/PSEN1 mice in hippocampal LTP, contextual fear conditioning, a radial arm water maze and a Morris water maze, but had no effect on Aβ load (Gong et al. 2004). Pyramidal hippocampal neurones from APP/PSEN1 mice given rolipram (0.03 mg/kg) daily for 3 weeks from either 3 or 15 months of age showed increased spine density, dendrite area and diameter, and spine head diameter compared with neurones from vehicle-treated APP/PSEN1 mice (Smith et al. 2009). Rolipram (0.1 mg/kg, i.p.) given 30 min before training also reversed the deficit of 9-month-old mutant APP-K670N,M671L transgenic mice in contextual fear conditioning, but had no effect on contextual conditioning in wild-type (WT) mice (Comery et al. 2005).

Several observations suggest that the beneficial effects of rolipram in rodent models of AD are mediated, at least in part, through PDE4B. The transcription of PDE4B is upregulated in area CA1 of the hippocampus after LTP induction (Ahmed and Frey 2005), suggesting a specific role for PDE4B in this form of synaptic plasticity. PDE4B transcription is also upregulated in microglial cells exposed to Aβ peptides, resulting in an increased production of TNFα, but rolipram markedly reduces the release of TNFα from Aβ-stimulated microglia cells (Sebastiani et al. 2006). In inflammatory cells from PDE4B, but not PDE4A or PDE4D, KO mice, TNFα production in response to an inflammatory stimulus (lipopolysaccharide, LPS) is reduced by over 50% (Jin and Conti 2002; Jin et al. 2005), while PDE4B-selective inhibitors (see Sect. 5.5.1) similarly blunt monocytic production of TNFα in vitro and in vivo (Naganuma et al. 2009; Suzuki et al. 2013). PDE4B mRNA expression across the brain remains stable throughout the ageing process in rats, whereas aged (25-month-old) rats show reduced expression of PDE4A and PDE4D mRNA in some brain regions (Kelly et al. 2014). Finally, PDE4B is the only subtype of PDE4 expressed in the locus coeruleus (Cherry and Davis 1999), a brain region affected by neurofibrillary degeneration early in the course of events leading to AD (Grudzien et al. 2007).

### *5.2.3 Frontotemporal Dementia*

Mutations in the *MAPT* gene encoding the tau protein cause frontotemporal dementia with parkinsonism-17 (FTDP-17), or Pick's disease, a rare neurodegenerative disorder that affects the frontal and temporal lobes of the brain. Clinical manifestations of FTDP-17, including behavioural and personality changes, cognitive impairment, and motor symptoms, usually become noticeable in a person's forties or fifties (Hutton et al. 1998). FTDP-17 is a tauopathy, characterized by an abnormal build-up of tau proteins in neurones, accumulating into spherical aggregations known as 'Pick bodies' (Piguet et al. 2011). Expression of human tau containing the most common FTDP-17 mutation, P301L (Hutton et al. 1998), in forebrain neurones under the control of the *Camk2a* promoter results in progressive age-related neurofibrillary

tangles, loss of neurones and generalized forebrain atrophy, and spatial reference memory impairments in rTg(tauP301L)4510 transgenic mice (Santacruz et al. 2005). At 3–4 months, these mice model early-stage disease; by 8 months they resemble a more severe stage of the human disease.

Tau can be degraded by both lysosomal processes, such as autophagy, and the ubiquitin-proteasome system (Lee et al. 2013). Cyclic AMP-dependent PKA phosphorylation of the proteasome enhances proteolytic activity (Asai et al. 2009). Exposure of cortical brain slices from rTg(tauP301L)4510 mice aged 3–4 months to rolipram for 8 h reduced the amounts of total and insoluble tau (Myeku et al. 2016). To assess the effect of rolipram on tauopathy-associated cognitive impairment, 3–4 month-old rTg(tauP301L)4510 mice were given rolipram (0.03 mg/kg, i.p., twice daily) for 21 days and then tested for spatial learning in the Morris water maze 6 h after the final administration. Untreated rTg(tauP301L)4510 mice showed an increased escape latency on the fourth (last) day of training, whereas the learning curve of rolipram-treated rTg(tauP301L)4510 mice was indistinguishable from that of WT mice (Myeku et al. 2016). Unfortunately, a probe trial to assess spatial memory retention at the end of learning was not undertaken. When experimental animals have deficits during probe trials, this dissociates memory from performance because measures recorded on probe trials are insensitive to swimming speed (Vorhees and Williams 2006).

PKA is known to phosphorylate tau at Ser214 (pS214) (Zhu et al. 2010). Brain extracts from rolipram-treated rTg(tauP301L)4510 mice showed a reduced total level of tau, but an increased level of pS214 tau, indicating that PKA activity was enhanced (Myeku et al. 2016). To assess proteome function in vivo, rTg(tauP301L)4510 mice were crossed with mice overexpressing a transgenic reporter for proteasome activity (ubiquitinated fragment fused to GFP). Normally, the GFP fusion protein is hydrolyzed rapidly by the proteasome, such that GFP is undetectable in normal cells (Peth et al. 2013). However, the transgenic rTg(tauP301L)4510-GFP mice showed accumulation of GFP puncta, indicating proteasome dysfunction, which increased with worsening tauopathy from 5 to 8 months. However, rolipram treatment reduced the levels of GFP in the hippocampus and cortex of rTg(tauP301L)4510-GFP mice (Myeku et al. 2016), indicating that rolipram improved proteasome activity in mice with tauopathy. This reinforces the concept that altered cAMP-PKA signalling is an important determinant of proteasome activity in vivo.

In 8–10-month-old rTg(tauP301L)4510 mice with more advanced tauopathy, rolipram treatment for 3 weeks had no significant benefits on proteasome activity, tau levels or cognition (Myeku et al. 2016). Nonetheless, given the ability of rolipram to promote proteasome activity, reduce tau and ameliorate spatial learning defects in mice during early-stage disease, it would be interesting to test whether enhancement of proteasomal function by rolipram treatment would enhance the clearance of pathogenic protein aggregates in animal models of other cerebral proteopathies, particularly Alzheimer's disease, which is considered a secondary tauopathy.

### 5.2.4 *Fragile X Syndrome*

Inhibition of PDE4 has also shown promise as a therapeutic strategy for cognitive impairment in a neurodevelopmental disorder. Fragile X syndrome (FXS) is the most common monogenic cause of intellectual disability and autism (Bagni and Oostra 2013; McCary and Roberts 2013). The *FMR1* gene contains a CGG repeat present in the 5′-untranslated region which can be unstable upon transmission to the next generation. The repeat is up to 55 CGGs long in the normal population. In patients with FXS, a repeat length exceeding 200 CGGs generally leads to methylation of the repeat and the promoter region, which is accompanied by silencing of the *FMR1* gene. The disease is a result of lack of expression of the fragile X mental retardation protein (FMRP) (Verkerk et al. 1991), leading to severe symptoms, including intellectual disability, autistic behaviours, hyperactivity and childhood seizures. FMRP is an RNA binding protein whose function is incompletely understood, but is believed to be involved in translational regulation (Bagni and Oostra 2013). This is of particular interest because new protein synthesis is required for LTP (Malenka and Bear 2004).

A key to understanding FMRP function is to identify its RNA targets. High-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation has been used to capture FMRP-mRNA interactions present in WT mouse brain, but not in *Fmr1* KO mouse brain, at postnatal days 11–25 (Darnell et al. 2011). This approach identified a robust set of 842 FMRP target transcripts that includes ADCY1 and ADCY5, encoding adenylyl cyclases, and PDE4B, but not the other PDE4 subtypes. Thus, FMRP regulates the expression of proteins involved in the regulation of cAMP levels. Consistent with this finding, overexpression of FMRP was found to increase the production of cAMP experimentally induced by the adenylyl cyclase activator forskolin or prostaglandin $E_1$ in neural cells (Berry-Kravis and Ciurlionis 1998).

It has been postulated that the ability of FMRP to repress translation of target mRNAs suggests that FXS may result from the overexpression of specific dosage-sensitive genes through loss of translational suppression (Darnell and Klann 2013). The expression levels of ADCY1, ADCY5 and PDE4B in brain from *Fmr1* KO mice or FXS patients are unknown, but it might be expected that upregulation of ADCY1/5 would increase cAMP synthesis, whereas upregulation of PDE4B would increase cAMP hydrolysis. Platelets, lymphoblastoid cells and neural cells from FXS patients have shown unaltered basal levels of cAMP, but decreased levels of cAMP production (Berry-Kravis and Huttenlocher 1992; Berry-Kravis and Sklena 1993; Berry-Kravis et al. 1995; Kelley et al. 2007). Consistent with these findings, platelets and cortex from *Fmr1* KO mice and heads from *fmr1* null *Drosophila* fruit flies showed unaltered basal cAMP levels, but decreased levels of cAMP production (Kelley et al. 2007), indicating that a robust defect in cAMP production in FXS is conserved across species. These observations have led to the ‘cAMP theory of FXS’, which posits that alterations in the cAMP pathway may result, in part, from dysregulated expression of proteins involved in the cAMP cascade (Kelley et al. 2008).

The human and *Drosophila* FMR1 genes are reported to contain CREB binding sites and to be regulated by CREB-mediated gene transcription (Hwu et al. 1997; Smith et al. 2006; Kanellopoulos et al. 2012), such that elevating cAMP levels might be expected to upregulate FMRP expression, via activation of PKA and CREB. Consistent with this, the provision of rolipram (200 μM) in the diet for 5–6 h both restored *fmr1* mRNA expression to WT levels and eliminated a robust olfactory learning deficit in heterozygous *fmr1* null flies (Kanellopoulos et al. 2012). Rolipram also abrogated a long-term (24-h) olfactory memory deficit in heterozygous *fmr1* null flies, but only when fed both before and after training (Kanellopoulos et al. 2012). In another fly study, chronic (9-day) dietary administration of rolipram at both low (50 μM) and high (500 μM) doses rescued immediate-recall (<2 min) and short-term (1 h) memory deficits of *fmr1* null flies in a conditioned courtship-associative memory test, but only the high dose rescued developmental malformation of the mushroom bodies (Choi et al. 2015), important structures for olfactory learning and memory in *Drosophila*. This finding suggests that the cognitive phenotype is not irreversibly determined by pathogenic developmental circuitry. Acute (12-h) dietary administration of rolipram at 50 μM also rescued the immediate-recall of *fmr1* null flies in the olfactory conditioning test (Choi et al. 2015).

*Fmr1* KO mice have exhibited impaired novel object recognition in several studies (Ventura et al. 2004; Restivo et al. 2005; Pacey et al. 2011), but the effect of rolipram on this cognitive phenotype has not been tested. However, administration of rolipram (0.03 mg/kg/day, s.c.) for 8 weeks, followed by a 3–5 week hiatus, has been shown to abrogate a robust neurophysiological phenotype, enhanced long-term depression (LTD), in *Fmr1* null mice (Huber et al. 2002; Choi et al. 2015).

## 5.3 Metabolic Effects of PDE4 Inhibition

The combined effects of less physical activity and the consumption of calorie-dense diets have led to unprecedented levels of overweight and obesity in affluent, sedentary societies (Baker 2015). As obesity rates rise among adults, metabolic complications of obesity, such as metabolic syndrome, are becoming more common. The main sign of metabolic syndrome is abdominal obesity, along with elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, and low high-density lipoprotein levels (Alberti et al. 2005). People with metabolic syndrome have a fivefold greater risk of developing type-2 diabetes (Stern et al. 2004) (see Sect. 5.3.2). Obesity can also exacerbate the age-related decline in physical and cognitive function in older people, and increases the risk for chronic diseases that result in poor quality of life, such as hypertension, coronary heart disease and stroke, osteoarthritis and cancer (Kopelman 2007; Wolf et al. 2007). Neuroimaging studies show increased brain atrophy in overweight and obese elderly subjects (Raji et al. 2010; Cherbuin et al. 2015). Obesity-related ill-health thus poses a serious, established and growing burden with consequences for individuals, healthcare services

and wider society (Wang et al. 2011b). This section gives an overview of inhibition of PDE4 as a potential therapeutic approach for obesity and diabetes.

### *5.3.1 Obesity*

The C57BL/6J and C57BL/6N mouse strains both develop obesity when allowed *ad libitum* access to a high-fat diet, but remain lean and physically normal when restricted to a standard diet (Collins et al. 2004; Nicholson et al. 2010; Podrini et al. 2013). Obesity in mice fed a high-fat diet leads to upregulation of pro-inflammatory processes (Pistell et al. 2010), increased production of reactive oxygen species (ROS) (Zhang et al. 2005), increased apoptosis and neurodegeneration, and decreased neurogenesis (Cai 2013), which together lead to decreased brain volumes, particularly of the hippocampus, and impaired memory and other cognitive deficits (Jeon et al. 2012).

When high-fat diet-fed C57BL/6J mice were treated with rolipram (2 mg/kg/day, oral gavage) for 12–14 weeks, they showed resistance to weight gain and had less body fat, even though their food intake was unaltered. These mice also showed an increased basal metabolic rate, with higher oxygen consumption and higher body temperatures in the fasting state, but unaltered physical activity levels. Furthermore, rolipram-treated mice had lower ROS levels in white adipose tissue (WAT), increased mitochondrial biogenesis in skeletal muscle, improved exercise tolerance on a treadmill, and increased glucose tolerance (Park et al. 2012). The dose of rolipram used (2 mg/kg) is greater than a dose (0.12 mg/kg) that showed sedative effects in mice in another study (Griebel et al. 1991). Consistent with the metabolic effects of rolipram, chronic obstructive pulmonary disease (COPD) patients treated with another pan-PDE4 inhibitor, roflumilast, showed reductions in body weight, with greater weight loss observed in individuals having a higher body mass index (BMI) (Gupta 2012). Roflumilast also shown an anti-obesity effect in women with polycystic ovary syndrome (PCOS), a disorder associated with insulin resistance and obesity. All of the women had been pretreated with metformin, the most widely used medication for type-2 diabetes (see Sect. 5.3.3), and were randomized to either continue on metformin alone, or in combination with roflumilast. Over 12 weeks, patients on metformin alone gained weight and showed increases in BMI and visceral adipose tissue (VAT) area, whereas those on metformin + roflumilast lost weight and showed reductions in BMI and VAT area (Jensterle et al. 2014).

Evidence that PDE4B may be a mediator of some of the metabolic effects of rolipram is provided by PDE4B KO mice, which are leaner, with lower fat pad weights, smaller adipocytes, and decreased serum leptin levels, when fed either a standard diet or a high-fat diet (Zhang et al. 2009), indicating that PDE4B deficiency can reduce adiposity. In WT mice, the high-fat diet decreased locomotor activity, but PDE4B deficiency almost completely blunted this reduction. PDE4B KO mice on a high-fat diet also showed lower levels of TNFα mRNA and macrophage infiltration in WAT, indicating that PDE4B deficiency can also suppress some

important obesity-induced inflammatory changes. Although the basal level of cAMP was unaltered in adipocytes from PDE4B KO mice, PDE4B-deficient adipocytes showed a greater accumulation of cAMP when experimentally exposed to the adenylyl cyclase activator isoproterenol, suggesting that PDE4B is involved in cAMP regulation in adipocytes. Nevertheless, insulin sensitivity was not improved in PDE4B KO mice because no differences from WT mice were observed in levels of fasting serum glucose and insulin or in glucose tolerance and insulin tolerance tests (Zhang et al. 2009). Cross-species support for a role for PDE4B in the regulation of adiposity comes from the finding that genetic variation in PDE4B affects subcutaneous fat thickness in both pigs and humans (Lee et al. 2011). These findings suggest that PDE4B inhibitors could have utility in the treatment of obesity and associated metabolic complications.

### *5.3.2 Alcoholic Fatty Liver*

Excessive alcohol consumption is associated with greater risk of obesity and large waist circumference (Arif and Rohrer 2005). In the liver, the first response to excessive alcohol consumption is the development of large fatty globules (hepatic steatosis). Although alcoholic fatty liver is reversible and typically has no associated symptoms, steatosis is the first stage of alcoholic liver disease, which can progress to alcoholic hepatitis and eventually cirrhosis (O'Shea et al. 2010). In the most widely used model for alcoholic liver injury, male C57BL/6J mice are fed the Lieber-DeCarli liquid diet containing ethanol [5% (v/v)] *ad libitum* for 4 weeks (Lieber et al. 1989). C57BL/6J is a strain of mice that voluntarily drink a large amount of ethanol (Yoneyama et al. 2008). In this alcoholic liver injury model, the mRNA levels for all PDE4 subtypes, PDE4A-D, are significantly upregulated 1–2 weeks after starting 5% ethanol compared with controls on an isocaloric liquid diet. This increase in PDE4 expression is accompanied by elevated PDE4 enzymatic activity and a decrease in cAMP and pCREB levels in the liver (Avila et al. 2016).

To examine the role of PDE4 in alcohol-induced hepatic steatosis, Avila et al. (2016) employed both pharmacological (rolipram; 5 mg/kg, 3 times/week, 4 weeks) and genetic (PDE4B KO) interventions to inhibit the activity of PDE4 and prevent the degradation of cAMP in the alcoholic liver injury model. Both rolipram and PDE4B KO led to significant reductions in fat accumulation and free fatty acid levels in the liver, and prevented the ethanol-mediated decrease in hepatic levels of cAMP and pCREB (Avila et al. 2016). The comparable effectiveness of both pan-PDE4 (rolipram) and specific PDE4B (PDE4B KO) inhibition suggests a predominant pathogenic role for PDE4B (among the PDE4 subtypes) in the development of alcohol-induced hepatic steatosis. PDE4B thus could serve as a therapeutic target in the treatment of alcoholic fatty liver disease. Although neither intervention for inhibiting PDE4 affected food consumption or ethanol metabolism (Avila et al. 2016), it is notable that lower doses of rolipram (0.5–1 mg/kg) were previously shown to

reduce ethanol intake in male C57BL/6J mice in 24-h two-bottle choice tests (Hu et al. 2011; Blednov et al. 2014).

### *5.3.3 Diabetes*

Diabetes mellitus in humans is a genetically and clinically heterogeneous group of glucose intolerance syndromes. Type-2 (non-insulin-dependent) diabetes is the more prevalent clinical form, in which obesity associated with progressively more severe insulin resistance is a common predictor of the pre-diabetic state. In patients with newly diagnosed type-2 diabetes, without COPD, roflumilast treatment led to reductions in glycated haemoglobin and fasting plasma glucose levels (Wouters et al. 2012). Consistent with this improvement in glycaemic variables, an animal model of type-2 diabetes, the *Lepr*$^{db/db}$ mouse (Hummel et al. 1966), showed reduced food and water consumption, reduced glycated haemoglobin levels, reduced blood glucose levels, increased fasted serum insulin levels, preserved pancreatic islet morphology and insulin production in pancreatic islet β cells, and minimal islet atrophy in response to 28-day treatment with roflumilast-*N*-oxide (3 mg/kg/day), the active metabolite of roflumilast (Vollert et al. 2012). Rolipram, roflumilast and roflumilast-*N*-oxide all increase serum levels of glucagon-like peptide-1 (GLP-1) (Park et al. 2012; Vollert et al. 2012; Ong et al. 2009), a cAMP-regulated gut hormone that increases insulin secretion from β cells (Gevrey et al. 2002; Holz and Habener 1992), and promotes satiety and suppresses energy intake in humans (Flint et al. 1998).

Type-1 (insulin-dependent) diabetes usually has an autoimmune T cell-mediated aetiology in which the pre-diabetic state is characterized by development of autoantibodies against certain proteins expressed by β cells, including insulin. The risk for development of type-1 diabetes is increased by obesity and may occur at an earlier age among obese individuals with a predisposition. Obesity also increases the risk for comorbidities among individuals with type-1 diabetes, especially metabolic syndrome, and microvascular and macrovascular diseases (Polsky and Ellis 2015). In an animal model of type-1 diabetes, the non-obese diabetic (NOD) mouse (Makino et al. 1980), treatment with rolipram (14 mg/kg, twice daily) for 4 weeks, from 12 to 16 weeks of age, had a lasting protective effect, associated with a significant reduction in the severity of insulitis, an inflammation of the islets of Langerhans (Liang et al. 1998). By 27 weeks of age, 80% of untreated NOD mice had diabetes, whereas only 20% of rolipram-treated mice were hyperglycaemic. Thus, 11 weeks after withdrawing drug therapy, the incidence of disease was still three to four times lower (Liang et al. 1998).

The inability of insulin to suppress hepatic glucose output is a major aetiological factor in the hyperglycaemia of type-2 and type-1 diabetic patients (DeFronzo et al. 1982). Biguanides such as metformin have an antihyperglycaemic action primarily by inhibiting hepatic glucose output via inhibition of glucagon-induced cAMP/PKA signalling (Miller et al. 2013). Although biguanides are known to phosphorylate AMP-activated protein kinase (AMPK), it is unclear how their glucose-lowering

effect is related to AMPK activation. However, it was recently demonstrated using a small-molecule AMPK activator (compound 991) that AMPK activation antagonizes glucagon signalling by S304 phosphorylation and activation of PDE4B, thereby lowering cAMP levels and decreasing PKA activation in intact hepatocytes. In hepatocytes from mice bearing a liver-specific deletion of the two AMPK catalytic subunits, these effects of compound 991 treatment were lost (Johanns et al. 2016). PDE4B activation could thus explain the reduction in glucagon-stimulated cAMP levels by biguanides (Miller et al. 2013).

## 5.4 Adverse Effects of PDE4 Inhibition

### *5.4.1 Role of PDE4D in Nausea and Emesis*

In principle, PDE4 inhibitors have considerable therapeutic potential. In practice, however, their clinical utility has been compromised by mechanism-associated side effects that limit maximally tolerated doses. Severe dose-limiting emesis following the administration of pan-PDE4 inhibitors has been observed humans and various animal species endowed with a vomiting reflex (Heaslip and Evans 1995; Robichaud et al. 2001). For example, in two separate macaque studies, the maximum tolerated doses of rolipram were 0.01 and 0.03 mg/kg, respectively, but the next highest dose of 0.05 mg/kg was not tolerated due to emesis (Ramos et al. 2003; Rutten et al. 2008). Although both PDE4B and PDE4D mRNAs are expressed in the area postrema, the chemosensitive trigger zone in the brain stem (Mori et al. 2010), PDE4D appears to be of particular importance in emesis.

As mice are unable to vomit, reduction in the duration of xylazine/ketamine-induced anaesthesia is used as a behavioural surrogate measure of emesis. Application of this procedure to PDE4B KO and PDE4D KO mice provided the following observations giving credence to the notion that PDE4D inhibition is responsible for the emetic side effect of pan-PDE4 inhibitors: (1) the duration of xylazine/ketamine-induced anaesthesia in PDE4D KO mice was significantly shorter compared with WT mice, whereas the anaesthetic affected PDE4B KO and WT mice to the same degree; (2) a PDE4 inhibitor significantly reduced the duration of anaesthesia in WT mice but not in PDE4D KO mice; (3) PDE4 activity in the brainstem of PDE4D KO mice was markedly lower compared with WT and PDE4B KO mice, indicating that PDE4D is the principle regulator of cAMP metabolism in the brainstem (Robichaud et al. 2002).

PDE4D-selective compounds that fully inhibit PDE4D activity have been found to potently reduce anaesthesia in the xylazine/ketamine test at doses (0.01 or 0.03 mg/kg) that showed maximal cognitive benefit in the novel object recognition test, thus corroborating the association of PDE4D inhibition with emesis. However, other PDE4D-selective compounds that only partially inhibit PDE4D had little or no effect on anaesthesia duration, even at a dose (3 mg/kg) 1000× that which showed

maximal cognitive benefit (0.003 mg/kg) (Burgin et al. 2010), suggesting that partial inhibitors of PDE4D are likely to have better tolerability with regard to emesis.

### *5.4.2 Vascular Injury*

In addition to the gastric adverse effects, a worrisome vascular toxicity has been observed in various organs during preclinical toxicology testing of pan-PDE4 inhibitors in laboratory animals including rats, dogs and monkeys (Giembycz 2005). Rats are particularly sensitive to the development of vascular injury following treatment with PDE4 inhibitors. In one study, rolipram was administered orally at a range of doses to rats for up 14 days, after which the animals were necropsied (Larson et al. 1996). No lesions were seen at a dose of 10 mg/kg, whereas inflammation and a necrotizing vasculitis in the mesentery and interstitial areas of the liver were clearly evident at a dose of 30 mg/kg. At the highest dose (100 mg/kg), rolipram was lethal (Larson et al. 1996). These findings demonstrate that repeated exposure to rolipram can cause vascular injury and death in rats, but it is noteworthy that the doses administered (≥30 mg/kg/day) are at least 60 times higher than the daily doses that have shown cognition-enhancing effects in rodent models of age-associated cognitive impairment (0.1 mg/kg; de Lima et al. 2008), Alzheimer's disease (0.03–0.5 mg/kg; Gong et al. 2004; Comery et al. 2005; Cheng et al. 2010; Wang et al. 2012) and frontotemporal dementia (0.06 mg/kg; Myeku et al. 2016) (see Sect. 5.2). Vascular toxicity has not been reported in humans, although mesenteric vasculopathy is difficult to monitor in human clinical trials (Burgin et al. 2010). To date, little is known about the role of the four individual PDE4 subtypes (A–D) in the development of vascular injury, but mesenteric vasculopathy has not been observed in initial studies of rats, mice and dogs treated with PDE4D inhibitors that partially inhibit enzyme activity (Burgin et al. 2010).

## 5.5 Selective Inhibition of PDE4B

### *5.5.1 Specific Inhibition of PDE4B in Mice*

Evidence supporting the notion that subtype-selective pharmacological inhibition of PDE4B might have pro-cognitive effects is provided by a study of mice with a missense mutation (Y358C) in the catalytic domain of PDE4B (McGirr et al. 2016). At the cAMP binding site, there is an interaction between the central phosphate group of cAMP and H406 in PDE4B1. Though the Y358 residue is located within the catalytic domain, it is neither at the site of cAMP binding nor rolipram binding (Richter et al. 2001). The Y358C mutation severely disrupts the docking position of cAMP, as the side chain of K282 bisects the binding site, resulting in partial (27%)

inhibition of enzymatic activity. This magnitude of PDE4 inhibition is proportionate with the physiological 33% inhibition of cAMP hydrolysis when PDE4B1 is phosphorylated by ERK2 (Baillie et al. 2000). This contrasts with the ≥70% inhibition of PDE4D7 elicited by D159687, a PDE4D partial inhibitor that has shown pro-cognitive effects in mice and rats (Burgin et al. 2010). Partial inhibition of PDE4B is likely to maintain spatial and temporal aspects of cAMP signalling. Indeed, hippocampal slices from homozygous PDE4B$^{Y358C}$ (*Pde4b*$^{enu1H}$) mice have similar basal levels of cAMP to WT mice, but show a greater accumulation of cAMP when experimentally exposed to forskolin, either alone or in combination with rolipram (McGirr et al. 2016).

Young adult (12-week-old) PDE4B$^{Y358C}$ mice show enhancements in spatial working memory in the Y-maze, in spatial memory acquisition, retention and reversal in the Morris water maze, and in object location recognition. The enhancement in object location recognition was greater when mice were tested in an aversive environment, suggesting that PDE4B$^{Y358C}$ mice were less anxious. Indeed, PDE4B$^{Y358C}$ mice exhibited lower anxiety and greater exploratory behaviour in the elevated plus maze, open field, light-dark box and hole-board tests. Additionally, PDE4B$^{Y358C}$ mice show enhanced LTP, but unaltered basal synaptic transmission and LTD, in hippocampal slices, as well as increased pCREB levels and dendritic spine density in both the hippocampus and amygdala, and enhanced neurogenesis in the adult dentate gyrus (McGirr et al. 2016).

Notwithstanding the obvious differences between a genetic manipulation from conception versus a pharmacological manipulation in adulthood, these results suggest potential outcome measures to investigate in mice treated with compound A, A-33 or other PDE4B-selective inhibitors. It remains to be established whether the cognitive enhancement exhibited by 12-week-old PDE4B$^{Y358C}$ mice persists into old age or when challenged with MK-801 (dizocilpine), an *N*-methyl-D-aspartate (NMDA) receptor antagonist that is used to model symptoms of schizophrenia (Zhang et al. 2000). It would also be interesting to challenge PDE4B$^{Y358C}$ mice with a high-fat diet, and to determine whether the Y358C mutation has cognitive benefits in mouse models of AD or FXS.

PDE4B KO mice (see Sect. 5.3.1), by contrast, show enhanced basal synaptic transmission and LTD, but unaltered LTP, in hippocampal slices (Rutten et al. 2011), and unaltered spatial memory acquisition and retention (Rutten et al. 2011; Siuciak et al. 2008; Zhang et al. 2008), but impaired reversal learning (Rutten et al. 2011), in the Morris water maze. PDE4B KO mice also exhibit a moderately anxiogenic behavioural profile with decreased exploratory activity in the hole-board and light-dark box tests (Zhang et al. 2008), decreased locomotor activity in some open field tests (Rutten et al. 2011; Siuciak et al. 2008; Zhang et al. 2008), but unaltered performance in the elevated plus maze (Siuciak et al. 2008). Like PDE4B$^{Y358C}$ mice, PDE4B KO mice have enhanced adult neurogenesis in the dentate gyrus (Zhang et al. 2008).

The neuronal signalling network is set up to maintain an optimal range for cAMP signalling in the brain, such that both the excessive activation and deactivation of the cAMP-PKA signalling pathway may induce the impairment of learning and

memory (Sato et al. 2004). Thus, the divergent behavioural phenotypes of the PDE4B$^{Y358C}$ and PDE4B KO mice may be due to the different magnitudes of PDE4B inhibition elicited by the KO mutation (full inhibition) versus the Y358C mutation (partial inhibition).

### *5.5.2 PDE4B-Selective Inhibitors*

Clinical studies of rolipram were limited by side effects including nausea and emesis that are thought to arise from inhibition of the PDE4D subtype. Various strategies have been employed to develop compounds with a reduced side effect profile (Maurice et al. 2014). The development of PDE4D partial inhibitors has been an effective strategy to achieve efficacy while reducing the side effect liabilities in preclinical species (Burgin et al. 2010). A complementary approach to overcome the tolerability issues of pan-PDE4 inhibitors is the development of PDE4B-selective inhibitors that capture therapeutic effects while avoiding PDE4D that mediates emesis and nausea (Srivani et al. 2008). In recent years, a number of compounds that selectively inhibit PDE4B over PDE4D have been developed for their use as therapeutic agents, as reviewed extensively elsewhere (Azam and Tripuraneni 2014). However, to date, in vivo tests have been reported for only two PDE4B-selective inhibitors.

Compound A (2-[4-[[2-(3-fluoro-4-methoxy-phenyl)-7,8-dihydro-6H-thiopyrano[3,2-*d*]pyrimidin-4-yl] amino]phenyl]acetic acid) has an $IC_{50}$ of 5.5 and 26 nM against human and mouse PDE4B2, respectively, and is selective for PDE4B2 over both human and mouse PDE4D2 (80- and 29-fold, respectively) (Suzuki et al. 2013). In mice, oral administration of compound A (3–100 mg/kg) had a dose-dependent anti-inflammatory effect by inhibiting the elevation of the plasma concentration of TNFα induced by injection of LPS. However, compound A was less effective against neutrophil accumulation in the lung induced by inhalation of LPS, showing a reduction only at the highest dose tested (300 mg/kg) (Suzuki et al. 2013).

Another compound, A-33 (2-(4-{[2-(5-chlorothiophen-2-yl)-5-ethyl-6-118 methylpyrimidin-4-yl]amino}phenyl)acetic acid), is reported to have $IC_{50}$ values of 19 and 32 nM against human PDE4B1 and 27 nM against PDE4B3 measured in vitro, and to be 113-fold and 49-fold more selective towards PDE4B1 as compared to PDE4D3 and PDE4D7, respectively (Naganuma et al. 2009; Fox et al. 2014; Titus et al. 2016). A-33 binds to a C-terminal regulatory helix termed CR3 (Control Region 3) that is present in all PDE4B isoforms, thereby locking the enzyme in an inactive ‘closed’ conformation (Fox et al. 2014). In mice, oral administration of A-33 (14 mg/kg) reduced LPS-induced TNFα production (Naganuma et al. 2009), while a median effective dose ($ED_{50}$) of 0.1 mg/kg had antidepressant effects in the forced swim test (Titus et al. 2016). The half-life of A33 in the mouse brain is 3.8–4.5 h (Titus et al. 2016). In ferrets, oral administration of A-33 (12.5 mg/kg) reduced LPS inhalation-induced neutrophil accumulation in the lungs by 44%,

while a much higher dose of 100 mg/kg was tolerated without emesis (Naganuma et al. 2009). In a rat model of traumatic brain injury, A-33 was recently shown to have pro-cognitive effects (Titus et al. 2016; see Sect. 5.5.3).

An alternative approach to activating the cAMP-PKA signalling pathway in the mammalian brain may eventually be provided by photoactivated adenylyl cyclase (PAC), an emerging optogenetic tool that has been shown to increase intracellular cAMP levels and alter behaviour in *Drosophila* flies and *Caenorhabditis elegans* nematodes upon stimulation by blue light (Schröder-Lang et al. 2007; Weissenberger et al. 2011).

### *5.5.3 Traumatic Brain Injury*

The majority of survivors of moderate and severe traumatic brain injury (TBI) have chronic neurobehavioural sequelae, including impairment in cognitive domains such as frontal executive functions, attention, short-term memory and learning, speed of information processing, and speech and language functions (Arciniegas et al. 2002). The high prevalence of cognitive impairment is due, in part, to the vulnerability of the hippocampus, which exhibits progressive bilateral atrophy even when not initially damaged (Bigler et al. 2002). There are currently no effective treatments to improve TBI-induced cognitive impairment (Wheaton et al. 2009), but potential therapeutic approaches have been evaluated in animal models of TBI.

In one of the oldest and most commonly used models, TBI is surgically induced in young adult male Sprague-Dawley rats by lateral fluid-percussion injury (Kabadi et al. 2010). In this rat lateral fluid-percussion model, hippocampal levels of cAMP show an acute decrease from 15 min to 4 h post-surgery (Atkins et al. 2007), associated with an elevated level of PDE4B2 (short isoform), but not PDE4B1/3/4 (long isoforms), between 1 and 24 h post-surgery (Wilson et al. 2016). Levels of TNFα, a pro-inflammatory cytokine mediated by PDE4B (Jin and Conti 2002), are enhanced maximally within 3–8 h post-surgery and return to non-injured levels by 24 h post-surgery (Titus et al. 2016). At 3 months post-surgery, the chronically injured hippocampus shows decreased levels of basal pCREB (Atkins et al. 2009), but PDE4B isoforms are not upregulated and TNFα is not detectable (Titus et al. 2016).

The potential utility of the PDE4B inhibitor A-33 (see Sect. 5.5.2) for cognitive manifestations of TBI has been evaluated in the rat lateral fluid-percussion model by Titus et al. (2016). *Ex vivo* assessment of synaptic plasticity in ipsilateral hippocampal slices from rats at 3 months post-surgery revealed a significant reduction in basal synaptic transmission and impaired expression of LTP. Bath application of A-33 (300 nM) in artificial CSF to hippocampal slices significantly reduced the deficits in basal synaptic transmission and rescued LTP expression (Titus et al. 2016).

To determine whether PDE4B inhibition by A-33 would restore cognitive function in vivo during the chronic TBI recovery period, rats received A-33 (0.3 mg/kg, i.p.) 30 min before cue and contextual fear conditioning, water maze training and a delayed match-to-place task in the water maze between 3–4 months post-surgery. Retention of learned behaviour or of learning was measured 1 day and 1 month after training. Treatment with A-33 significantly reversed the TBI-induced deficits in cue and contextual fear conditioning and water maze retention, but did not enhance the performance of non-injured rats (Titus et al. 2016).

When the rats were killed at 5 months post-surgery, after behavioural testing, the intermittent A-33 treatment (eight doses over 4 weeks) was found to have had no effect on the significant atrophy and microglia accumulation (quantified using Iba1 immunostaining) observed in the cortex and hippocampus (Titus et al. 2016). A-33 administered 3–4 months post-surgery thus appears to have pro-cognitive benefits in injured rats, without reversing the pathology caused by TBI. Similarly, rolipram (0.03 mg/kg, i.p.) was previously shown to rescue the cognitive deficits of TBI rats in the fear conditioning and water maze tests, but had no effect on hippocampal atrophy in TBI rats at 8 weeks post-surgery (Titus et al. 2013). As the administration of A-33 (0.3 mg/kg, i.p.) at 5 h post-surgery was shown to reduce TNFα levels at 6 h post-surgery (Titus et al. 2016), it would be interesting to test whether the administration of A-33 within 24 h post-surgery, when TNFα levels are higher, would reduce the TBI-related neural damage.

To evaluate the feasibility of A-33 for inhibiting PDE4B in the injured brain, brain concentrations of A-33 at the dose used for behaviour assessment (0.3 mg/kg) were measured. Rats received A-33 at 3 months post-surgery and were decapitated 30 min later for the collection of trunk blood and brain tissue. Quantification of A-33 levels in plasma and brain tissue (cortex and hippocampus combined) by mass spectrometry revealed that A-33 has low brain distribution, with a similar B/P (brain/plasma) ratio of 3.2% in TBI rats and 2.4% in non-injured controls (Titus et al. 2016). Despite its low brain penetration, brain levels of A-33 were four to fivefold higher than the $IC_{50}$ against PDE4B measured in vitro (see Sect. 5.5.2), suggesting that A-33 can attain relevant concentrations in the brain against the PDE4B target. A caveat is that the rats were not perfused before decapitation, so the brain tissue analysed contained cerebral blood. The ability of systemically administered A-33 to cross the blood-brain barrier and reach its site of action thus requires further investigation.

A-33 treatment was found to rescue the decrease in pCREB levels in the hippocampus of TBI rats (Titus et al. 2016), suggesting that a signalling pathway in the brain known to be regulated by PDE4B is inhibited with A-33. This finding provides support for the notion that A-33 improved learning and memory in TBI rats by altering cAMP signalling rather than the pathology caused by TBI. However, because inhibition of PDE4B in the brain after A-33 treatment was not directly measured by Titus et al. (2016), it is currently unknown whether brain-specific inhibition of PDE4B was the underlying mechanism for the improvements in learning and memory.

## 5.6 Concluding Remarks

Affluent, sedentary societies are becoming increasingly elderly and overweight, thus presenting a major challenge to prolong health in an ageing population. Indeed, 'Adding life to years as well as years to life' has become something of a mantra in health policy circles (World Health Organization 2002). Inhibition of PDE4 in preclinical rodent models has shown promise as a therapeutic strategy for age-related neurodegenerative conditions and obesity-related metabolic diseases, as well as for FXS, the most common inherited cause of intellectual disability. But the clinical application of pan-PDE4 inhibitors has been hampered by adverse side effects that limit maximally tolerated doses. The findings of cognitive enhancement in PDE4B$^{Y358C}$ mice (McGirr et al. 2016), pro-cognitive effects of a non-emetic PDE4B-selective inhibitor (A-33) in a rat model of TBI (Naganuma et al. 2009; Titus et al. 2016), and resistance to obesity in PDE4B KO mice (Zhang et al. 2009) highlight the PDE4B subtype as a promising target for the development of therapeutic interventions in both neurological and metabolic disorders.

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

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# Part II
# PDEs in Cognition of Aging and 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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**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.

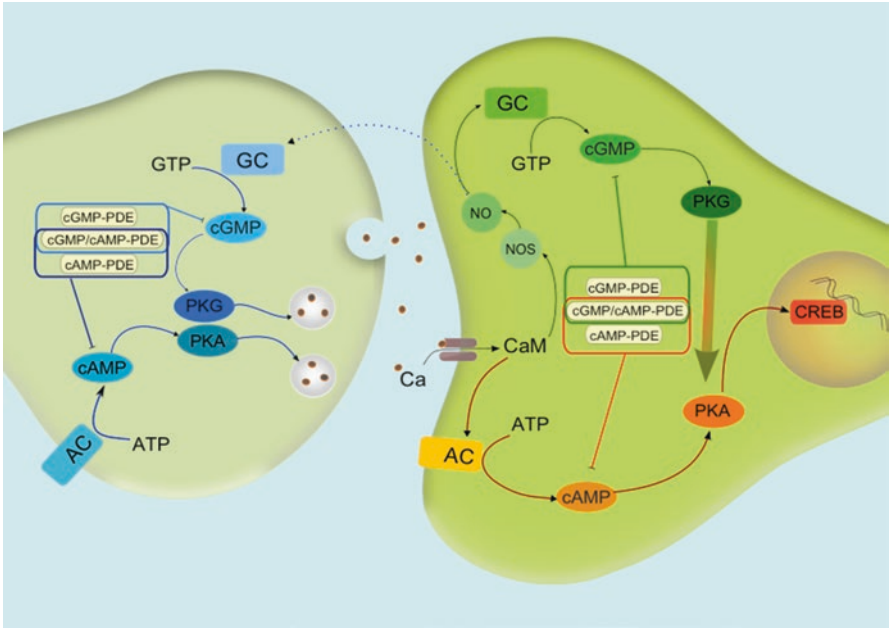


**Fig. 6.1** Schematic diagram of pre- and postsynaptic cellular processes related to the second messengers cAMP and cGMP involved in LTP-related signal transduction (Heckman et al. 2015a; reprinted with permission)

**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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# Chapter 7
# The Past, Present, and Future of Phosphodiesterase-4 Modulation for Age-Induced Memory Loss

**Rolf T. Hansen III and Han-Ting Zhang**

**Abstract** The purpose of this chapter is to highlight the state of progress for phosphodiesterase-4 (PDE4) modulation as a potential therapeutic for psychiatric illness, and to draw attention to particular hurdles and obstacles that must be overcome in future studies to develop PDE4-mediated therapeutics. Pathological and non-pathological related memory loss will be the focus of the chapter; however, we will at times also touch upon other psychiatric illnesses like anxiety and depression. First, we will provide a brief background of PDE4, and the rationale for its extensive study in cognition. Second, we will explore fundamental differences in individual PDE4 subtypes, and then begin to address differences between pathological and non-pathological aging. Alterations of cAMP/PDE4 signaling that occur within normal vs. pathological aging, and the potential for PDE4 modulation to combat these alterations within each context will be described. Finally, we will finish the chapter with obstacles that have hindered the field, and future studies and alternative viewpoints that need to be addressed. Overall, we hope this chapter will demonstrate the incredible complexity of PDE4 signaling in the brain, and will be useful in forming a strategy to develop future PDE4-mediated therapeutics for psychiatric illnesses.

**Keywords** Phosphodiesterase-4 (PDE4) • Aging • Memory • Cognition • cAMP signaling

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## 7.1 Introduction and Background of PDE4

Phosphodiesterases (PDEs) are a collective group of 11 enzyme families that hydrolyze the second messengers cAMP and/or cGMP. The PDE families are differentiated from one another through unique protein structure, tissue expression, cyclic nucleotide specificities, and distinct genes located on different chromosomes (Zhang et al. 2002; Conti et al. 2003; Houslay and Adams 2003; Shepherd et al. 2003; O'Donnell and Zhang 2004). Among the 11 PDE families, PDE4 has received much focus in regards to psychiatric illness due to its strong expression in the brain, in particular, the brain areas involved in psychiatric illness such as the prefrontal cortex, striatum, hippocampus, and amygdala.

PDE4 was first characterized as an enzyme capable of cAMP phosphodiesterase activity from rat liver plasma membranes (Marchmont and Houslay 1980); but, its involvement in cognition and behavior was first identified through mutations in the *Drosophila melanogaster* dunce PDE4D gene, which led to deficient cAMP-specific PDE activity and abnormal memory (Kauvar 1982; Qui et al. 1991). PDE4 was later discovered in both rodents (Davis et al. 1989; Swinnen et al. 1989) and humans (Bolger et al. 1993) to consist of a small family of enzymes encoded through four different genes, which are now referred to as PDE4-A, B, C, and D (Reeves et al. 1987; Wang et al. 1997; Houslay and Adams 2003; O'Donnell and Zhang 2004). These PDE4 subtypes are not expressed equally throughout the body. PDE4C has been shown to be largely deficient in the brain (Cherry and Davis 1999; Perez-Torres et al. 2000; Lakics et al. 2010). In regards to cognitive research, PDE4-A, B, and D are the predominant subtypes which are focused upon (Zhang 2009).

The canonical protein structure of PDE4 is divided into several domains along the N′ to C′ termini that each play unique functions in the role of the enzyme. The individual PDE4 splice variants have unique N′ termini that are encoded through alternative promoters, and are involved with cellular localization and binding to unique binding partners (Beard et al. 1999, Beard et al. 2002; Jang et al. 2010). The PDE4 enzyme is contrasted from other PDEs in that it contains two upstream conserved regions (UCRs) termed UCR1 and UCR2 located immediately downstream of the N′-terminus region of the protein. These UCR regions play an important regulatory role, modulating both activity of the enzyme (Sette et al. 1996; Hoffmann et al. 1999; Houslay and Baillie 2003; Burgin et al. 2010) and dimerization with other PDE4 units (Richter and Conti 2002, 2004; Bolger et al. 2015). Within the C′-terminus lies the catalytic domain of the PDE4 enzyme which is responsible for the hydrolysis of cAMP to 5′-AMP (Saldou et al. 1998; Conti et al. 2003; Houslay and Adams 2003; Zhang 2009; Houslay 2010). From this canonical structure, there are then numerous PDE4 splice variants within each subtype that are produced through alternative promoters and splicing events. Twenty-five PDE4 splice variants have been identified to date, including 6 PDE4A splice variants, 5 PDE4B variants, 3 PDE4C variants, and 11 PDE4D variants (Bolger et al. 1996, Bolger et al. 1997, Bolger et al. 2003b; Huston et al. 1997; Sullivan et al. 1998; Rena et al. 2001; Miró et al. 2002; Shepherd et al. 2003; Wallace et al. 2005; Richter et al. 2005;

Chandrasekaran et al. 2008; Mackenzie et al. 2008; Zhang 2009). This alternative splicing results in the formation of different length PDE4s that are classified as long form, short form, super-short form, and dead-short form. Long-form PDEs are full length and contain no truncation, short forms lack a UCR1 region, super-short forms lack a UCR1 and have a truncated UCR2, and dead-short lack both UCR regions and also contain a truncated catalytic domain that render the PDE4 functionally inactive (O'Donnell and Zhang 2004; Zhang 2009; Richter et al. 2013). Interestingly, this alternative splicing has been shown to be increased in mammals and humans in parallel with increasing levels of cognitive capacity (Johnson et al. 2010). The following sections will describe the expression and broad behavioral roles of the individual PDE4 subtypes.

### *7.1.1 PDE4A*

The PDE4A subtype has six splice variants; PDE4A1 is short (Sullivan et al. 1998), PDE4A7 is the dead-short form (Horton et al. 1995; Johnston et al. 2004), and PDE4A5 (PDE4A4 is the human orthologue) (McPhee et al. 1995; Huston et al. 2000; Beard et al. 2002), PDE4A8 (Bolger et al. 1996), PDE4A10 (Rena et al. 2001), and PDE4A11 (Wallace et al. 2005) are long forms (Bolger et al. 1997; Owens et al. 1997; Conti et al. 2003; O'Donnell and Zhang 2004; Cheung et al. 2007; Houslay et al. 2007; Chandrasekaran et al. 2008; Lynex et al. 2008; Zhang 2009). The broad PDE4A subtype has been found to be strongly expressed in the olfactory bulbs, deep layers of the cortex, amygdala, hypothalamus, hippocampus, piriform cortex, and cerebellum (Engels et al. 1995; Cherry and Davis 1999; Perez-Torres et al. 2000; McPhee et al. 2001; D'Sa et al. 2005; Lakics et al. 2010; Johansson et al. 2012; Kelly et al. 2013).

PDE4A is one of the few PDE4 subtypes where differential splice-variant expression patterns have been looked at. McPhee and colleagues found that the long PDE4A5/10 isoforms were present throughout most of the cortex, CA1, and CA2 in overlapping patterns. However, PDE4A10 was present in the islands of Calleja while PDE4A1 and PDE4A5 were not. In addition, PDE4A5 and PDEA10 were present in the medial nucleus of the amygdala where PDE4A1 expression was absent. PDE4A1 had a different expression pattern than the longer isoforms, with very strong expression in the glomerular layer of the olfactory bulbs, CA3 of the hippocampus, the cerebellum, and low staining in the brain stem (McPhee et al. 2001). D'Sa and colleagues also looked at PDE4A expression, and observed *slightly* different patterns. Specifically, they saw that PDE4A1 was high in the medial septum, diagonal band, olfactory system, hippocampus and cerebellum; PDE4A5 was highly expressed in the olfactory nuclei, deep cortical layers, and the DG and CA1 of the hippocampus; PDE4A10 was highly expressed in the DG and CA1; and PDE4A8 expression was absent in the brain (D'Sa et al. 2005).

PDE4A1 has also been found to be unique, in that it is inserted into lipid bilayers through a TAPAS-1 (tryptophan anchoring phosphatidic acid selective-binding

domain 1); these bilayers are typically found on golgi that traffic out to the membrane (Baillie et al. 2002). PDE4A5 is unique from the remaining subtypes in that it can interact with SH3 domains on proteins through its N′ Terminal region (O'Connell et al. 1996). This SH3 binding plays a significant role in the targeting of PDE4A5, which has been found to be expressed in perinuclear regions, and also within membrane ruffles (Beard et al. 2002). This expression pattern of PDE4A5 has been shown to be disrupted in apoptotic cells by cleavage of caspase 3 at its SH3 binding sites, and overexpression of PDE4A5 protects against apoptosis (Huston et al. 2000). Another unique trait of PDE4A5 is that it can bind to an immunophilin known as XAP2 which partially inhibits the enzyme up to 60%, and increases its sensitivity to the broad PDE4 inhibitor rolipram (Bolger et al. 2003b). Interestingly, PDE4A can also be activated by the P70S6 kinase in adipocytes during adipogenesis, which suggests it could play a role in obesity (MacKenzie et al. 1998). Lastly, PDE4A5 is the only member of the PDE4A family which can be phosphorylated by MAPK and subsequently attenuate its activation by PKA (MacKenzie et al. 2011). The PDE4A7 and PDE4A8 splice variants are exclusively found in the testis in rodents, however in humans PDE4A8 has undergone an evolutionary change which causes it to be expressed in the brain (Mackenzie et al. 2008).

The role of PDE4A in behavior is at a very preliminary level, however recent studies have shown that PDE4A knock out (KO) mice display increased anxiety-like behavior and enhancement in fear memory (Hansen et al. 2014). Other studies have also shown that PDE4A may play a role in memory deficits caused by sleep deprivation (Vecsey et al. 2009). To date, there are no known PDE4A mutations implicated in the etiology of any human disease, although PDE4A expression has been shown to be altered in the brains of autistic individuals (Fatemi et al. 2010). At this time, there are still no PDE4A selective pharmacological modulators.

### *7.1.2 PDE4B*

PDE4B has five splice variants identified PDE4B1–5; PDE4B1, 3, and 4 are long isoforms, PDE4B2 is short, and PDE4B5 is super short (Bolger et al. 1997; Conti et al. 2003; O'Donnell and Zhang 2004; Cheung et al. 2007; Houslay et al. 2007; Chandrasekaran et al. 2008; Lynex et al. 2008; Zhang 2009).

Most of the PDE4 subtype expression patterns overlap a good bit and vary with different studies, but PDE4B is unique in the fact that it is consistently the highest PDE4 subtype expressed in the striatum, globus-pallidus, nucleus accumbens, hypothalamus, and amygdala (Engels et al. 1995; Cherry and Davis 1999; Perez-Torres et al. 2000; McPhee et al. 2001; D'Sa et al. 2005; Lakics et al. 2010; Johansson et al. 2012; Kelly et al. 2013). PDE4B is also unique in that mutations in PDE4B are strongly believed to play a role in schizophrenia through its interaction with disrupted in schizophrenia 1 protein (DISC1) (Millar et al. 2005; Fatemi et al. 2008; Numata et al. 2008, Numata et al. 2009; Kähler et al. 2010). Interestingly, it

was observed that this PDE4B mutation results in a decrease of PDE4B expression (Fatemi et al. 2008).

PDE4B KO mice studies have shown that PDE4B is the main PDE4 subtype responsible for the release of tumor necrosis factor-α (TNF-α) in response to lipopolysaccharide injections (LPS). In particular, these studies found that PDE4B KO reduced the amount of TNF-α released in response to LPS and PDE4A/PDE4D did not play a role (Jin and Conti 2002; Jin et al. 2005a). PDE4B was also specifically found to be altered in the brain after systemic LPS infusions, while PDE4A and PDE4D displayed no changes (Johansson et al. 2011). Thus, it appears that PDE4B may be one of the main PDE4 subtypes involved with regulation of microglia (Pearse and Hughes 2016), as PDE4B has also been shown to be involved with Amyloid beta induced microglia activation (Sebastiani et al. 2006). Additional studies have shown that PDE4B KO is anxiogenic and increases corticosterone levels, and also decreases depressive-like behavior in mice (Zhang et al. 2008).

Traditionally, it was not possible to target individual PDE4 subtypes pharmacologically due to the lack of highly selective inhibitors of specific PDE4 subtypes; however, recent studies have demonstrated feasibility for specific inhibition of PDE4B by targeting the Leu674 residue in PDE4B region control region 3 (CR3) (Fox et al. 2014; Hagen et al. 2014).

### *7.1.3 PDE4D*

PDE4D has 11 identified splice variants termed PDE4D1–11; PDE4D1/2 are short forms, PDE4D6 is super short, and the remaining splice variants are all full length PDE4 proteins (Bolger et al. 1997; Conti et al. 2003; O'Donnell and Zhang 2004; Richter et al. 2005; Cheung et al. 2007; Houslay et al. 2007; Chandrasekaran et al. 2008; Lynex et al. 2008; Zhang 2009; Maurice et al. 2014).

PDE4D has a diffuse pattern of expression throughout the brain, with noticeable expression in the hippocampus, cortex, thalamus, area postrema, periaqueductal grey, brain stem, and cerebellum (Engels et al. 1995; Cherry and Davis 1999; Perez-Torres et al. 2000; McPhee et al. 2001; D'Sa et al. 2005; Lakics et al. 2010; Johansson et al. 2012; Kelly et al. 2013). Initial studies have demonstrated some differential expression of PDE4D splice variants in the brain, with PDE4D2 displaying distinct expression in the dorsal and median raphe nuclei, PDE4D1 in white matter cells, and PDE4D1 and 2 in the area postrema (Miró et al. 2002). PDE4D8 is predominantly expressed outside of the central nervous system, in particular in cardiac myocytes (De Arcangelis et al. 2009; Raymond et al. 2009; Mika and Conti 2015).

In addition, PDE4D also has specific binding partners such as myomegalin protein (Verde et al. 2001), beta arrestins (Baillie et al. 2003, Baillie et al. 2007; Bolger et al. 2003a; Lynch et al. 2005, Lynch et al. 2007; Li et al. 2009), RACK1 (Conti et al. 2003), SH3-domain regions (Beard et al. 1999) and the A-Kinase anchoring proteins (AKAPS), Erk, and exchange protein directly activated by cAMP (EPAC) (Dodge et al. 2001; Dodge-Kafka et al. 2005).

PDE4D has also been extensively studied using PDE4D KO mice designed by homolgous recombination (Jin et al. 2005b) and through viral manipulation. PDE4D KO mice exhibit improved memory (Li et al. 2011), increased synaptic long-term potentiation (LTP) (Rutten et al. 2008), reduced levels of depressive-like behavior (Zhang et al. 2002), elevated neurogenesis (Li et al. 2011), and impaired growth and fertility (Jin et al. 1999). This is supported by studies showing that RNAi or inhibitors with relatively high selectivity for PDE4D also produce memory-enhancing effects (Burgin et al. 2010; Bruno et al. 2011; Li et al. 2011), reverse memory deficits induced by beta-amyloid peptide 1–42 (Zhang et al. 2014) and/or produce antidepressant activity (Wang et al. 2013). Furthermore, mice deficient in PDE4D have been shown to be less senitive to the antidepressant effect of rolipaim (Zhang et al. 2002). Together, these studies have strongly implicated PDE4D in memory as well as depressive-like behavior. Unfortunately, PDE4D KO also causes emetic-like behavior, the major side effect of broad PDE4 inhibitors such as rolipam (Robichaud et al. 2002a, b). Upon further investigation this data makes sense, as PDE4D has the highest expression of any of the PDE4 subtypes in the area postrema which is the emetic center of the brain (Cherry and Davis 1999; Miró et al. 2002; Mori et al. 2010).

PDE4D single nucleotide polymorphisms (SNP) have also been associated with stroke (He et al. 2013; Heyer et al. 2013; Liu et al. 2013a, b), however the results have been inconsistent (Shao et al. 2014). In a meta-analysis, Yan and colleagues found that PDE4D SNP83 is higher in Asian and Chinese populations, but not among Caucasians (Yan et al. 2014). PDE4D mutations have also been associated with skeletal dysplasia and intellectual disability (Lindstrand et al. 2014).

While it was previously very difficult to make a PDE4 subtype-specific inhibitor, x-ray structure of the PDE4 proteins (Wang et al. 2007a) revealed a phenylalanine-196 residue that was only present on PDE4D, which was able to be targeted by allosteric inhibitors (Burgin et al. 2010). These PDE4D allosteric inhibitors were able to reduce the emetic effects of PDE4D inhibition as measured through duration of anesthesia induced by ketamine/xylazine (Robichaud et al. 2002a, b) while maintaining the beneficial nootropic effects (Burgin et al. 2010). The memory enhancing effects of PDE4D dysruption have also been supported by other recently developed PDE4D selective inhibitors (Bruno et al. 2011, 2014; Ricciarelli et al. 2017; Sierksma et al. 2013).

## 7.2 Differences Between Normal Aging and Pathological Aging

Contrary to public belief, normal aging does not necessitate a general decline of overall cognitive ability. Indeed, normal aging usually affects specific cognitive abilities such as spatial memory, working memory, reaction time, and long-term memory (Podtelezhnikov et al. 2011; Hansen and Zhang 2013). Not all individuals are affected in normal aging, and certain forms of cognition such as short-term

memory remain unchanged, while other forms of memory such as semantic memory can actually improve with age (Glisky 2007).

It was initially believed that with normal aging there is a natural loss of neurons in the brain (Brody 1955; Shefer 1973; Henderson et al. 1980); however, it is now believed there is no significant neuronal death that occurs with normal aging (Peters et al. 1998). The previous misconceptions were likely due to artifacts involved with the processing of aged brains (Haug 1985). Young brains shrink more during the fixation process, giving them the appearance of increased neuronal density (Peters et al. 1998). Once this confounding variable was accounted for through the use of unbiased stereological methods, the aged brain displays no differences in neuronal number (Giannaris and Rosene 2012).

One change normally observed in health aging is a decrease in brain volume (Skullerud 1985; Scahill et al. 2003; Driscoll et al. 2009) which may be due to decreases in neuronal size and somatic density (Terry et al. 1987). An alternative theory is that there is a loss of synapses in the aged brain (Geinisman 1999; Terry and Katzman 2001); however, others have observed no change of synapses in the aged brain (CA1 of the rat hippocampus), suggesting that memory deficits observed in senescence may be *functional* in nature (Geinisman et al. 2004). This decreased functional hypothesis is supported through observations that columns in the rhesus monkey brain display attenuated strength despite the fact that neuronal density and number are not changed (Cruz et al. 2004). These changes observed in normal aging are much different from what is seen in pathological aging.

Pathological aging or dementias such as Alzheimer's Disease (AD) are a significant diversion from the aging process, and have many differences from normal aging. Unlike normal aging which displays no significant changes in neuronal death, AD is characterized by massive atrophy in the brain which results in cognitive deficits much more severe than normal aging (Scheltens et al. 1995; Jack et al. 1997; Drachman 2006; Glisky 2007; Herrup 2010; Podtelezhnikov et al. 2011). The exact mechanisms and etiology for AD are still unknown, but in sporadic or non-familial cases (which account for the majority of AD cases) age is the main risk factor. Individuals over the age of 85 have a 50% chance of contracting this disease, and after contracting the disease there is an average survival of 5–10 years (Drachman 2006; Herrup 2010; Podtelezhnikov et al. 2011).

It has been suggested that AD could represent an accelerated aging process of the brain (Podtelezhnikov et al. 2011) and that the neurons could be undergoing massive functional transformation. Indeed, AD brains display significant changes in genes involved with lipid metabolism, the stress response, and inflammation. Interestingly, they also have changes in genes involved with cell adhesion, migration, and morphogenesis that bear a striking resemblance to patterns of gene expression also observed in epithelial mesenchymal transition (EMT) tissues (Podtelezhnikov et al. 2011). These findings reveal a similarity between AD brains and EMT that suggest a major transformation in brain tissue physiology and receptor signaling is occurring in AD. When the data obtained from the normal aged patients was extrapolated, it was predicted that in non-pathological aging, normal subjects would not reach cognitive decline comparable to AD subjects until

130–140 years of age (Podtelezhnikov et al. 2011). Other studies have confirmed that massive gene changes take place in AD. While some of these gene changes also occur in normal aging, the unique changes only in AD reinforce that pathological and normal again processes are different (Miller et al. 2008). We will next focus on alterations that occur with cAMP and PDE4 with age, and discuss the correlations that this has with cognitive decline.

## 7.3 Alterations of cAMP/PDE4 Signaling in Normal Aging

There are many components to the cAMP pathway that could be altered with aging. The immediate downstream effector of cAMP is protein kinase A (PKA), which releases from autoinhibition upon cAMP binding, and then translocates to the nucleus where it phosphorylates cAMP response element binding protein (CREB). CREB is a 43 kDa transcription factor that binds to cAMP response element (CRE) promoter sites (Carlezon et al. 2005) located on specific CRE-mediated genes such as brain derived neurotrophic factor (BDNF) (Finkbeiner et al. 1997; Tao et al. 1998); however, thousands of other CREB targets have been identified (Conkright et al. 2003; Zhang et al. 2005). CREB has been highly implicated in learning and memory (Dash et al. 1990; Bourtchuladze et al. 1994) and long term potentiation (LTP), a putative mechanism for the formation of memory (Impey et al. 1996). Other downstream effectors for cAMP include exchange protein directly activated by cAMP (EPAC) (de Rooij et al. 1998; Kraemer et al. 2001; Bos 2006; Gloerich and Bos 2010); or hyperpolarization-activated cyclic nucleotide-gated (HCN) channels which are expressed throughout the brain (Moosmang et al. 1999) and can be activated by cAMP resulting in significant influences on neuronal activity strength and behavior (Ramos et al. 2006; Wang et al. 2007b; Arnsten et al. 2010). It is important to note that as a controller of cAMP, PDE4 and its subtypes therefore also regulate these downstream targets, and changes in PDE4 activity, expression, or even localization could all have significant effects downstream.

When addressing what effects normal aging has on cAMP levels there are many variables to take into consideration. First, it should not be expected that there would be a global increase or decrease of cAMP throughout the entire body or even within the brain itself; therefore, it's possible nuanced changes might be occurring within discrete brain regions or nuclei where one region displays increased cAMP and another displays decreased cAMP. It is also important to differentiate between baseline and stimulated levels of cAMP. Although baseline levels may remain unchanged, stimulated levels which would occur through application of a neurotransmitter such as dopamine or norepinephrine may reveal a different finding. Also, one needs to take into consideration the model organism, and the method with which the tissue was collected and cAMP measured. It has been suggested that cAMP and cGMP in vivo are notoriously difficult to study and can change quickly after death; because of this, some would argue that the only "accurate" way to measure cAMP from a living animal would be to perform microwave fixation (Stavinoha 1993;

Delaney and Geiger 1996; O'Callaghan and Sriram 2004; Murphy 2010). Microwave fixation is able to achieve near instantaneous denaturing of proteins (rendering them inactive) in the brain such as adenylyl cyclases (AC), proteases, phosphatases, and PDEs, all of which could contribute to confounding changes in cAMP levels after death in animals. Microwave fixation essentially allows the researcher to save a "snapshot" of the brain comparable to when the animal was alive, and avoids most of the confounds observed with various other methods of sacrificing (O'Callaghan and Sriram 2004).

With these variables in mind, there have been several studies which have sought to determine how cAMP changes with maturation and normal aging (Hansen and Zhang 2013). One of the first studies showed that in the cerebral cortex of aged rats there was a noticeable decrease in baseline cAMP from 26 to 2 pmol/mg at 2–6 months of age, respectively (Zimmerman and Berg 1974); however, levels were unchanged during the last 18 months of life. This is consistent with other studies that have shown decreased cAMP in aged rat cerebellum (Austin et al. 1978) and striatum (Sugawa and May 1994), senescent human lymphocytes (Birkenfeld and Ben-Zvi 1984), and the aged gerbil hippocampal CA1 region and cerebral cortex (Hara et al. 1992). Schmidt and Thornberry showed that in aged rats from 3–24 months of age, there was no decline of baseline cAMP levels in the hypothalamus, cortex, hippocampus, or brain stem; however, there was a 44% decrease of norepinephrine-stimulated cAMP levels in the cerebellum (Schmidt and Thornberry 1978). Others have also shown no change in baseline levels of cAMP with aging, yet observed attenuated dopamine activation of adenylyl cyclases (Puri and Volicer 1977; Sugawa and May 1994). Contradicting these results, elevations of cAMP have been observed in the striatum of aged rats (Sugawa and May 1993; Sugawa and May 1994). The data on how baseline levels of cAMP change with aging have yet to come to agreement; however, the effect of aging on activated levels of cAMP seems to be more consistent. It has been observed that adenylyl cyclase activity (Makman et al. 1980; O'Connor et al. 1981; Sugawa and May 1994) and expression (O'Connor et al. 1983; Araki et al. 1995; Ramos et al. 2003; Mons et al. 2004) decrease with age, suggesting that there may be a loss of hormonal sensitivity with aging (Boas et al. 1973). Baseline levels of adenylyl cyclase are actually increased in the aged caudate and cerebellum, while activated levels of AC are lower in all the brain regions tested (Boas et al. 1973). It is interesting that these two regions have altered baseline activity of adenylyl cyclase, as it was previously mentioned that both the striatum and cerebellum have been noted to have consistent changes in cAMP with aging. One possible explanation is that different brain regions age differently. While increased baseline adenylyl cyclase activity in the cerebellum could be a compensatory mechanism to account for a loss of cAMP (Austin et al. 1978; Schmidt and Thornberry 1978), increased baseline activity in the caudate could be the direct reason for elevated levels of cAMP observed in the aged striatum (Sugawa and May 1993; Sugawa and May 1994). Although these studies fail to converge on an overall unifying trend of how cAMP changes with aging in the brain, they at least suggest cAMP levels are altered, and these alterations may be specific to different brain regions.

Changes in cAMP observed with aging could be due to differences in neurotransmitter levels and altered PDE4 activity. Indeed, neurotransmitters such as dopamine, norepinephrine, acetylcholine, and glutamate have been observed to be decreased in the aged brain (Austin et al. 1978; Kaiser et al. 2005; Tomobe et al. 2007). Though there have been no consistent results on how PDE4 changes in the healthy aged brain, accumulating evidence suggests altered PDE4 activity and expression may contribute to changes in cAMP. PDE4 activity has been shown to be increased in the cortex, hypothalamus, and hippocampus of aged rats (Stancheva and Alova 1991); meanwhile decreases of PDE4 activity have been observed in the aged *Macaca mulatta* striatum and frontal cortex, and rat brain (Tohda et al. 1996). PDE4 expression was shown to be unchanged in the aged mouse hippocampus (Kelly et al. 2013), however it was decreased in the cerebral cortex which is in agreement with other studies (Tohda et al. 1996). Interestingly, PDE4A expression has also been shown to be decreased in the striatum (Kelly et al. 2013), which parallels the increase cAMP observed in this area (Sugawa and May 1993; Sugawa and May 1994). PDE4D is significantly decreased in the aged mouse cerebellum (Kelly et al. 2013), which has consistently shown age-related changes in cAMP levels (Austin et al. 1978; Schmidt and Thornberry 1978). One would expect decreased PDE4D expression in the cerebellum to result in increased cAMP levels. However, this decrease may be compensatory in nature to adapt to the falling levels of cAMP, which may be due to a loss of hormonal sensitivity or adenylyl cyclase activity. Other studies have not observed age-related changes of PDE4 in the brain (Puri and Volicer 1977; Tohda et al. 1996; Ramos et al. 2003). Overall, these findings highlight the incredible complexity of PDE4 signaling. Regulation of PDEs are elaborate, and the very molecule (cAMP) they hydrolyze also regulates their expression in a negative feedback loop (Liu et al. 2000; O'Donnell and Xu 2012). Interestingly, elevations of cAMP can increase PDE4 expression through a CREB dependent mechanism (D'Sa et al. 2002), thus downstream targets of cAMP also have further feedback regulation of its own activity.

Alterations of cAMP signaling should result in changes in downstream targets. Decreases of PKA activity have been seen in the aged fruit fly brain (Laviada et al. 1997) and hippocampus and frontal cortex of aged rats (Karege et al. 2001a, b). Decreases in PKA activity resulting from altered cAMP signaling should subsequently diminish phosphorylated activation of CREB (pCREB). Indeed, decreases of CREB signaling have been observed in the aged brain (Yamamoto-Sasaki et al. 1999; Hattiangady et al. 2005; Kudo et al. 2005; Porte et al. 2008; Xu et al. 2010). CREB is an important mediator of LTP and memory (Dash et al. 1990; Bourtchuladze et al. 1994; Yin et al. 1994; Impey et al. 1996; Johannessen et al. 2004; Carlezon et al. 2005; Brightwell et al. 2007) and enhancement of pCREB activation in the aged brain improves memory (Xu et al. 2010; Zhao et al. 2013). These results show that while cAMP signaling may be diminished in certain brain areas, restoration of downstream signaling may be able to rescue the behavioral deficits caused by this alteration in function. Together, this suggests that cognitive deficits which develop with normal aging are not permanent, and offers hope these deficits are functional in nature with the possibility of being treated.

## 7.4 Alterations of cAMP/PDE4 Signaling in Pathological Aging

Pathological aging such as AD also results in significant alterations of cAMP signaling. Alzheimer's patients display increased cAMP in their cerebrospinal fluid (CSF) (Martınez et al. 1999) and microvessels of the brain, particularly the hippocampus (Hernandez et al. 2001). BACE1 is the enzyme responsible for the proteolytic cleavage of amyloid-β protein that results in the hallmark neuritic plaques of AD. Interestingly, in addition to its contribution to the formation of amyloid plaques, BACE1 directly interacts with adenylyl cyclase via its transmembrane domain, and overexpression of BACE1 results in the reduction of cAMP signaling and downstream targets such as PKA and pCREB (Chen et al. 2012). Adenylyl cyclase activity and expression are also decreased in AD brains (Ohm et al. 1989, 1991; Cowburn et al. 1992; O'Neill et al. 1994; Schnecko et al. 1994; Yamamoto et al. 2000).

Altered adenylyl cyclase activity would most likely immediately affect PKA activation. Administration of amyloid-β to hippocampal neurons decreases PKA activity; when given the selective PDE4 inhibitor rolipram, this effect was able to be reversed (Vitolo et al. 2002). Interestingly, in both human AD and animal models there is a loss of LTP and spatial memory before neuronal death and morphological changes occur (Vitolo et al. 2002). These observations suggest biochemical changes precede anatomical changes, and might represent a potential site for therapeutic intervention. Increased PKA-regulatory subunit expression has also been observed in AD subjects (Blalock et al. 2003). Increased expression of the PKA-regulatory subunit could be predicted to decrease PKA activity and downstream signaling.

In addition to altered adenylyl cyclase activity, altered PDE4 signaling could directly result in cAMP alterations. McLachlan and colleagues observed decreased expression of PDE4 isoforms in the hippocampus of AD subjects, however, the PDE4D1 isoform doubled in expression (McLachlan et al. 2013). PDE4 expression also changes dynamically with the severity of the disease. During stage 1 through 2 of the Braak scale, AD brains display increased expression of PDE4A and PDE4B mRNA in the entorhinal cortex, and an increase of PDE4A mRNA in the frontal cortex (Pérez-Torres and Mengod 2003). Progression to Braak stages 3–4, results in decreased PDE4A expression in the frontal cortex and CA2 region of the hippocampus, and increased PDE4D expression in the putamen (Pérez-Torres and Mengod 2003). It is possible that in the early stages of AD or Braak stages 1–2, increased levels of PDE4 are a direct effect of amyloid-β plaques or oligomers, causing a decrease in cAMP levels and subsequent decrease in the cAMP/CREB pathway. However, in the later stages of the disease, the decrease in PDE4A expression seen in the frontal cortex and CA2 could be a compensatory mechanism due to chronically reduced levels of cAMP (O'Donnell and Xu 2012). Application of amyloid-β to rat microglial cells increases PDE4B expression and TNF-α production (Sebastiani et al. 2006). It is interesting to note that inflammation may be a major mediator of Alzheimer's disease (Rubio-Perez and Morillas-Ruiz 2012), and

that PDE4B is highly implicated in inflammation processes (Jin and Conti 2002; Jin et al. 2005a; Pearse and Hughes 2016). Inhibition of PDE4B and PDE4D may represent novel therapeutic targets for Alzheimer's disease (Gurney et al. 2015; Pearse and Hughes 2016).

Diminished cAMP signaling should manifest downstream through decreased phosphorylation of CREB. Phospho-CREB levels are reduced in the Alzheimer's Tg2576 mouse model, and exposure of rat primary hippocampal neurons to amyloid-β decreases CREB promoter activity (Pugazhenthi et al. 2011). Application of amyloid-β to hippocampal cultures also reduces glutamate-invoked pCREB levels (Vitolo et al. 2002), and rats infused with amyloid-β into the hippocampus display decreased pCREB and memory (Wang et al. 2012). Alzheimer's brains display an inverse correlation between amyloid-β levels and CREB (Pugazhenthi et al. 2011). Familial Alzheimer's mutations in amyloid precursor protein (APP) negatively regulate CRE-mediated transcription (Ikezu et al. 1996; Giambarella et al. 1997). Indeed, diminished pCREB has been observed in Alzheimer's brains (Yamamoto-Sasaki et al. 1999). However, other enzymes involved in the formation of amyloid-β such as APP and the presenilins do not always result in reduced pCREB and subsequent CRE mediated gene expression. Mutations in the presenilin gene which is observed in familial AD result in constitutive over-activation of CREB (Müller et al. 2011). The amount of amyloid-β present may also fundamentally affect how the CREB pathway is altered. For instance, moderate levels of intracellular amyloid-β increase CRE-mediated gene expression (Echeverria et al. 2005). However, when these levels are significantly raised beyond normal levels, or are aggregated into fibrils and plaques like in AD, there is a significant decrease in CRE-mediated gene expression (Echeverria et al. 2005; Arvanitis et al. 2007). This suggests the relationship between amyloid-β and CREB modulation may be more complicated than a simple linear relationship, and in fact may be more accurately represented by the "inverted U" model. Non-pathological levels of amyloid-β may increase CREB activity, while pathological levels decrease CREB activity. Both elevations and decreases in CREB-mediated transcription have been observed in AD brains, suggesting that CREB dysregulation as a whole is altered in AD pathology. CREB-mediated signaling is altered in the first stages of incipient AD, suggesting it could be one of the first pathways to change in the start and progression of the disease (Satoh et al. 2009). Taking into consideration the aforementioned studies of how amyloid-β is able to both enhance and impair CREB, these observations should not be surprising. CREB has been shown to regulate thousands of gene transcription products (Johannessen et al. 2004; Carlezon et al. 2005). This dysregulation could represent both direct pathological manifestations of the disease and compensatory mechanisms aimed at combatting it.

## 7.5 Evidence for PDE4 Modulation as a Therapeutic Target in Pathological and Non-Pathological Aging

The literature suggests that cAMP signaling is altered in both normal aging and pathological aging; however, the question remains whether restoration of this signaling can improve cognitive function. Recent preclinical studies have shown that modulation of the cAMP pathway may attenuate cognitive deficits.

Amyloid-β decreases PKA and CREB activity in neuronal culture, and this effect is reversed through pretreatment with rolipram (Vitolo et al. 2002). Rolipram is also able to attenuate amyloid-β induced elevations of TNF-α and PDE4B in rat microglial cells (Sebastiani et al. 2006). This highlights the potential effects of PDE4 inhibition on inflammation, which is thought to play a main role in the disease (Frankola et al. 2011; Galimberti and Scarpini 2011; Clark et al. 2012; Montgomery and Bowers 2012; Rubio-Perez and Morillas-Ruiz 2012), and that targeting PDE4B may represent a novel therapeutic target for Alzheimer's disease (Pearse and Hughes 2016). In mouse models of AD, rolipram reverses deficits in LTP and memory, and pCREB levels in the hippocampus (Gong et al. 2004). These effects appear to be long lasting, as one course of chronic rolipram treatment was able to improve LTP and memory in AD mice 2 months after the end of their treatment (Gong et al. 2004). In other animal studies where amyloid-β was infused into the hippocampus, rolipram treatment was able to attenuate the effects of amyloid-β by improving memory and increasing pCREB levels (Cheng et al. 2010), and also reversed apoptotic responses (Wang et al. 2012). Reversal of apoptotic responses is particularly important in regards to pathological aging due to the large loss of neurons observed. PDE4 inhibition is also able to reverse morphological changes in AD models. Supporting this, Smith and colleagues found that treatment with rolipram reversed decreases in dendritic spine density in the hippocampus of transgenic AD-APP mice (Smith et al. 2009). The effects of rolipram have even been investigated against the deficits caused by iron loading in the brain, a process that occurs with aging and has been suggested to be responsible for age-induced cognitive impairments and neurodegeneration. Rolipram administration was able to reverse the effects of iron deposition on object recognition memory (de Lima et al. 2008). Rolipram and drugs that enhance cAMP signaling also rescue memory and LTP deficits in aged WT C57Bl/6J mice (Bach et al. 1999).

More recently there have been advances in the development of PDE4B and PDE4D subtype specific inhibitors (Burgin et al. 2010; Bruno et al. 2011, 2014; Azam and Tripuraneni 2014; Fox et al. 2014). The PDE4D specific inhibitor GEBR-7b enhances memory without the standard emetic side effects observed with rolipram (Miró et al. 2002; Mori et al. 2010; Bruno et al. 2011). GEBR-7b was also able to reverse the spatial memory deficits observed in AD APPswe/PS1dE9 mice, although pCREB levels were unchanged (Sierksma et al. 2013). Knock down of the long form PDE4D splice variants also reversed the memory deficits caused by amyloid-β (Zhang et al. 2014). These findings are in agreement with earlier studies

suggesting PDE4D is the predominant subtype involved with memory processes (Burgin et al. 2010; Li et al. 2011).

Resveratrol is another compound that hints at the potential of PDE4 for aging related phenotypes; however it might work in a slightly different manner than rolipram. Resveratrol is a polyphenol compound typically found in red wine of which the nootropic neurotrophic effects have been known for some time (Tredici et al. 1999); however, it was recently discovered that resveratrol is a non-specific PDE inhibitor able to mimic the effects of caloric restriction, and reverse metabolic aging-like phenotypes (Park et al. 2012). Most recently, we found that resveratrol reversed Aβ1-42-induced cognitive deficits via PDE4 inhibition and its subsequent activation of cAMP-CREB-BDNF signaling (Wang et al. 2016). The other pathway whereby resveratrol benefits aging is through a downstream cAMP effector known as EPAC, which is a guanine nucleotide exchange factor (GEF) for the small GTPases RAP1/2 (de Rooij et al. 1998; Kraemer et al. 2001; Bos 2006; Roscioni et al. 2008; Gloerich and Bos 2010) that exhibits implications for memory enhancement (Ouyang et al. 2008) and nerve growth (Murray and Shewan 2008; Murray et al. 2009). Reversal of age-induced metabolic phenotypes by resveratrol and EPAC was further found to be through the Sirtuin (SIRT) family (Park et al. 2012), which has been highly implicated in longevity and aging (Kim et al. 2007; Cristòfol et al. 2012). The effects of resveratrol were further explored in normally aged mice, and it was found that resveratrol was able to improve both memory and LTP following infusions into the cerebral ventricles (Zhao et al. 2013). Interestingly, the effect of resveratrol was not seen in SIRT1 KO mice. Further examination into the mechanisms of resveratrol action suggest downregulation of microRNAs 134 and 124, which are thought to regulate CREB expression (Zhao et al. 2013). These findings once again implicate CREB as one of the major potential downstream targets that might benefit from PDE4 inhibition and increased cAMP signaling.

The discovery of EPAC in downstream cAMP signaling has opened up new doors for therapeutic intervention. Interestingly, in the brains of AD patients, EPAC expression is altered (Mcphee et al. 2005). Typically, the two forms of EPAC (EPAC1 and EPAC2) have contrasting expression in the brain with EPAC2 more highly expressed in the CNS, and EPAC1 in the periphery; however, in AD brains EPAC1 expression is elevated, while EPAC2 expression is decreased (Mcphee et al. 2005). Other studies have also implicated EPAC in AD; activation of EPAC increased production of sAPPα (Maillet et al. 2003; Zaldua et al. 2007). This is a small neurotrophic molecule formed from the breakdown of APP in the non-amyloidogenic pathway. Thus, it is possible that activation of EPAC could shift the breakdown of APP away from the amyloidogenic pathway and the production of pathogenic levels of amyloid-β, towards the non-amyloidogenic pathway and production of therapeutic levels of the neurotrophic sAPPα.

## 7.6 Pitfalls and Side Effects of PDE4 Inhibition

Although targeting PDE4 for age-related memory loss seems promising, there are many side effects and pitfalls which need to be considered. The idea of using PDE4 as a target for psychiatric illness is not a novel one. Since PDE4 was first discovered, it was found shortly thereafter that the broad PDE4 inhibitor rolipram significantly improved memory (Randt et al. 1982; Egawa et al. 1997; Imanishi et al. 1997; Barad et al. 1998), and reversed depressive-like behavior in rodents (Wachtel 1983; Wachtel and Schneider 1986). Because of these initial findings, rolipram made it to clinical trials as a potential antidepressant and showed both promising (Zeller et al. 1984; Guiot-Goffioul et al. 1987; Laux et al. 1988) and inconsistent results (Bertolino et al. 1988). However, it was soon discovered that rolipram had a major flaw; several studies began confirming that rolipram induced severe nausea and emesis (Hebenstreit et al. 1989; Scott et al. 1991). The failure of rolipram in clinical trials leads to an important question, why *was* rolipram destined to fail? It has since been discovered that PDE4 is highly expressed in the area postrema (Cherry and Davis 1999) which is the emetic center of the brain (Mori et al. 2010). Inhibition of the PDE4 in this region "activates" the area postrema, and produces the emetic response typical of rolipram (Heaslip and Evans 1995; Robichaud et al. 2001; Richter et al. 2013). This emesis is most likely mediated by PDE4D, which has the highest expression levels in the area postrema (Cherry and Davis 1999; Miró et al. 2002; Mori et al. 2010). Subtype-specific inhibition would be one strategy to avoid the side effects caused by broad PDE4 inhibition. Recently subtype-specific inhibitors for PDE4B (Fox et al. 2014; Hagen et al. 2014) and PDE4D (Burgin et al. 2010; Bruno et al. 2011) have been developed that may avoid the strong emetic effects plaguing previous PDE4 inhibitors.

The development of broad PDE4 inhibitors for treating cognitive disorders in the future will be a difficult venture and is most likely not the most appropriate strategy. PDE4 is distributed throughout the body (Johansson et al. 2012), so treatment of a cognitive disorder with a broad PDE4 inhibitor would most likely result in many off-target effects. In addition, the multiple PDE4 subtypes and splice variants display differential expression in various brain regions and within individual neurons themselves. Within individual neurons distinct N′ termini of the individual splice variants results in unique compartmentalization, which is important for the control of different transduction messages, and allows the neuron to retain specificity over different pathways (Catherine Jin et al. 1998; Houslay and Adams 2003; Martin and Cooper 2006; Baillie 2009; Houslay 2010; Oliveira et al. 2010; Blackman et al. 2011; Vincent et al. 2012). This suggests the PDE4 subtypes and splice variants have non-redundant functional roles, and further demonstrates that broad inhibition is not the most appropriate strategy.

In addition, it can't be assumed that elevations of cAMP/CREB signaling is beneficial in all brain regions. Although elevation of cAMP and pCREB enhances memory in the hippocampus, increasing cAMP in the amygdala could promote anxiety-like behavior or thoughts. In vitro studies demonstrate the anxiogenic

corticotrophin releasing factor (CRF) elevates cAMP signaling, whereas the anxiolytic compound neuropeptide Y (NPY) decreases cAMP signaling (Mulchahey et al. 1999; Sheriff et al. 2001). This parallels in vivo studies that show stress and anxiety are correlated with increases in pCREB (Adamec et al. 2011) and BDNF levels (Lakshminarasimhan and Chattarji 2012). Furthermore, overexpression of pCREB in the amygdala increases anxiety-like behavior (Wallace et al. 2004), and PDE4A and PDE4B KO mice display significant increases in anxiety-like behavior that correlate with an increase in cAMP signaling (Zhang et al. 2008; Hansen et al. 2014). Elevation of pCREB signaling in the nucleus accumbens can also have additional detrimental effects. Stress in rats produces an increase in CREB activation in the nucleus accumbens, and overexpression of CREB in this area leads to depressive-like anhedonia; dominant-negative infusion of CREB displays the opposite behavioral effect (Muschamp et al. 2011). Also, elevated cAMP/PKA activity in the prefrontal cortex impairs working memory (Taylor et al. 1999; Ramos et al. 2003), and aged rats have elevated levels of CREB and pCREB in the PFC as they age (Ramos et al. 2003; Vandesquille et al. 2013). It should come as no surprise that working memory and long-term memory (LTM) are affected differently by cAMP signaling as working memory "requires the continuous and dynamic updating of memory buffers, whereas long-term memory consolidation involves changes that are static and long lasting" (Arnsten et al. 2005).

One other pitfall is that the effects of altering cAMP levels on different pathological targets remain to be fully understood. While the majority of research shows amyloid-β decreases cAMP/CREB signaling, there are some studies showing the opposite (Echeverria et al. 2005; Satoh et al. 2009; Müller et al. 2011). CREB is one of the principal transcription factors affected in AD. A complete dysregulation of CREB signaling has been observed with some transcripts being up regulated and others down regulated (Blalock et al. 2003). Eliciting pathological changes from compensatory changes observed in this altered CREB signaling are a significant hurdle as cAMP/PDE4 signaling is notorious for being incredibly compensatory (O'Donnell and Xu 2012). Globally raising cAMP could have detrimental effects contributing to the pathology of AD. For instance, raising cAMP levels causes an increase in APP protein expression, and activation of both the amyloidogenic and non-amyloidogenic pathways (Canepa et al. 2013). Assuming the "amyloid hypothesis" of AD is correct, increasing APP could have detrimental effects on the amount of amyloid-β plaques produced and subsequent cognitive performance. Increased cAMP signaling also results in hyper-phosphorylation of tau protein, one of the hallmark pathologies of AD (Litersky and Johnson 1992; Jicha et al. 1999).

## 7.7 The Future of PDE4 for Therapeutic Intervention

Although much has been learned about PDE4 in cognition, there is still much to be discovered. First, the field needs to consistently characterize where individual PDE4 splice variants are expressed in the brain, and the roles these variants play in

behavior and cognition. This is one of the most complicated challenges that need to be overcome due to the large number of splice variants in the PDE4 family, unique expression of these splice variants in different brain regions, and localized expression of these variants within individual neurons which leads to microdomains and compartmentalization of signaling. Progress has been made with the development of PDE4B and PDE4D inhibitors (Burgin et al. 2010; Bruno et al. 2011; Fox et al. 2014); however, there are still no PDE4A specific inhibitors, nor are there drugs capable of targeting specific PDE4 splice variants. An optional approach to using drugs to identify the functional roles of PDE4 specific splice variants would be to use genetic approaches such as viral-mediated knockdown (Li et al. 2011; Wang et al. 2013; Zhang et al. 2014). Due to the incredible number of PDE4 splice variants, this will take time and patience. Characterization of unique splice variant compartmentalzation is a fundamental issue that needs to be addressed in future research, as this may allow for targeted intervention of unique downstream targets for the individual splice variants that may not be able to be resolved through subtype inhibition.

Inhibition of PDE4 may also not be appropriate in all scenarios. One should recognize the incredibly delicate balance of cAMP signaling in the brain, and that global activation of cAMP through broad PDE4 inhibition might not be the best approach. In regards to working memory, it appears that decreases in cAMP levels in the PFC would actually improve memory. This also might hold true in the nucleus accumbens and amygdala, as overexpression of CREB in these regions has been shown to produce anhedonia (Muschamp et al. 2011) and anxiety, respectively (Wallace et al. 2004). There may need to be a paradigm shift in the way we think about PDE4 modulation as a therapeutic for cognitive disorders; in particular, researchers may need to begin thinking about PDE4 activation as a possible therapeutic strategy for the future. This makes sense in regards to anxiety or working memory. However, in regards to hippocampus-dependent memory PDE4 inhibition may still be the best approach.

Despite these drawbacks facing PDE4 as a therapeutic for aging and AD, PDE4 cognitive therapy remains promising. Many of the initial mechanisms that are disrupted in normal aging are also present in the early and later stages of AD. If animal models hold true, PDE4 inhibition may reverse morphological and functional behavioral changes that occur both in senescent memory and the initial pathological decline in AD. One of the main differences between AD and normal aging is the large number of neurons are killed as pathogical aging progresses, whereas no signficant neuronal loss occurs with normal aging. Thus, it seems that in normal aging the biological mechanisms may become stagnant, which shows great promise for PDE4 modulation as a therapeutic to “wake up” the cAMP signaling pathways. In regards to AD, the most promise for PDE4 modulation would be at the beginning of the disease before neuronal death begins to occur, and the signal transduction pathways are beginning to be changed. Once neuronal death begins to occur, the best hope for PDE4 therapies would be to stop further progression of the disease through increased neurogenesis or decreased apoptosis. It is unknown if PDE4 modulation would be able to restore lost cognitive ability derived from neuronal

loss; however, elevations of cAMP signaling increases neurogenesis and enhances memory (Li et al. 2011), indicating a role of PDE4 in cognitive deficits caused by neuronal loss. Overall, PDE4 modulation could represent a prophylactic therapeutic for AD and other CNS diseases with cognitive deficits. However, more research is needed into the field to characterize the consequences of altering cAMP levels in a disease as severe as AD.

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

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# Chapter 8
# A Role for Phosphodiesterase 11A (PDE11A) in the Formation of Social Memories and the Stabilization of Mood

**Michy P. Kelly**

**Abstract** The most recently discovered 3′,5′-cyclic nucleotide phosphodiesterase family is the Phosphodiesterase 11 (PDE11) family, which is encoded by a single gene PDE11A. PDE11A is a dual-specific PDE, breaking down both cAMP and cGMP. There are four PDE11A splice variants (PDE11A1-4) with distinct tissue expression profiles and unique N-terminal regulatory regions, suggesting that each isoform could be individually targeted with a small molecule or biologic. PDE11A4 is the PDE11A isoform expressed in brain and is found in the hippocampal formation of humans and rodents. Studies in rodents show that PDE11A4 mRNA expression in brain is, in fact, restricted to the hippocampal formation (CA1, possibly CA2, subiculum, and the adjacently connected amygdalohippocampal area). Within the hippocampal formation of rodents, PDE11A4 protein is expressed in neurons but not astrocytes, with a distribution across nuclear, cytoplasmic, and membrane compartments. This subcellular localization of PDE11A4 is altered in response to social experience in mouse, and in vitro studies show the compartmentalization of PDE11A4 is controlled, at least in part, by homodimerization and N-terminal phosphorylation. PDE11A4 expression dramatically increases in the hippocampus with age in the rodent hippocampus, from early postnatal life to late aging, suggesting PDE11A4 function may evolve across the lifespan. Interestingly, PDE11A4 protein shows a three to tenfold enrichment in the rodent ventral hippocampal formation (VHIPP; a.k.a. anterior in primates) versus dorsal hippocampal formation (DHIPP). Consistent with this enrichment in VHIPP, studies in knockout mice show that PDE11A regulates the formation of social memories and the stabilization of mood and is a critical mechanism by which social experience feeds back to modify the brain and subsequent social behaviors. PDE11A4 likely controls behavior by regulating hippocampal glutamatergic, oxytocin, and cytokine signaling, as well as protein translation. Given its unique tissue distribution and relatively selective effects on behavior, PDE11A may represent a novel therapeutic target for

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neuropsychiatric, neurodevelopmental, or age-related disorders. Therapeutically targeting PDE11A4 may be a way to selectively restore aberrant cyclic nucleotide signaling in the hippocampal formation while leaving the rest of the brain and periphery untouched, thus, relieving deficits while avoiding unwanted side effects.

**Keywords** PDE11A • PDE11 • Phosphodiesterase 11 • cAMP • cGMP • Psychiatric illness • Brain • Endocrine • Immune • Hippocampus • Memory • Inflammation • Lithium • Tissue expression • Social Behavior • Oxytocin

## 8.1 Introduction

The most recently discovered phosphodiesterase (PDE) family is PDE11, which hydrolyzes cAMP and cGMP equally well (Hetman et al. 2000; Fawcett et al. 2000; Yuasa et al. 2000; Yuasa et al. 2001a; Weeks et al. 2007). The PDE11 family is comprised of a single gene, PDE11A (Hetman et al. 2000; Fawcett et al. 2000; Yuasa et al. 2000; Yuasa et al. 2001a; Yuasa et al. 2001b). As with most PDE families, the N-terminal domain of PDE11A serves a regulatory function, whereas the C-terminal domain encompasses the catalytic domain (Weeks et al. 2007). The longest isoform of mouse, rat and human PDE11A (a.k.a. PDE11A4) demonstrates ~95% protein sequence homology, suggesting results obtained in preclinical rodent models will be applicable to the human condition. As extensively reviewed elsewhere (Kelly 2015), literature findings surrounding the tissue distribution pattern for the various PDE11A isoforms are highly disparate, in part due to a number of poor quality commercially-available antibodies, but well-controlled studies consistently show select PDE11A isoforms being expressed in brain (particularly the hippocampal formation), the adrenal gland, and the prostate. Studies examining tissues from PDE11A wild-type (WT) and knockout (KO) mice find abundant PDE11A4 protein in the hippocampal formation of brain but not in any of 23 peripheral tissues assessed; whereas, PDE11A1 was found in prostate, PDE11A3 in the seminal vesicles, and PDE11A1 and PDE11A3 in spleen (Kelly 2015). Consistent with this restricted tissue distribution profile, PDE11A appears to regulate brain function, tumor physiology, and, possibly, inflammation (Kelly 2015; Fatemi et al. 2010a; Couzin 2008; Wong et al. 2006; Luo et al. 2009a; Kelly et al. 2010; Hegde et al. 2015; Pathak et al. 2016; Hegde et al. 2016b; Mertens et al. 2015 but see Laje et al. 2009; Perlis et al. 2010; Kelly 2015; Alevizaki and Stratakis 2009; Faucz et al. 2011; Greene et al. 2010; Carney et al. 2010; Almeida and Stratakis 2011; Vezzosi et al. 2012; Horvath et al. 2009; Horvath et al. 2006a; Horvath et al. 2006b; Libe et al. 2008; Libe et al. 2011 but see Bimpaki et al. 2009; Kelly 2015; Pathak et al. 2016; DeWan et al. 2010; Oki et al. 2011; Witwicka et al. 2007; Bazhin et al. 2010). Here, we review how PDE11A may regulate brain function to determine whether or not PDE11A4 holds promise as a future therapeutic target, particularly in the context of neuropsychiatric, neurodevelopmental and/or age-related disease.

## 8.2 Molecular Features of PDE11A

The PDE11A gene on chromosome 2q31.2 contains 23 exons and yields four splice variants PDE11A1-4 (Hetman et al. 2000; Fawcett et al. 2000; Yuasa et al. 2000; Yuasa et al. 2001a; Yuasa et al. 2001b), which have been recently schematically reviewed in depth (Kelly 2015). Isoform-specific promoters, coupled with alternative splicing, yield unique N-terminal domains for each PDE11A isoform (Yuasa et al. 2001b) and likely account for the differences in tissue expression that are seen with each PDE11A isoform (Kelly 2015). While the C-terminal region encompasses the catalytic domain of PDE11A, the N-terminal region encompasses various regulatory domains and sites of post-translational modification. Within the N-terminal region are 2 cGMP binding PDE, Anabaena adenylyl cyclase and *E. coli* FhlA (GAF) domains (Makhlouf et al. 2006). Perhaps not surprisingly, phylogenetic analyses suggests PDE11A is most closely related to other GAF-domain containing PDEs, particularly PDE5A and PDE6A-C as well as PDE2A and PDE10A (Yuasa et al. 2001b; Kelly 2015). As we will see below, the four PDE11A isoforms hydrolyze cAMP and cGMP, and each has a unique N-terminal region with differential representation of the GAF-A domain, which binds cGMP (Gross-Langenhoff et al. 2008; Gross-Langenhoff et al. 2006; Jager et al. 2012; Matthiesen and Nielsen 2009), and the GAF-B domain, which is required for homodimerization.

Although each of the four variants exhibits a unique N-terminal regulatory domain (c.f., Kelly 2015), the C-terminal domain is consistent across the 4 PDE11A isoforms. Exons 8–23 are included in each isoform and encode not only the C-terminal catalytic domain but also a portion of the N-terminal regulatory GAF-B domain (Makhlouf et al. 2006). In addition to exons 8–23, PDE11A1 also uniquely includes exon 7; thus, PDE11A1 includes only a truncated GAF-B domain within its N-terminus (Yuasa et al. 2001b). PDE11A2 includes exons 5 and 6, but not 7, in addition to exons 8–23; thus, PDE11A2 includes a truncated GAF-A domain and a full GAF-B domain. (Hetman et al. 2000; Makhlouf et al. 2006). The PDE11A3 transcript is encoded by exons 1, 2, 4, 5, and 6 in addition to exons 8–23; however, translation of PDE11A3 does not begin until exon 2 (Yuasa et al. 2001b). Thus, similar to PDE11A2, PDE11A3 contains a truncated GAF-A domain and a full GAF-B domain upstream of the C-terminal catalytic domain. PDE11A4 is the only isoform to include exon 3 along with the shared exons 4–6 and 8–23 (Yuasa et al. 2001b). PDE11A4, the isoform expressed in brain, is the longest isoform. Therefore, it is at times erroneously referred to as "full-length" PDE11A, despite the fact that it lacks exons 2 and 7. Importantly, exon 3 contains two validated phosphorylation sites (S117 and S162) and the beginning of the GAF-A domain (Yuasa et al. 2000); thus, PDE11A4 is the only isoform to include the GAF-A domain in its entirety. As we will review in greater detail below, these phosphorylation sites and GAF domains may be important for intramolecular signaling (Weeks et al. 2007; Gross-Langenhoff et al. 2008) and may provide a mechanism by which it will be possible to therapeutically target a single PDE11A isozyme with high selectivity (Kelly 2015). Indeed, isozyme-specific targeting of PDE11A activity has already been demonstrated as vinpocetine can inhibit PDE11A3 but not PDE11A4 (Yuasa et al. 2000).

Under physiological conditions, all PDE11A isoforms hydrolyze both cAMP and cGMP (cf. Makhlouf et al. 2006). As extensively reviewed elsewhere (Kelly 2015), PDE11A1 and PDE11A2 have higher substrate affinities relative to PDE11A3 and PDE11A4 (Yuasa et al. 2001a; Weeks et al. 2007); however, PDE11A3 and PDE11A4 have significantly higher catalytic rates relative to PDE11A1 and PDE11A2 (Yuasa et al. 2001a; Weeks et al. 2007). A comparison of rat PDE11A found similar results to those obtained using *Homo sapiens* PDE11A (Yuasa et al. 2001a). The net effect of the relative differences in substrate affinities versus turnover rates is that the four PDE11A isoforms hydrolyze cAMP and cGMP comparably well (Yuasa et al. 2001a; Weeks et al. 2007). Site-directed mutagenesis shows that Q869 is required for substrate binding of the PDE11A catalytic domain (Weeks et al. 2009); however, in absence of a resolved crystal structure the full implications of these results remain to be determined. Such knowledge would benefit designing compounds capable of selectively targeting the PDE11A catalytic domain over its nearest neighbor PDE5A.

The N-terminal region of PDE11A clearly regulates catalytic function of the enzyme. For example, as the length of the N-terminus increases, the affinity of the catalytic domain for cAMP and cGMP decreases (Weeks et al. 2007). That is, PDE11A4 has the lowest substrate affinity for cAMP and cGMP and PDE11A1 and PDE11A2 have the highest substrate affinities for cAMP and cGMP (Weeks et al. 2007). Similarly, in a chimeric protein consisting of the PDE11A4 N-terminus linked to the catalytic domain of a bacterial adenylyl cyclase, deletion of the first 196 amino acids (i.e., all amino acids upstream of the GAF-A domain) increases basal catalytic activity (Gross-Langenhoff et al. 2008). Together, these studies support a regulatory role for the PDE11A N-terminal region.

As mentioned above, PDE11A4 is the only PDE11A variant that includes S117 and S162, 2 serines phosphorylated by protein kinase A, protein kinase G (PKA/PKG) (Yuasa et al. 2000) and possibly other enzymes (Kelly 2015). In vitro, PKA and PKG fail to phosphorylate PDE11A3, which lacks S117 and S162, but do phosphorylate PDE11A4 (Yuasa et al. 2000). Further, most of the PKA and PKG-induced phosphorylation of PDE11A4 is ablated by mutating S117 and S162 to phosphomutant alanines (Yuasa et al. 2000). The remaining PKA and PKG-induced phosphorylation observed in the S117A/S162A PDE11A4 mutant likely reflects phosphorylation of S124, which is predicted to be phosphorylated by PKA and PKG (Kelly 2015) and is phosphorylated along with S117 and S162 in COS-1 cells (Pathak et al. 2015).

Emerging evidence suggests these N-terminal phosphorylation sites may play an important role in regulating PDE11A4 function. Gross-Langenhoff *et al.* used a chimeric protein consisting of the PDE11A4 N-terminus linked to the catalytic domain of a bacterial adenylyl cyclase to explore the functional role S117/S162 phosphorylation (Gross-Langenhoff et al. 2008). In that chimeric protein, the phosphorylation state of the N-terminal regulatory region affected intramolecular regulation of the GAF-A domain. Specifically, when both S117 and S162 were phosphorylated, or were replaced with amino acids that mimicked the phosphorylated state, the downstream GAF-A regulatory domain had an increase in affinity for cGMP (Gross-Langenhoff et al. 2008). Interestingly, deleting the first 176 amino

acids similarly increased cGMP affinity for the GAF-A domain (Gross-Langenhoff et al. 2008), suggesting that phosphorylation of S117/S162 may produce a conformational change that exposes the cyclic nucleotide binding pocket of the GAF-A domain. In COS-1 and HEK293T cells, we have shown that introducing a phosphomimic mutation (aspartate, D) to S117 and S124 (S117D, S124D) traffics PDE11A4 into punctate structures and shifts PDE11A4 from the cytosol to the membrane (Pathak et al. 2015). In contrast, a S162D mutation has the completely opposite effect, distributing PDE11A4 throughout the cytosol and shifting PDE11A4 from the membrane to the cytosol (Pathak et al. 2015). Interestingly, S162D completely blocks the effects of S117D/S124D (Pathak et al. 2015). The fact that S162D blocks the effect of S117D in the context of a full-length recombinant protein, yet potentiates the effect of S117D in the context of the chimeric protein, may suggest a complex interplay between these N-terminal phosphorylation events and the C-terminus. Consistent with the fact that S162D shifts PDE11A4 to the cytosol and blocks the ability of S117D/S124D to shift PDE11A4 toward the membrane, we find that wild-type PDE11A found in the cytosol can be phosphorylated at any of the three serines; whereas, PDE11A4 in the membrane shows phosphorylation at serines 117 and 124 but not 162 (Pathak et al. 2015). It will be important to determine whether the ability of these phosphorylation events to control the subcellular compartmentalization of PDE11A4 is related to conformational changes in the enzyme, changes in cGMP binding of the GAF-A domain, and/or the prevention or promotion of other post-transtional modifications (e.g., palmitoylation, ubiquitination, sumoylation).

The functional consequence of cyclic nucleotide binding of a PDE GAF domain differs by PDE family. cGMP binding the GAF-A domain can directly increase catalytic activity (PDE2A, Martinez et al. 2002; Beavo et al. 1971), increases substrate affinity (PDE5A, Francis et al. 2011), indirectly inhibit catalytic activity by strengthening specific inhibitory protein-protein interactions (PDE6, D'Amours and Cote 1999), or have no effect at all (PDE10A, Jager et al. 2012; Matthiesen and Nielsen 2009). Several laboratories have reported that cGMP binds the PDE11A4 GAF-A, but not GAF-B, domain (Gross-Langenhoff et al. 2008; Gross-Langenhoff et al. 2006; Jager et al. 2012; Matthiesen and Nielsen 2009), likely with a lower $K_i$ versus the catalytic domain (Matthiesen and Nielsen 2009). Although cGMP binding of the PDE11A4 GAF-A domain did stimulate catalytic activity of the PDE11A4-cyclase chimeric protein described above (Gross-Langenhoff et al. 2008; Gross-Langenhoff et al. 2006), it did not impact catalytic activity of recombinant full-length PDE11A4 (Jager et al. 2012; Matthiesen and Nielsen 2009). That said, it is interesting to note that the ability of cGMP to allosterically stimulate catalytic activity of the PDE11A4-cyclase chimera was dependent on the presence of an aspartate within a conserved NKFDE motif that is present in all mammlian PDE GAF domains (Gross-Langenhoff et al. 2006). Mutating D355 to an alanine abolished the intramolecular signaling events triggered by cGMP. It remains to be determined whether mutating D355 simply prevents cGMP from binding the GAF-A domain or prevents some downstream consequence of cGMP binding (e.g., a conformational change; Gross-Langenhoff et al. 2006). Although cGMP does not appear to allosterically regulate catalytic activity of PDE11A4, a cGMP analog

Rp-8-pCPT-PET-cGMPs does appear to allosterically increase the catalytic activity of both recombinant and native PDE11A4 (Jager et al. 2012). Unfortunately, Rp-8-pCPT-PET-cGMPs inhibits PKG and, likely, many other cGMP-binding molecules; thus, its use as a tool compound is limited. That said, the fact that Rp-8-pCPT-PET-cGMPs is capable of allosterically stimulating PDE11A function provides a key proof-of-concept for developing PDE11A4 activators.

GAF domains not only bind cyclic nucleotides, they also bind proteins, including other molecules of PDEs themselves. All PDE11A isoforms, indeed all PDEs (Francis et al. 2011; Heikaus et al. 2009), exist as oligomers (Weeks et al. 2007). The C-terminal portion of the GAF-B domain is required for dimerization of all PDE11A isoforms (Weeks et al. 2007). PDE11A1, with the shortest N-terminal region, tetramerizes; whereas, PDE11A2-4 homodimerize. It is not completely understood how oligomerization affects PDE11A function. Weeks *et al.* found that the dimeric PDE11A2 and the tetrameric PDE11A1 have nearly identical substrate affinities for cAMP and cGMP, suggesting that quaternary structure does not greatly impact affinity of the cyclic nucleotides for the catalytic site (Weeks et al. 2007). We have shown that homodimerization controls the subcellular compartmentalization of PDE11A4 (Pathak et al. 2016). A mutation within the GAF-B domain found in select mouse strains that strengthens homodimerization shifts PDE11A4 from the cytosol towards the membrane; whereas, disrupting homodimerization shifts PDE11A4 from the membrane to the cytosol (Pathak et al. 2016). Disrupting homodimerization also increases proteolytic degradation of PDE11A4 (Pathak et al. 2016). Although the mechanism responsible remains to be determined, it is interesting to note that PDE11A4 is predicted to be ubiquitinated and sumoylated (Kelly 2015). Thus, it is possible that disruption of homodimerization may make PDE11A4 more vulnerable to these particular posttranslational modifications that tag a protein for degradation. It remains to be determined whether or not homodimerization directly affects PDE11A4 catalytic activity as well. In vitro studies examining the PDE2, PDE4, and PDE5 families argue that dimerization is not required for catalytic activity; however, dimerization may influence post-translational modifications of the enzymes, thereby altering subcellular localization and/or catalytic activity (Francis et al. 2011; Keravis and Lugnier 2010). Clearly, much work remains to understand the functional role of PDE11A oligomerization. That said, these studies suggest that homodimerization may be a novel mechanism by which we could therapeutically target PDE11A function, particularly in disease states where the enzyme may become mislocalized.

## 8.3 Tissue Expression Patterns for PDE11A

Understanding the tissue expression profile of an enzyme is key when considering it as a therapeutic target. As noted above, tissue expression profiling studies for PDE11A have proven highly inconsistent. Poor tools are likely to blame for many of these discrepant reports. We have shown that Western blots of peripheral tissues

probed with various commercially-available PDE11A antibodies demonstrate an abundance of non-specific bands at molecular weights that are consistent with the various PDE11A isoforms (Kelly 2015). The fact that the bands do not disappear in tissue from a PDE11A KO mouse shows these signals are non-specific. Some discrepancies may also be related to the fact that studies have not examined the correct sub-compartment of certain organs. For example, initial studies failed to detect PDE11A expression in brain (Fawcett et al. 2000; Yuasa et al. 2000). Subsequent studies, however, have convincingly shown that PDE11A4 is, in fact, expressed within a small, restricted region of the brain (particularly the ventral hippocampal formation, Kelly et al. 2010; Jager et al. 2012; Kelly et al. 2014). Finally, discrepancies could be related to species differences or a differential expression profile under normal versus disease conditions.

As we have extensively reviewed elsewhere (Kelly 2015), tissue expression profiling studies to date suggest PDE11A expression is relatively restricted throughout the body. PDE11A1 and/or PDE11A2 may be expressed at a moderate level in prostate, pancreas and kidney. PDE11A3 is likely expressed within specific compartments of the testes and seminal vesicles at moderate levels. PDE11A4 is certainly expressed at high levels in the ventral hippocampal formation of brain, and may be expressed at moderate levels in the human—but not mouse—adrenal gland. Limited evidence points to low levels of PDE11A4 being expressed in liver, heart, and pituitary, and moderate levels being expressed in human prostate, but our own studies employing 23 peripheral tissues from PDE11A KO mice as negative controls fail to detect PDE11A4 outside of brain (Kelly 2015). With such a limited tissue expression profile, a PDE11A-targeted therapeutic would stand to impact very selective systems of interest without eliciting wide-ranging toxicological side effects.

PDE11A4 mRNA in brain is almost exclusively expressed in neurons of CA1, possibly CA2, the subiculum and the adjacently connected amygdalohippocampal area (Kelly 2015; Kelly et al. 2010; Hegde et al. 2016a; Kelly et al. 2014; Kelly 2014) (Fig. 8.1). Within these neuronal populations, PDE11A4 protein is found in the cell bodies, axons, and select dendrites (Hegde et al. 2016a) (Fig. 8.2, 8.3, 8.4 and 8.5). Trafficking of PDE11A4 protein to the axons explains why PDE11A4 protein can be detected at miniscule levels in brain regions that receive VHIPP projection but, themselves, lack PDE11A4 mRNA (e.g., prefrontal cortex and striatum) (Kelly 2015; Hegde et al. 2016a). PDE11A4 is the only PDE whose expression in brain originates solely from the hippocampal formation (c.f., Kelly et al. 2014; Kelly 2014; Kelly and Brandon 2009; Xu et al. 2011). In the rodent hippocampus, PDE11A4 expression is minimal on postnatal day 7 but dramatically increases with each postnatal week, reaching young adulthood levels by postnatal day 28 (Hegde et al. 2016a). PDE11A expression stabilizes for a short period of time during young adulthood, but then again significantly increases between young, middle, and late adulthood (Kelly et al. 2014). The fact that PDE11A expression is restricted to the hippocampal formation, at least during young adulthood, suggests a PDE11A-targeted therapeutic may be capable of selectively targeting hippocampal function whilst leaving the rest of the brain undisturbed.

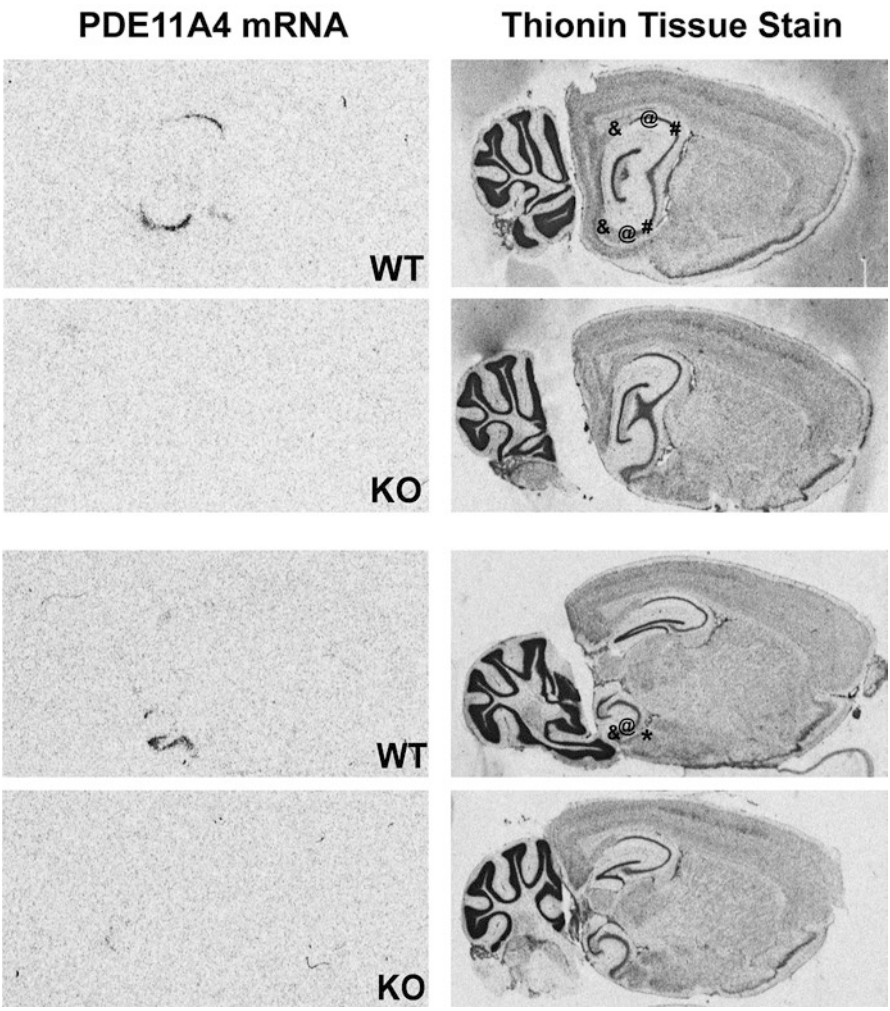


**Fig. 8.1** PDE11A4 mRNA expression is restricted to the hippocampal formation, with enrichment in ventral versus dorsal hippocampus. Autoradiographic *in situ* hybridization was conducted using two $^{35}$S-labeled antisense oligonucleotide probes with identical patterns of signal (shown: 5′-gccacctgtctggagatctcccacggtttggtcacggc-3′ recognizing nucleotides 2538-2501 of NM_001081033.1; not shown: 5′-cgcatcaagtaatcttcaaacaactctgggtgcct-3′ recognizing nucleotides 129-95 of NM_001081033.1). Note that no signal is observed in sections from PDE11A knockout (KO) mice, suggesting specificity of the signal detected in sections from PDE11A wild-type (WT) mice. When comparing the autoradiograph to the thionin tissue stain, it is clear that PDE11A mRNA expression is restricted to the hippocampal formation (CA1$^{@}$, possibly CA2$^{\#}$, subiculum$^{\&}$ and the adjacently connected amygdalohippocampal area*—regions indicated on thionin stain with corresponding symbols), with a two to tenfold enrichment in ventral versus dorsal hippocampus. *Top panels* show sections from ~2.76–2.88 mm lateral from Bregma, and *bottom panels* show sections from ~2.28–2.40 mm lateral from Bregma. Contrast and brightness were adjusted to enhance the graphical clarity of the images

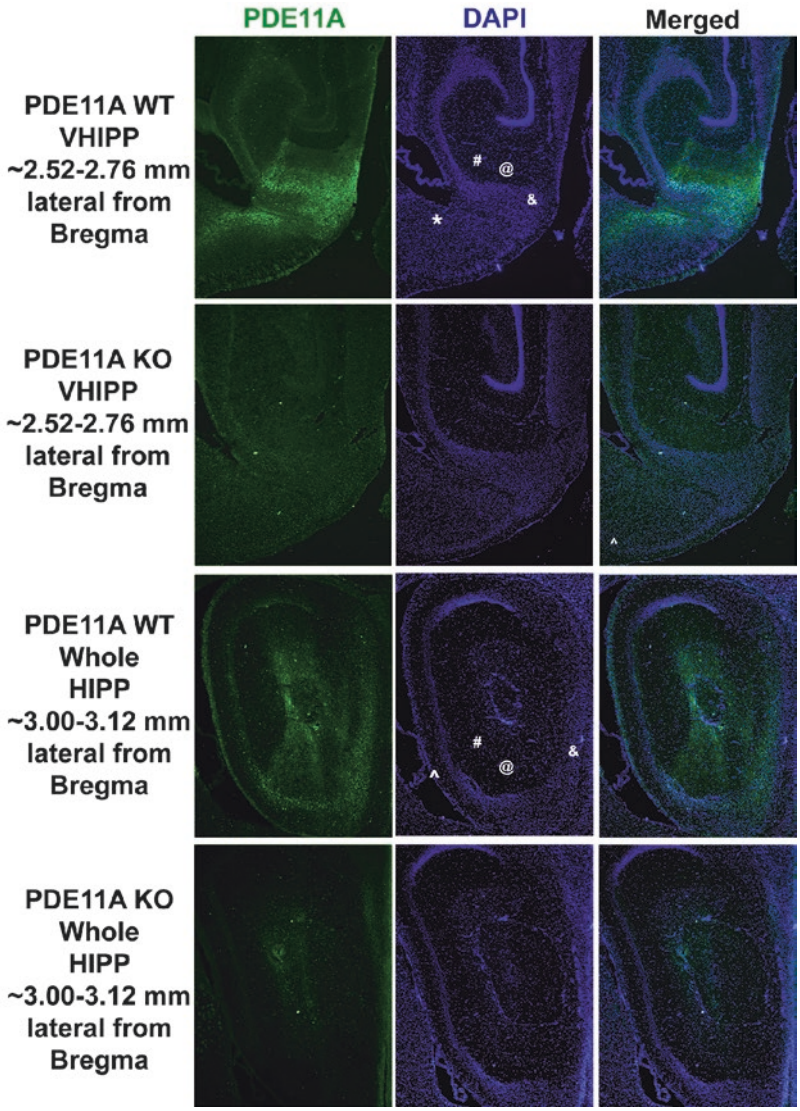


**Fig. 8.2** PDE11A4 protein expression is enriched in the ventral hippocampal formation, with expression throughout the stratum pyramidale, stratum radiatum, and fimbria. Identical staining for PDE11A was obtained in sections from PDE11A wild-type (WT) mice when sections were processed for immunofluorescence using an antibody that recognized all PDE11A isoforms (Fabgennix PD11-112 1:100, shown) or an antibody recognizing only PDE11A4 (AVES PDE11#1 1:5000; Hegde et al. 2016a). Note that minimal staining is observed in sections from PDE11A knockout (KO) mice, suggesting specificity of the signal detected in WT tissue. PDE11A protein is distinctly expressed in CA1$^{@}$, possibly CA2$^{\#}$, subiculum$^{\&}$, the adjacently connected amygdalo-hippocampal area*, and the fimbria^), with a two to threefold enrichment in ventral versus dorsal hippocampus. Images collected using a 4× objective. Histogram stretch and gamma were corrected to enhance the graphical clarity of the images. *Green*—PDE11A, *Blue*—DAPI nuclear stain

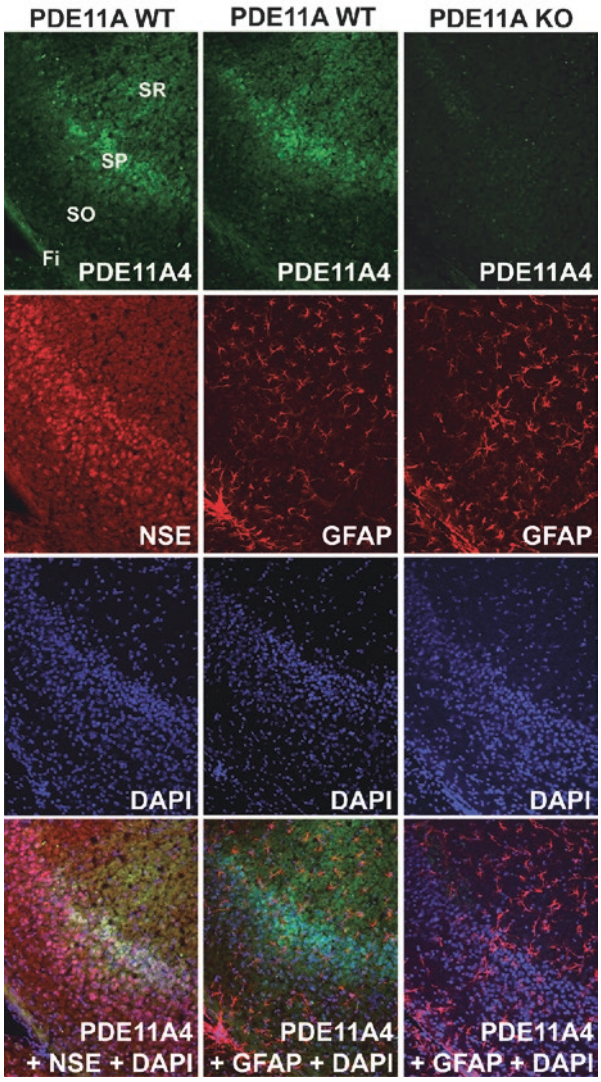


**Fig. 8.3** PDE11A4 protein expression within CA1 is found within a subset of neurons, not astrocytes. Immunofluorescence was conducted on sections from PDE11A wild-type (WT) and knockout (KO) mice collected ~2.76–2.88 mm lateral from Bregma. PDE11A protein expression (Fabgennix PD11-112 1:100) clearly colocalizes with a marker of neuronal cytoplasm (AVES neuronal specific enolase (NSE) 1:500) within a subset of neurons in CA1, and possibly CA2, stratum pyramidale (SP; i.e., cell body layer). Note: colocalization of *green* + *red* = *yellow*. PDE11A protein does not appear to colocalize with a marker of astrocytes (AVES glial acidic fibrillary protein (GFAP) 1:500) in SP, stratum radiatum (SR; i.e., the dendritic layer), stratum oriens (SO; i.e., axonal projection layer), or the fimbria (Fi; i.e., projecting axonal bundle). Images collected using a 20× objective. Histogram stretch and gamma were corrected to enhance the graphical clarity of the images. *Green*—PDE11A, *Blue*—DAPI nuclear stain, *Red*—either NSE or GFAP as indicated

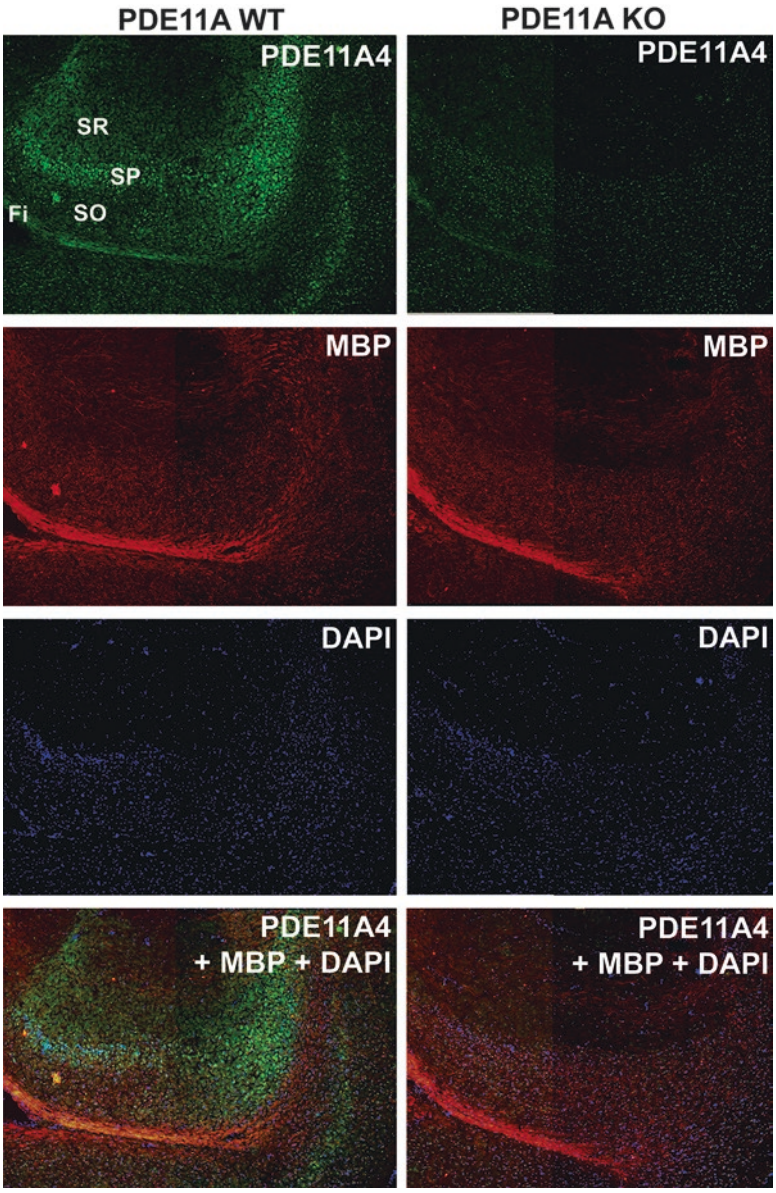


**Fig. 8.4** PDE11A4 protein expression is seen in the fimbria, a structure mostly composed of hippocampal efferents. Immunofluorescence was conducted on sections from PDE11A wild-type (WT) and knockout (KO) mice collected ~2.76 mm lateral from Bregma. PDE11A protein expression (Fabgennix PD11-112 1:100) is clearly found in the fimbria, as indicated by colocalization with a marker of myelin that labels axon bundles (AVES myelin basic protein (MBP) 1:100). Note: colocalization of *green* + *red* = *yellow*. Two images per section were collected using a 10× objective and then stitched together using Adobe Photoshop. Histogram stretch and gamma were corrected to enhance the graphical clarity of the images. *Green*—PDE11A, *Blue*—DAPI nuclear stain; *Red*—MBP

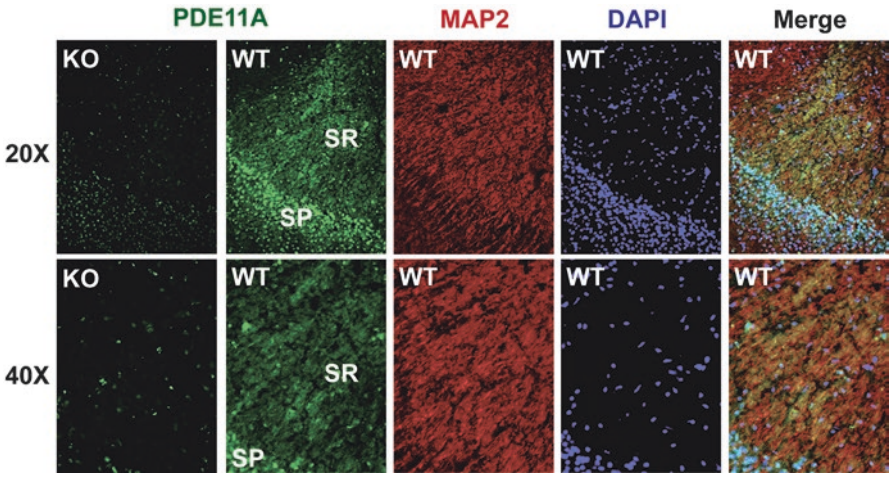


**Fig. 8.5** PDE11A4 protein expression is detected within a subset of dendrites in stratum radiatum. Immunofluorescence was conducted on sections from PDE11A wild-type (WT) and knockout (KO) mice collected ~3.00 mm lateral from Bregma. Punctate PDE11A protein expression (Fabgennix PD11-112 1:100) is clearly found in stratum pyramidale (cell body layer), as indicated by colocalization with a subset of DAPI staining. Fibril-like PDE11A labeling can be found in the stratum radiatum, a structure mostly composed of CA dendrites. Consistent with the fibril-like pattern of PDE11A staining in the stratum radiatum, PDE11A protein expression colocalizes with a marker of dendrites (Neuromics microtubule associated protein 2 (MAP2) 1:2000). Note: colocalization of *green* + *red* = *yellow*. Images were collected using a 20× or 40× objective, as indicated. Histogram stretch and gamma were corrected to enhance the graphical clarity of the images. *Green*—PDE11A, *Blue*—DAPI nuclear stain; *Red*—MAP2

Upon biochemical fractionation of the hippocampus, PDE11A4 can be found in nuclear, cytosolic and membrane compartments (both soluble and insoluble). This compartmentalization of PDE11A4 is modifiable in response to behavioral, genetic, and biochemical manipulations. Relative to single-housed mice, group-housed mice exhibit increased PDE11A4 protein expression in the soluble membrane compartment but not the cytosolic compartment of the VHIPP (Hegde et al. 2016b). Similarly, BALB/cJ mice express significantly more PDE11A4 protein than C57BL/6J mice in the soluble membrane but not cytosolic compartment of VHIPP (Pathak et al. 2016). Although it remains to be determined how social experience elicits such compartment-specific effects on PDE11A4 protein expression, the compartment-specific difference in PDE11A4 protein expression that is observed between C57BL/6J and BALB/cJ mice appears to be largely accounted for by a single point mutation at amino acid 499 within the GAF-B homodimerization domain (Pathak et al. 2016). In the C57BL/6J, 499 is a non-phosphorylatable alanine; however, in the BALB/cJ 499 is a phosphorylatable threonine (Pathak et al. 2016). We showed that the BALB/cJ 499T and corresponding phosphomimic mutation (T499D) shifts PDE11A4 from the cytosol to the membrane, relative to the C57BL/6J 499A, perfectly replicating the PDE11A4 protein expression differences noted between these mouse strains in VHIPP (Pathak et al. 2016). The ability of the

499T mutation to drive PDE11A4 into the membrane appears to be driven by an ability to increase homodimerization of PDE11A4, as 499T increases homodimerization of PDE11A4 relative to 499A and disrupting homodimerization has the opposite effect of the 499T mutation, shifting PDE11A4 from the membrane to the cytosol (Pathak et al. 2016). PDE11A4 can also be shifted between the cytosol and membrane in response to N-terminal phosphorylation events. We have shown that phosphorylation of serines 117 and 124 shifts PDE11A4 from the cytosol to the membrane, while phosphorylation of serine 162 keeps PDE11A4 in the cytosol (Pathak et al. 2016). The ability to therapeutically control the subcellular localization of PDE11A4 may become highly relevant as bipolar disorder, lithium responsiveness, and Alzheimer's disease have all been associated with subcellular compartment-specific differences in cyclic nucleotide signaling (Rahman et al. 1997; Fields et al. 1999; Chang et al. 2003; Mori et al. 1998; Jensen and Mork 1997; Casebolt and Jope 1991; Bonkale et al. 1999).

Not only is PDE11A4 expression restricted to the hippocampal formation, it is uniquely enriched three to tenfold in ventral versus dorsal hippocampus (Kelly 2015; Kelly et al. 2010; Hegde et al. 2016a; Kelly et al. 2014; Kelly 2014). The extent of the VHIPP versus DHIPP enrichment—that is, whether it is closer to three versus tenfold—depends on the mouse strain examined (Pathak et al. 2016). The ventral hippocampal formation in rodents is analogous to the anterior hippocampal formation in primates (Moser and Moser 1998). As such, we will use the term "ventral-anterior" hippocampal formation (VHIPP) and "dorsal-posterior" hippocampal formation (DHIPP) throughout the remainder of this chapter for consistent referencing of these anatomically and functionally distinct subregions across species.

The VHIPP and DHIPP are functionally, anatomically, and biochemically segregated (e.g., (Roman and Soumireu-Mourat 1988; Papatheodoropoulos and Kostopoulos 2000; Gusev et al. 2005; Bast and Feldon 2003; Fanselow and Dong 2010). While the VHIPP modulates sociality, emotion/affect, motivation, stress, behavioral flexibility, and sensorimotor gating, the DHIPP regulates learning, memory, contextual representation, and spatial navigation (c.f., Moser and Moser 1998; Bast and Feldon 2003; Fanselow and Dong 2010; Tseng et al. 2008; Behrendt 2011) also see (Roman and Soumireu-Mourat 1988; Marquis et al. 2008; Gruber et al. 2010). These separable functions are reflected in, if not driven by, differences in the extrahippocampal connectivity of VHIPP versus DHIPP. Whereas the VHIPP is highly interconnected with the prefrontal cortex, amygdala, olfactory bulb, hypothalamus, and the shell of the nucleus accumbens, the DHIPP is highly interconnected with thalamic subregions, sensory-related cortices, and the core of the nucleus accumbens (c.f., Bast and Feldon 2003; Fanselow and Dong 2010). Biochemical segregation of VHIPP versus DHIPP can easily be visualized when mapping expression of numerous gene products (Fanselow and Dong 2010), including PDE11A4 (Fig. 8.1). Consistent with its enrichment in the VHIPP, emerging evidence from human and rodent studies suggests PDE11A4 is a key regulator of social behaviors and mood stabilization.

## 8.4 A Role for PDE11A in Brain Function

Human studies to date largely implicate PDE11A dysfunction in mood disorders (Kelly 2015). Several studies associate PDE11A single nucleotide polymorphisms (SNPs) with major depressive disorder (Wong et al. 2006; Luo et al. 2009b; Cabanero et al. 2009) and 1 study associates an inactivating mutation in PDE11A with suicide risk (Coon et al. 2013). PDE11A has also been nominally associated with fluoxetine and desipramine response (Wong et al. 2006; Luo et al. 2009b), but not with citalopram or duloxetine response (Perlis et al. 2010; Cabanero et al. 2009). Whether the inconsistencies in the latter findings are related to differences in patient populations, differences in the specific antidepressants studied, or some unknown factor remains to be determined. PDE11A SNPs have also been associated with lithium responsiveness in both a retrospective and prospective cohort of patients with bipolar disorder (Couzin 2008; Kelsoe 2010). Further, lithium decreases PDE11A mRNA expression in IPSC-derived hippocampal neurons originating from lithium-responsive patients but not lithium-unresponsive patients (Mertens et al. 2015). As reviewed below, studies in rodents suggest PDE11A may be highly relevant to the social deficits that are associated with mood disorders, and may be particularly important for regulating lithium responsivity.

A link between PDE11A and lithium responsivity is consistent with studies that associate changes in the cAMP cascade with bipolar disorder (Rahman et al. 1997; Fields et al. 1999; Chang et al. 2003; Avissar et al. 1997; Avissar and Schreiber 2006; Schreiber and Avissar 1991; Schreiber et al. 1991; Alda et al. 2013; Dowlatshahi et al. 1999; Fatemi et al. 2010b; Fatemi et al. 2008), including cyclic nucleotide alterations restricted to specific subcellular compartments (Rahman et al. 1997; Fields et al. 1999; Chang et al. 2003) and changes associated with lithium responsivity (Alda et al. 2013; Sun et al. 2004). The link between PDE11A and lithium responsivity is also particularly intriguing in light of the study associating PDE11A SNPs and suicide risk (Coon et al. 2013) because lithium uniquely reduces suicide risk in patients with bipolar disorder (Malhi et al. 2013).

Deletion of PDE11A in mice elicits very selective behavioral deficits. PDE11A KO mice show no differences in basal anxiety- or depression-related behaviors (but see below for drug-induced changes in these behaviors), including elevated plus maze, stress-induced hyperthermia, four-plate, tail suspension test, and forced swim test (Kelly et al. 2010), as well as sucrose preference (WT, 80.3 ± 3.5%; KO, 78.2 ± 3.3%; n = 16-17/genotype; effect of genotype: $F(1,28) = 0.022$, $P = 0.883$). They also show no differences in contextual or cued fear conditioning or the motoric response to an acute dopaminergic challenge (Kelly et al. 2010). While single-housed PDE11A KO mice show increased locomotor activity in an open field and normal prepulse inhibition (PPI) of acoustic startle relative to WT littermates (Kelly et al. 2010), group-housed PDE11A KO mice show normal locomotor activity in an open field (WT,11,187 ± 455 cm; KO, 10,978 ± 486 cm; n = 21–25/genotype; effect of genotype ($F(1,41) = 0.098$, $P = 0.756$) and modestly lower PPI at the lowest

prepulse intensity relative to PDE11A WT mice (PPI at 4 db above background: WT, 16.49 ± 2.76%; KO, 3.85 ± 2.92%; n = 50–55/genotype; genotype × prepulse intensity: $F(2207) = 3.59$, $P = 0.029$; *Post hoc* WT vs. KO within 4 db above background, $P = 0.02$). The most notable and reliable phenotypes exhibited by PDE11A KO mice, however, are impairments in social behaviors (Kelly et al. 2010; Hegde et al. 2016a; Hegde et al. 2016b).

PDE11A is required for intact social interactions and is a key mechanism by which social experience, particularly social isolation, shapes the brain. Using Brodkin's version of the 3-chamber social approach assay, we found that deletion of PDE11A reduces social approach behaviors towards stranger mice but not towards cagemates (Kelly et al. 2010; Hegde et al. 2016b). The reduced social approach observed in PDE11A heterozygous (HT) and knockout (KO) mice appears somewhat sensitive to the effects of social buffering and depends on the social context (i.e., the strain of the stimulus mouse). Presence of a cagemate improves social approach behavior of PDE11A KO mice towards a novel mouse from the colony but not towards a novel C57BL/6J; whereas, presence of a cagemate improves social approach behavior of PDE11A HT mice towards a C57BL/6J but not a novel mouse from the colony (Hegde et al. 2016b). Other mice appear to be capable of detecting abnormalities in the social behavior of PDE11A KO mice, because male and female C57BL/6J spend significantly more time with a sex-matched PDE11A WT mouse vs its KO littermate (Hegde et al. 2016b), suggesting the PDE11A KO mice may be socially isolated. We believe this finding is particularly intriguing given the PDE11A SNP that has been associated with increased suicide risk in a Utah pedigree is an inactivating mutation (Coon et al. 2013). As noted above, social isolation decreases PDE11A4 protein expression within the membrane fraction of VHIPP samples relative to group-housed mice, and this isolation-induced decrease in PDE11A4 expression is sufficient to impair subsequent social behavior—both social approach and social memory formation (Hegde et al. 2016b). Consistent with these behavioral phenotypes, RNA sequencing of VHIPP from PDE11A KO and WT littermates shows that PDE11A regulates gene expression in the oxytocin signaling pathway (Hegde et al. 2016b), a key signaling cascade underlying social behaviors (c.f. Ebstein et al. 2012; Viero et al. 2010; Lukas and Neumann 2013; Stoesz et al. 2013).

We have also shown that PDE11A is required for the formation of social memories (Kelly et al. 2010; Hegde et al. 2016a). Although PDE11A KO mice show intact short-term memory for social odor recognition (SOR) or social transmission of food preference (STFP), PDE11A KO mice fail to show any long-term memory for SOR or STFP 24 h following training. Such a pattern suggests PDE11A KO mice retain the ability to learn and retrieve each memory but fail in their ability consolidate these memories to a long-lasting form (Hegde et al. 2016a). In contrast, PDE11A KO mice show perfectly intact contextual fear conditioning (Kelly et al. 2010) and non-social odor recognition memory 24 h after training (Hegde et al. 2016a), suggesting the SOR and STFP memory consolidation impairments are selective and due to the social nature of the stimuli employed. There are several possible explanations for why PDE11A KO mice exhibit such a selective memory deficit. As noted above, the social deficits present in the PDE11A mutant mice are consistent with the

fact that PDE11A4 is enriched in the VHIPP. The social deficits are also consistent with our RNA sequencing study showing that PDE11A KO mice exhibit significantly altered gene expression in the oxytocin pathway (Hegde et al. 2016b). Indeed, like our PDE11A KO mice, oxytocin KO mice similarly show an inability to form social memories while their ability to form non-social memories remains intact (Ferguson et al. 2000). The memory impairments observed in PDE11A KO mice may also be related to altered glutamatergic signaling. Previously, we showed that PDE11A KO mice are more sensitive to the hyperactivating effects of the NMDA receptor antagonist MK-801 (Kelly et al. 2010). We have also shown that deleting PDE11A impairs *de novo* protein synthesis, which is required to transition memory from a short-term to a long-term state. PDE11A KO mice show significantly reduced expression of the ribosomal S6 kinase 2 (RSK2) and phosphorylation of the ribosomal protein S6 at serines 235/236 relative to WT littermates (Hegde et al. 2016a). It will be of interest to future studies to determine which of these mechanisms accounts for social memory deficits in PDE11A KO mice and if those deficits can be reversed.

Interestingly, the manifestation of social memory deficits precedes the manifestation of social approach deficits in PDE11A mutant mice. That is, adolescent PDE11A KO mice already have an inability to form social long-term social memories (Hegde et al. 2016a) but show no differences in their social approach behavior, relative to WT littermates (Hegde et al. 2016b). This raises the interesting possibility that adolescent PDE11A mutant mice have a fundamental inability to form long-term memories for what is and what is not a socially-acceptable behavior, which ultimately leads to alterations in social approach behavior at a later age. Such a relationship has been observed in humans where impaired social skills early in development predict lower quality friendships in adolescents with intellectual disabilities (Tipton et al. 2013). It will be of interest to future studies to determine if social interaction deficits observed in adult PDE11A mutant mice are, in fact, directly related to their inability to form social memories.

As noted above, PDE11A has been genetically and functionally associated with lithium responsivity in patients with bipolar disorder (Couzin 2008; Mertens et al. 2015; Kelsoe 2010). In mice, we have shown that PDE11A4 negatively regulates lithium responsivity (Pathak et al. 2016). PDE11A4 mRNA and protein expression negatively correlate with lithium responsivity such that C57BL/6J mice that respond well to lithium exhibit lower levels of PDE11A4 expression in hippocampus than do BALB/cJ mice that respond poorly to lithium (Pathak et al. 2016). Further, using PDE11A mutant mice, we have shown that decreasing PDE11A expression is, in fact, sufficient to increase lithium response (Pathak et al. 2016). At face value, this may suggest that a PDE11A inhibitor might augment the therapeutic effects of lithium. Indeed, AKT—an enzyme whose activation is required for lithium's behavioral effects (Pan et al. 2011)—is predicted to phosphorylate PDE11A at serines 117 and 124 (Kelly 2015). As noted above, phosphorylation of S117/S124 would shift PDE11A4 towards the membrane compartment (Pathak et al. 2016), thereby removing it from the cytosol where AKT is selectively located. Shifting PDE11A from the cytosol to the membrane could serve to extend the activation of AKT (i.e., its phosphorylation),

because PDE11A4 would no longer be in a position to negatively regulate the cytosolic pools of cAMP that lead to phosphorylation of AKT in neurons via Epac (Nijholt et al. 2008). Of course, deleting PDE11A4 would artificially augment such feedforward signaling. That said, it is equally possible that lowered PDE11A expression may simply trigger a specific pathophysiology that happens to be particularly well-treated by lithium (Pathak et al. 2016). Indeed, both PDE11A KO mice and IPSC-derived hippocampal neurons from bipolar patients exhibit altered signaling in the neuroactive-ligand receptor pathway and increased neural activity (Kelly et al. 2010; Hegde et al. 2016b; Mertens et al. 2015). Further, decreasing PDE11A4 expression is sufficient to upregulate the proinflammatory cytokine IL-6 (Pathak et al. 2016), increased signaling of which has been repeatedly measured in patients with mania, depression, and suicidal ideation (Dowlati et al. 2010; Maes et al. 1997; Maes et al. 1995; Brietzke et al. 2009; Simon et al. 2008; Niculescu et al. 2015; Khandaker et al. 2014) and is reversed by lithium (Watanabe et al. 2014). Thus, studies in mice are most consistent with the notion that lowered levels of PDE11A trigger a specific pathophysiology that is readily treated by lithium.

PDE11A inactivating mutations have been identified in patients with adrenocortical tumors (Vezzosi et al. 2012; Horvath et al. 2006a; Horvath et al. 2006b; Libe et al. 2008; Libe et al. 2011) but see (Bimpaki et al. 2009; Louiset et al. 2010), so it is also worth noting that one study has examined the anterior hippocampus in patients with Cushing Syndrome, albeit not patients genotyped with regard to PDE11A mutations. Maheu and colleagues (Maheu et al. 2008) found heightened functional activation of the VHIPP during encoding of an emotional-faces recognition test in patients with Cushing Syndrome. As noted above, PDE11A KO mice show deficits in social odor recognition memory and social transmission of food preference (Kelly et al. 2010; Hegde et al. 2016a). PDE11A KO mice also show heightened activation of ventral CA1 relative to WT littermates, as indicated by increased expression of the activity-regulated immediate-early gene Arc (Kelly et al. 2010). In this context, it is important to note that our PDE11A KO mice do not appear to develop any adrenal tumor pathology (Kelly et al. 2010), consistent with the fact that the mouse adrenal gland does not express PDE11A (Kelly 2015), suggesting it is the loss of PDE11A4 in the brain, as opposed to adrenal dysfunction, that is driving the neurocognitive deficits of PDE11A KO mice. Studies in patients with Cushing Syndrome have correlated overall hippocampal function with dysregulated cortisol levels (Starkman et al. 1999; Starkman et al. 1992; Forget et al. 2000; Starkman et al. 2001); however, a potential role for aberrant PDE11A signaling within the VHIPP has not yet been examined. Unfortunately, to our knowledge, hippocampal function has not been assessed in patients genotyped with PDE11A inactivating mutations. Thus, it remains to be determined whether hippocampal deficits observed in patients with Cushing Syndrome are simply related to the indirect effects of rising peripheral cortisol levels or the direct effects of PDE11A inactivation within the hippocampus.

Ectopic PDE11A expression in brain regions outside of the hippocampus may also be an important facet of disease pathophysiology. Fatemi and colleagues reported an increase in PDE11A mRNA expression in the cerebellum of patients

with bipolar disorder relative to controls (Fatemi et al. 2010a). We are unable to detect PDE11A expression in cerebellum taken from healthy humans or rodents, suggesting that a disease state may drive ectopic expression of PDE11A outside of the hippocampal formation (Kelly 2015). Our recent findings and ongoing studies in the lab suggest that aging drives ectopic expression of PDE11A4 outside of the hippocampal formation (Kelly et al. 2014), with negative consequences. If this holds true, it may be important to not simply modulate catalytic activity of PDE11A4 but rather to restore proper physiological control over the mechanisms controlling PDE11A4's unique tissue-specific expression profile.

## 8.5 PDE11A Pharmacological Tools

As extensively reviewed elsewhere (Kelly 2015), progress has been made in identifying pharmacological activators and inhibitors of PDE11A catalytic activity. Jager and colleagues used an in vitro FRET-based assay to screen compounds for their ability to bind to and initiate a conformational change in a fragment of PDE11A4 that included both GAF domains (Jager et al. 2012). While cAMP failed to bind the PDE11A4 GAF construct, both cGMP and the cGMP analog Rp-8-pCPT-PET-cGMPS did (Jager et al. 2012). In a catalytic activity assay, cGMP failed to stimulate PDE11A4 catalytic activity. In contrast, Rp-8-pCPT-PET-cGMPS stimulated PDE11A catalytic activity of both recombinant human PDE11A4 as well as native mouse PDE11A4 enriched from hippocampus approximately four to fivefold (Jager et al. 2012). Interestingly, Rp-8-pCPT-PET-cGMPS failed to activate PDE11A when the 196 amino acids upstream of the PDE11A4 GAF-A domain were deleted (Jager et al. 2012). This suggests that it will be important for PDE11A compound screens to employ full-length PDE11A4, as opposed to some truncated version of the protein. Although Rp-8-pCPT-PET-cGMPS does not appear to bind the GAF domains of PDE5A or PDE2A (Jager et al. 2010), suggesting specificity in its ability to target the GAF domains of PDE11A4, it is well known to have significant off-target activities, including inhibition of PKG and cGMP-gated ion channels (see product insert for Rp-8-pCPT-PET-cGMPS on www.biolog.de). Although this off-target activity severely limits the use of Rp-8-pCPT-PET-cGMPS as a tool PDE11A activator, this study provides critical proof-of-concept that it is possible to stimulate PDE11A4 catalytic activity.

Ceyhan *et al.* identified four PDE11A4 inhibitors using a yeast-based high-throughput assay (Ceyhan et al. 2012). All four compounds were fairly potent (IC50s = 0.11–0.33 μM) and BC11-28 and BC11-38, in particular, were highly selective for PDE11A4 versus other PDE families (>350-fold selective for PDE11A) (Ceyhan et al. 2012). One strength of this screen was the fact that it employed full-length PDE11A4, as opposed to an isolated catalytic domain; therefore, these compounds are likely to inhibit the native protein. That said, because the screen was conducted with the full-length PDE11A4, it is not possible to determine whether the inhibitors compete for the substrate binding pocket or modulate catalytic activity

via binding to an allosteric site (e.g., the GAF-A domain) (Ceyhan et al. 2012). PDE11A cGMP hydrolytic activity was inhibited by all four compounds in both the yeast-based assay and an enzyme assay. In an H295R cell-based assay, however, only BC11-38 was able to inhibit PDE11A cAMP hydrolytic activity (Ceyhan et al. 2012). The fact that all four compounds were able to inhibit cGMP-PDE11A activity in the yeast-based assay but only one was able to inhibit cAMP-PDE11A activity in the H295R-based assay may simply be related to differential availability of the four PDE11A inhibitors in the two different media employed in the respective assays (i.e, differences in solubility or freely available compound) (Ceyhan et al. 2012). An alternative possibility, reviewed elsewhere in great detail (Kelly 2015), is that certain compounds may be functionally selective in their ability to inhibit cAMP-PDE11A versus cGMP-PDE11A catalytic activity. While many compounds, such as IBMX, zaprinast, dipyrimadole, and a few PDE5A-preferring inhibitors, inhibit cAMP-PDE11A and cGMP-PDE11A activity with equal potency, several other PDE5A-preferring inhibitors require much higher concentrations to inhibit the cAMP-hydrolyzing activity of PDE11A versus its cGMP-hydrolyzing activity (Yuasa et al. 2000; Ahmed et al. 2011; Ahmed et al. 2012; Mohamed et al. 2011). Clearly the BC compounds are membrane-penetrant; however, their ability to cross the blood-brain-barrier has not been determined. Indeed no studies have yet reported in vivo activities of the compounds or their pharmacokinetic profiles. Although it remains to be determined whether these PDE11A inhibitors will prove useful for in vivo experiments, their identification certainly proves it is possible to inhibit PDE11A with a high degree of selectivity versus other PDEs.

## 8.6 Future Considerations for PDE11A Research

In recent years we have learned much about PDE11A4 function in the brain, but many questions remain. Considering the number of conflicting findings in the field regarding the tissue expression profile of PDE11A, it will be critical to continue carefully assessing which tissues and cell types express the various PDE11A isoforms, and how those expression patterns may change with age or disease state. Given how dramatically PDE11A4 expression increases in the hippocampus across the lifespan (Kelly et al. 2010; Kelly et al. 2014), the "when and where" of PDE11A4 expression will be critically important when evaluating the efficacy and side effect potential of a PDE11A4-targeted therapeutic. It will also be key in understanding whether PDE11A4 may be considered a therapeutic target in the context of neurodevelopmental, adulthood, or age-related disease. Although emerging evidence shows that phosphorylation events and homodimerization can alter the subcellular compartmentalization of PDE11A4 (Pathak et al. 2016; Pathak et al. 2015), it is not yet clear if these mechanisms also affect catalytic activity and/or whether they can be therapeutically exploited to restore aberrant subcellular localization of the enzyme (e.g., in the case of social isolation; Hegde et al. 2016b). That said, the GAF domains are highly intriguing from a drug target perspective because they are found

in no other mammalian proteins other than PDEs (Francis et al. 2011), and the PDE11A4 GAF domains are less than 50% homologous to those in other PDE families (Viero et al. 2010). This, together with the fact that PDE11A4 is the only PDE11A isoform with a full GAF-A domain, suggests it might not only be possible to selectively target PDE11A4 over other PDE families, but also selectively target PDE11A4 relative PDE11A3, A2 or A1. With such specific targeting, it might be possible to reduce, if not eliminate, side effect liability associated with targeting other PDEs or PDE11A signaling outside the brain. Finally, we need to gain a far better understanding of signaling events that lie upstream and downstream of PDE11A, and how coupling to those signaling events may change with age, disease, and acute versus chronic manipulations.

## 8.7 Summary

Evidence is mounting that PDE11A critically regulates select aspects of brain function. Our studies in PDE11A KO mice show that PDE11A plays a crucial role in the formation of social memories and lithium responsiveness and is a key mechanism by which social experience feeds back to shape the brain (Kelly et al. 2010; Hegde et al. 2016a; Pathak et al. 2016; Hegde et al. 2016b). These finding are consistent with the fact that human studies associate PDE11A with lithium responsiveness, MDD, and suicide risk (Couzin 2008; Wong et al. 2006; Mertens et al. 2015; Luo et al. 2009b; Cabanero et al. 2009; Coon et al. 2013; Kelsoe 2010; Aitchison et al. 2009). As extensively reviewed elsewhere (Kelly 2015), there are a large number of studies reporting VHIPP dysfunction as well as cyclic nucleotide dysregulation in patients with neuropsychiatric disease, particularly schizophrenia and Alzheimer's disease (Lee et al. 2004; Pegues et al. 2003; Suddath et al. 1990; Rametti et al. 2007; Schobel et al. 2009a; Shenton et al. 1992; Nesvaderani et al. 2009; Ghose et al. 2009; Zhou et al. 2008; Goldman et al. 2011; Schobel et al. 2009b; Jessen et al. 2003; Hall et al. 2010; Rajarethinam et al. 2001; Rusch et al. 2008; Laakso et al. 2000; Leube et al. 2008; Jansen et al. 1993; Quiroz et al. 2010; Yakushev et al. 2011a; Yakushev et al. 2011b; Yakushev et al. 2010; Rahman et al. 1997; Avissar et al. 1997; Garver et al. 1982; Kafka et al. 1979; Kafka et al. 1986; Kanof et al. 1986; Kafka and van Kammen 1983; Ofuji et al. 1989; Kaiya 1992; Kaiya et al. 1990; Kanof et al. 1989; Kang 1990; Kanof et al. 1987; Bowers and Study 1979; Belmaker et al. 1978; Gattaz et al. 1983; Ebstein et al. 1976; Turetsky and Moberg 2009; Memo et al. 1983; Avissar et al. 2001a; Avissar et al. 2001b; Edmunds et al. 2008; Young et al. 1991; Young et al. 1993; Young et al. 1994; Cowburn et al. 1994; Gurguis et al. 1999a; Gurguis et al. 1999b; Gurguis et al. 1997; Bonkale et al. 1996; Shanahan et al. 1997; Yamamoto et al. 2000; Fowler et al. 1995; Ohm et al. 1989; Baltrons et al. 2004; Ohm et al. 1991; Martinez et al. 1999). Certainly, a PDE11A-targeted therapeutic would be positioned to address both. Clearly, future studies should determine which, if any, neuropsychiatric disorders are associated with alterations in PDE11A expression, localization, and/or catalytic activity or if a

PDE11A-targeted therapeutic could compensate for upstream or downstream insults (e.g., at the AMPA receptor). Clearly more studies examining PDE11A in patient tissue are needed to drive a therapeutic indication for a PDE11A modulator, but we believe the data to date suggest PDE11A holds potential as a future therapeutic target (Kelly 2015).

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

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# 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

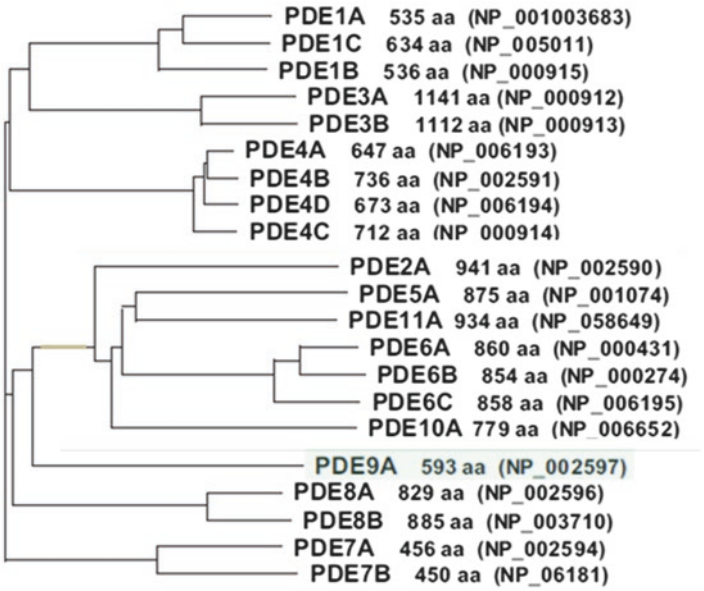



**Fig. 9.1** Phylogenetic tree of human PDE families, adapted from Omori and Kotera 2007

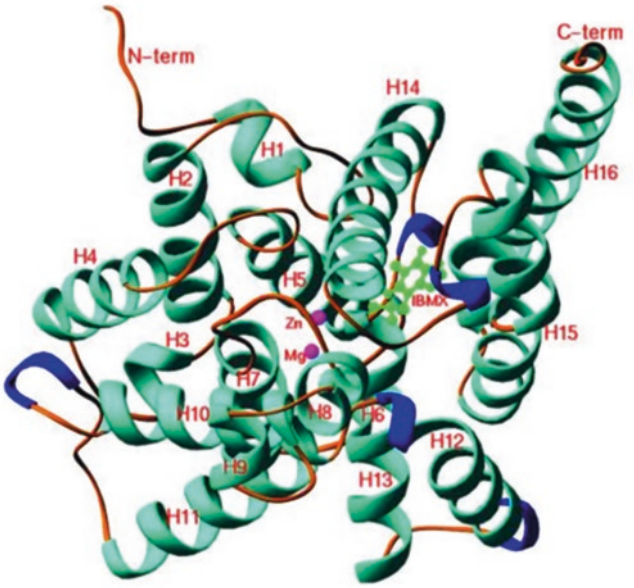



**Fig. 9.2** X-ray structure of PDE9, adapted from Huai et al. 2004

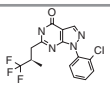
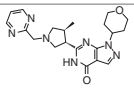
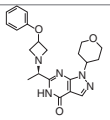
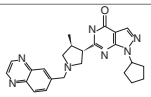
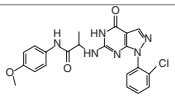
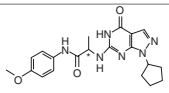
presumably is zinc as in other PDEs, the nature of the second ion however is unknown. Biochemical studies have shown that *in vitro* manganese ($Mn^{2+}$) activated PDE9 twice as much as magnesium or calcium (Fisher et al. 1998). Maximal activity of PDE9 is achieved at $Mn^{2+}$ concentration of 1–10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

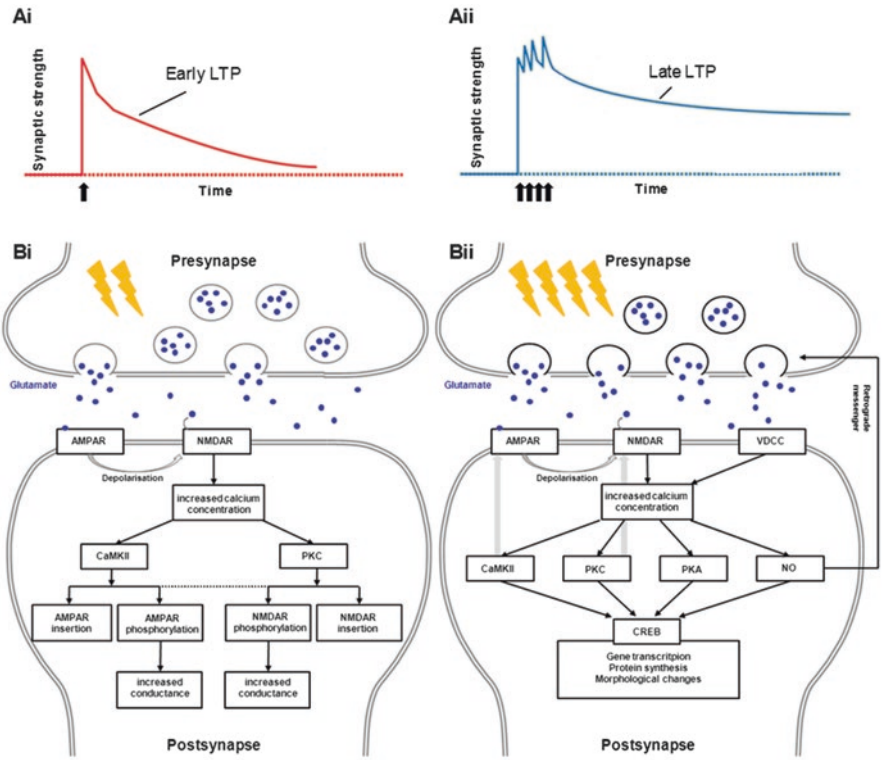



**Fig. 9.3** Mechanisms underlying early and late LTP in the CA1 region. (**a**) The durations of early (i) and late (ii) LTP are shown schematically. *Arrow/s* indicate/s the different stimulation protocols. Adopted from Huang 1998. (**b**) Schematic drawing of the mechanisms of early (i) and late (ii)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

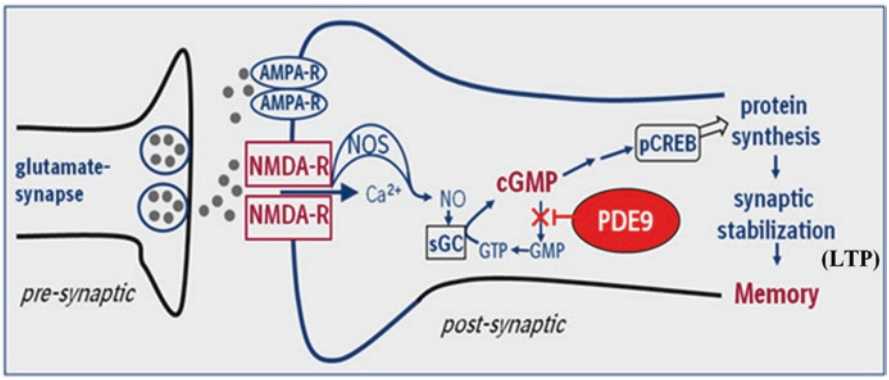



**Fig. 9.4** Putative mechanism of action of PDE9 inhibition for strengthening synaptic plasticity

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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# Part III
# PDEs in Parkinson's and Huntington's Diseases

# Chapter 10
# Regulation of Striatal Neuron Activity by Cyclic Nucleotide Signaling and Phosphodiesterase Inhibition: Implications for the Treatment of Parkinson's Disease

**Fernando E. Padovan-Neto and Anthony R. West**

**Abstract** Cyclic nucleotide phosphodiesterase (PDE) enzymes catalyze the hydrolysis and inactivation of cyclic nucleotides (cAMP/cGMP) in the brain. Several classes of PDE enzymes with distinct tissue distributions, cyclic nucleotide selectivity, and regulatory factors are highly expressed in brain regions subserving cognitive and motor processes known to be disrupted in neurodegenerative diseases such as Parkinson's disease (PD). Furthermore, small-molecule inhibitors of several different PDE family members alter cyclic nucleotide levels and favorably enhance motor performance and cognition in animal disease models. This chapter will explore the roles and therapeutic potential of non-selective and selective PDE inhibitors on neural processing in fronto-striatal circuits in normal animals and models of DOPA-induced dyskinesias (LIDs) associated with PD. The impact of selective PDE inhibitors and augmentation of cAMP and cGMP signaling on the membrane excitability of striatal medium-sized spiny projection neurons (MSNs) will be discussed. The effects of cyclic nucleotide signaling and PDE inhibitors on synaptic plasticity of striatonigral and striatopallidal MSNs will be also be reviewed. New data on the efficacy of PDE10A inhibitors for reversing behavioral and electrophysiological correlates of L-DOPA-induced dyskinesias in a rat model of PD will also be presented. Together, these data will highlight the potential of novel PDE inhibitors for treatment of movement disorders such as PD which are associated with abnormal corticostriatal transmission.

**Keywords** Phosphodiesterase • cAMP • cGMP • Dopamine • Striatum • Medium spiny neuron • Parkinson's disease • L-DOPA-induced dyskinesia

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## 10.1 Cyclic Nucleotide Synthesis

Neurotransmitters in the central nervous system (CNS) such as norepinephrine, dopamine (DA), adenosine, and others exert their actions via heterotrimeric membrane-bound G-protein coupled receptors (GPCRs). Heterotrimeric G proteins are composed of α, β, and γ subunits and in the inactive form, the α subunit is bound to guanosine diphosphate (GDP) resulting in an inactive αβγ-complex (Gilman 1987). Neurotransmitter-induced activation of GPCRs dissociates the αβγ-complex and starts a biochemical reaction that modifies target proteins and initiates a series of intracellular signaling cascades. These signaling cascades are linked to the regulation of second messenger synthesis and degradation. The first second messenger to be discovered was 3′-5′-cyclic monophosphate (cAMP) (Berthet et al. 1957). Today, we know that many other molecules including cyclic guanosine monophosphate (cGMP), nitric oxide (NO), and calcium are second messengers in the CNS with important functions in a variety of biological processes (Seifert et al. 2015). Adenylyl cyclase (AC) is the effector enzyme that triggers the generation of cAMP via adenosine triphosphate (ATP). A total of nine isoforms of membrane-bound AC and one isoform of soluble AC synthesize cAMP. AC can be modulated by signals other than GPCRs like calcium and protein kinases, suggesting that cAMP production is complex and requires the integration of extracellular and intracellular signals (Mons et al. 1998; Seifert et al. 2015). The distinct localization of ACs in neuronal sub-compartments likely results in cAMP production within spatially confined distribution zones which are functionally coupled to various regulatory processes. A variety of GPCRs are coupled to membrane-bound AC and the production of cAMP is facilitated by neurotransmitter-induced activation of stimulatory ($G_s$, $G_{olf}$) GPCRs (e.g., norepinephrine (NE) β-adrenergic receptors, DA D1-like receptors, adenosine A2A receptors) and inhibited by neurotransmitter coupling to inhibitory ($G_i$) GPCRs (e.g., DA D2-like receptors, M4 muscarinic receptors). cAMP modulates many physiological processes via activation of protein kinase A (PKA) and cyclic nucleotide-gated channels (CNGC) (Fig. 10.1).

The next second messenger to be identified was cGMP (Ashman et al. 1963). Synthesis of cGMP occurs via activation of either membrane-bound particulate guanylyl cyclase (pGC) or soluble guanylyl cyclase (sGC) by natriuretic peptides and the neuromodulator NO, respectively (Boehning and Snyder 2003; Bredt 2003; Bredt et al. 1990; Garthwaite 2008). NO is a gaseous free radical with an unpaired electron that does not require energy to disperse through the cells. Due to its short half-life, NO signaling is limited to the modulation of biochemical processes confined within 10–20 cell diameters (i.e. 100–300 μm) (Murad 2011). NO can be produced by three different isoforms of NO synthase (NOS; neuronal NOS (nNOS), inducible NOS (iNOS), endothelial NOS (eNOS)) (Alderton et al. 2001; Garthwaite 2008). Unlike other classical neurotransmitters, NO is not stored in vesicles and upon release can act on both pre- and post-synaptic terminals to modulate synaptic plasticity, including short and long-term changes in the efficacy of excitatory and inhibitory synaptic transmission (see below). In the CNS, the NO-sensitive sGC is

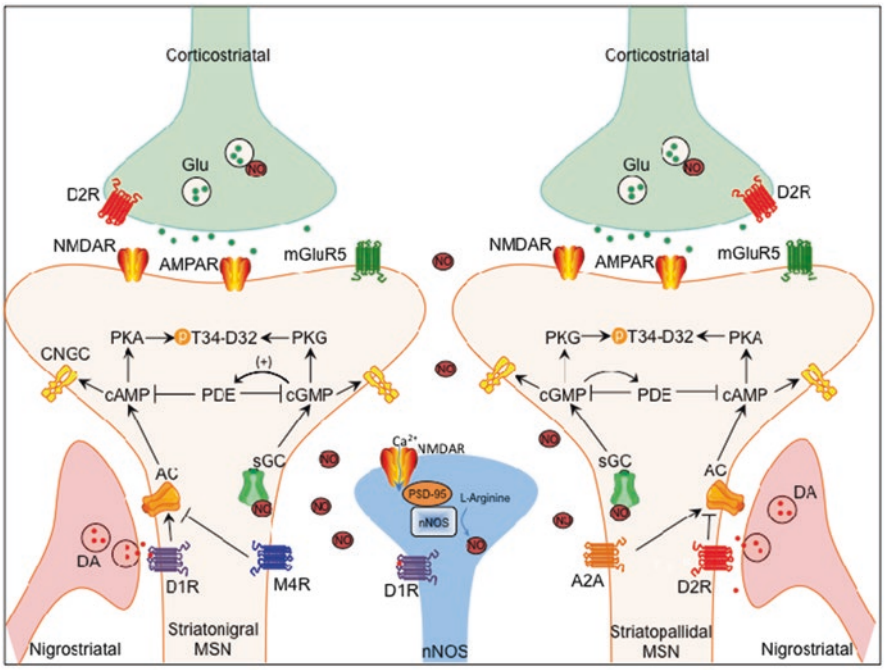


**Fig. 10.1** Schematic diagram of the roles of DA-AC-cAMP-PKA and NO-sGC-cGMP-PKG signaling and PDE function in the regulation of MSN membrane excitability. DA released from nigrostriatal terminals binds to D1-like or D2-like DA receptors on the postsynaptic MSN and leads to either stimulation or inhibition of AC, respectively. Increases in cAMP tone will activate PKA, which can phosphorylate DARPP-32 at threonine 34 to have complex effects on downstream signaling pathways, neurotransmitter receptors, and voltage-gated ion channels. cAMP (along with cGMP) can also stimulate CNGCs in the plasma membrane which can lead to cation influx and membrane depolarization. Tonic NO signaling increases glutamatergic transmission across corticostriatal synapses potentially via nitrosylation of presynaptic release proteins or a sGC-cGMP-dependent mechanism. NO transmission can also activate sGC in the postsynaptic MSN and stimulate cGMP production. cGMP can activate CNGC, stimulate PDEs, or activate PKG. Transient activation of NO-sGC-cGMP signalling in the intact striatum increases the responsiveness of MSNs to excitatory glutamatergic drive and facilitates short-term potentiation of corticostriatal synaptic transmission. Together these studies suggest that like DA D1/5 receptor modulation, the net impact of NO-sGC-cGMP-PKG signalling on membrane excitability may depend on the steady-state membrane potential of MSNs and interactions with glutamateric drive and NMDA receptor activation (modified from Threlfell and West 2013)

the major receptor for NO. NO binds to the heme domain on sCG and increases enzyme activity approximately 200–400-fold (Stone and Marletta 1994). sGC catalyzes the conversion of guanosine 5′-triphosphate (GTP) to cGMP. cGMP modulates many biological processes via activation of cGMP-gated ion channels, cGMP-dependent protein kinases (protein kinase G, PKG), cyclic nucleotide-dependent phosphodiesterases (PDEs) and others (Fig. 10.1).

## 10.2 Impact of Striatal Dopamine Signaling on Striatal Neuronal Excitability

In the striatum, five GPCRs (D1–D5) regulate cAMP production through DA signaling. D1-like receptors (D1 and D5) stimulate $G_{s/olf}$ proteins, allowing the $\alpha_s$ subunit to dissociate from the βγ-complex and stimulate AC to produce cAMP from ATP. D2-like receptors (D2, D3 and D4) stimulate $G_i$ proteins and the $\alpha_i$ subunit inhibits AC and reduces cAMP production. It is now accepted that there is a segregation of dopaminergic receptors and peptide neurotransmitters in striatonigral and striatopallidal GABAergic medium-sized spiny projection neurons (MSNs). Although there is some degree of coexpression (Perreault et al. 2010), the majority of striatonigral MNSs exclusively express D1 dopaminergic receptors, as well as the neuropeptides dynorphin, and substance P, whereas the majority of the striatopallidal MSNs express D2 dopaminergic receptors and enkephalin (Gerfen and Young 1988). Therefore, upon activation of D1- or D2-like receptors, DA can increase or decrease the production of cAMP inducing opposite effects on signaling pathways in striatonigral and striatopallidal MSNs. cAMP can affect numerous downstream signaling pathways via the activation of PKA. Once activated, PKA has numerous cellular targets including voltage-gated ion channels, transcription factors, glutamate receptors and the DA- and cAMP –regulated phosphoprotein MW 32 kDA (DARPP-32) (Greengard et al. 1999; Hemmings et al. 1984). DA facilitates corticostriatal transmission via activation of D1-like receptors and stimulation of postsynaptic AC-cAMP-PKA signaling. Conversely, D2-like receptor activation produces the opposite effect in part by suppressing AC-cAMP-PKA signaling cascades in both pre- and postsynaptic elements.

DARPP-32 is a potent inhibitor of protein phosphatase-1 (PP-1) which is involved in the regulation of AMPA and NMDA receptor function and trafficking, expression of transcription factors such as CREB and ΔFosB, and neural plasticity (Svenningsson et al. 2004). DARPP-32 is expressed in areas receiving massive dopaminergic nerve terminals from the substantia nigra compacta (SNc) and ventral tegmental area (VTA), such as the striatum and nucleus accumbens, respectively (Hemmings and Greengard 1986; Ouimet et al. 1992, 1998). Immunohistochemical studies demonstrated that DARPP-32 is expressed in the perikarya including the dendritic spines, axon terminals, and the nucleus (Ouimet and Greengard 1990). Given that DARPP-32 is one of the primary targets of the cAMP-PKA signaling pathway, the cellular localization of this protein suggests that cAMP can regulate important cellular processes through DARPP-32. Via PKA activation, cAMP can transfer the extracellular pre-synaptic signal to DARPP-32 proteins located in the axon terminals and impact on the signal output of neurons and regulate long-term neural plasticity processes such as long-term depression (LTD) and long-term potentiation (LTP) (Calabresi et al. 2000). In the nucleus, DARPP-32 may also participate in the regulation of gene expression by increasing the phosphorylation of histone H3 (Stipanovich et al. 2008). cAMP-PKA signaling also stimulates cAMP

response element binding protein (CREB) phosphorylation, which regulates the expression of immediate early genes (IEGs) including members of the Fos and Jun family (Svenningsson et al. 2004).

## 10.3 Role of Striatal NO-cGMP Signaling in the Modulation of Striatal Neuron Excitability

NO is a gaseous neuromodulator implicated in the regulation of numerous physiological and pathophysiological processes in both the peripheral and central nervous system (Garthwaite 2008). NO is produced in the striatum by a subclass of GABAergic interneurons that co-express nNOS, neuropeptide Y and somatostatin (Kawaguchi et al. 1995). The synthesis of NO requires concurrent NMDA and DA D1 receptor activation (West and Tseng 2011). These interneurons are aspiny having 12–25 mm in diameter, fusiform or polygonal somas, and comprise less than 3% (~21,000 cells) of the total neuronal population of the striatum (West et al. 1996). The NO effector enzyme sGC is highly expressed in MSNs of both the direct and indirect pathways (Ariano 1983; Ding et al. 2004) and its activity is reported to be higher in the striatum than in any other region of the brain (Hofmann et al. 1977; Matsuoka et al. 1992). The nNOS-NO-sGC-cGMP-PKG pathway can phosphorylate DARPP-32 on the amino acid threonine 34 (Thr-34) (Tsou et al. 1993; Calabresi et al. 2000) and affect many other molecular signaling pathways. Studies performed in brain slices from rats and mice have shown that the nNOS-NO-sGC-cGMP-PKG signaling pathway mediates a rapid, transient increase in DARPP-32 phosphorylation at Thr-34 residue in striatal MSNs that is dependent on glutamatergic stimulation of NMDA, AMPA and metabotropic glutamate subtype 5 receptors, as well as an increase in intracellular calcium levels (Nishi et al. 2005).

Early work by our group demonstrated that intrastriatal infusion of the NO generator SNAP increased firing rate and burst duration of striatal neurons (West et al. 2002). On the other hand, systemic administration of NOS inhibitors decreased spontaneous firing activity of striatal MSNs and attenuated striatal NO efflux evoked by train stimulation of the frontal cortex (Ondracek et al. 2008). Intrastriatal infusion of the NO scavenger CPT-I0 decreased the responsiveness of striatal neurons to intracellular current injection (West et al. 2002), and also decreased the amplitude of excitatory postsynaptic potentials (EPSPs) evoked during electrical stimulation of the prefrontal cortex (West and Grace 2004). Consistent with this, we have recently demonstrated that genetic disruption of nNOS decreased the subpopulation of striatal neurons that exhibited spontaneously firing activity by approximately 50%, suggesting that tonic NO-cGMP levels play an important role in facilitating MSN activity in the striatum (Padovan-Neto et al. 2015b). Additionally, cortically-evoked responses were depressed in nNOS knockout mice as compared to wild type controls, showing that NO-cGMP signaling mediates synaptic facilitation during stimulation of corticostriatal afferents (Padovan-Neto et al. 2015b). Similar effects

were observed following manipulation of sGC activity. Thus, systemic administration of the sGC inhibitor ODQ reduced cortically-evoked MSN spike activity (Sammut et al. 2010) and the non-specific NOS/sGC inhibitor methylene blue attenuated striatal NO efflux evoked by train stimulation of the frontal cortex (Sammut et al. 2007). Taken together, these findings demonstrate that in the intact system, striatal NO signaling enhances the membrane excitability of striatal MSNs and facilitates corticostriatal transmission via a cyclic nucleotide-dependent mechanism (Padovan-Neto et al. 2015b; West and Grace 2004).

## 10.4 Phosphodiesterase Control of Striatal Neuronal Excitability

Numerous studies have shown that under physiological conditions, elevations in cyclic nucleotide levels facilitate MSN excitability (Threlfell and West 2013). Corticostriatal transmission is facilitated by drugs that increase cAMP and cGMP synthesis as well as agents which decrease their degradation (e.g., PDE inhibitors or cyclase activators). Conversely, corticostriatal transmission is depressed by drugs that produce opposite effects (e.g., cyclase inhibitors). Early studies using brain slice preparations demonstrated that bath application of the AC activator forskolin or a PKA activator enhanced the amplitude and duration of EPSPs evoked by electrical stimulation of cortical fibers, suggesting that AC-cAMP-PKA signaling pathway participates in the regulation of glutamatergic excitatory synaptic transmission in the striatum (Colwell and Levine 1995).

In agreement with the above observations, boosting intracellular cyclic nucleotide levels with PDE inhibitors or cGMP analogues (e.g. 8-Br-cGMP) has a facilitatory effect on membrane excitability and responsiveness of MSNs to corticostriatal inputs (West and Grace 2004; Threlfell et al. 2009; Sammut et al. 2010; Padovan-Neto et al. 2015b). The intracellular application of the PDE inhibitor zaprinast (non selective inhibitor of PDEs 5, 6, 9, 10 and 11) induced longer up-state durations compared with controls, an effect that was also observed with intracellular injection of cGMP (West and Grace 2004). In addition to this, zaprinast robustly depolarized the membrane of MSNs and increased the spontaneous spike activity of these cells (that is driven by glutamatergic inputs) (West and Grace 2004). Likewise, inhibition of intracellular cyclic nucleotide metabolism with the PDE10A inhibitor papaverine increased the duration of the depolarized up states and depolarized the average steady-state membrane potential (Threlfell and West 2014). Also, inhibition of PDE10A with papaverine or TP-10 increased responsiveness of MSNs to cortical inputs (Threlfell et al. 2009; Padovan-Neto et al. 2015b). This facilitatory effect of PDE10A inhibition was abolished via local sGC inhibitor infusion and absent in nNOS knockout mice (Padovan-Neto et al. 2015b). Taken together, these results indicate that PDE10A activity may act to dampen asynchronous or weak cortical input so that only strongly coherent corticostriatal transmission is capable of

producing spike activity in MSNs. Thus, attenuation of this filtering capacity of PDE10A with selective inhibitors is expected to increase intracellular cAMP/cGMP tone, and therefore the responsiveness of MSNs to glutamatergic corticostriatal transmission. Given its key role in controlling corticostriatal drive, manipulating the filtering capacity of PDE10A using pharmacological tools represents a promising strategy for treating neurodegenerative disorders like Parkinson's disease (PD), while perhaps, minimizing the side effects of DA-replacement therapies (see below).

## 10.5 Phosphodiesterase Expression and Control of Striatal Striatonigral and Striatopallidal Projection Pathways

PDEs are subdivided into 11 families and encoded by 21 genes resulting in more than 100 functionally distinct enzymes produced by alternate splicing (Menniti et al. 2006; Conti and Beavo 2007). The dual substrate enzymes PDE1B, PDE2A, and PDE10A are all highly enriched in the striatum. The cAMP specific enzymes PDE3A, PDE3B, PDE4D, PDE7B, and PDE8B are also prominently expressed in striatum. The cGMP specific enzyme PDE9A is moderately expressed in the striatum (Bender and Beavo 2006). PDEs have a significant modulatory role in second-messenger signaling due to their critical metabolic control over cAMP and cGMP. The large number of PDE isoforms expressed in striatum allows for precise temporal and spatial control over the function of individual MSNs, thereby enabling the coordination of electrical and chemical activity in the striatonigral and striatopallidal subpopulations of MSNs. The following sections will review our current state of knowledge on the role of PDE isoforms in the regulation of striatal processing and MSN output.

### *10.5.1 Dual-Substrate Phosphodiesterases*

**PDE1B:** PDE1B is soluble and is abundantly expressed in the striatum (Polli and Kincaid 1994). PDE1B knockout mice have enhanced exploratory activity (Reed et al. 2002) and also increased DA turnover (Siuciak et al. 2007). The administration of the DA D1 receptor agonist SKF81297 to striatal slices obtained from PDE1B knockout mice increased phosphorylation of DARPP-32 at Thr34 and the GluR1 AMPA receptor at Ser845 (Reed et al. 2002). These findings suggest that PDE1B acts to filter cyclic nucleotide signaling mediated by DA D1 receptor stimulation in striatonigral MSNs. This filtering property of PDE1B is lost in the knockout mice and the abnormal cAMP signal amplification may result in the hyperphosphorylation of downstream target signaling proteins and increased activation of the direct striatonigral (i.e., go) pathway.

**PDE2A:** The PDE2A family (PDE2A1 – soluble; PDE2A2 and PDE2A3 – membrane bound) is abundantly expressed in the mammalian forebrain with particularly strong immunoreactivity exhibited in the striatum (Russwurm et al. 2009; Stephenson et al. 2009). PDE2A knockout mice cannot be used for behavioral studies because they die at gestational day 17–18 (Stephenson et al. 2009). Recent experiments have demonstrated however, that selective PDE2A inhibitors increased cAMP levels in response to stimulation of AC with forskolin, specifically in striatopallidal MSNs, whereas no effect was observed in striatonigral MSNs (Polito et al. 2013). This dichotomy disappeared when the broad-spectrum PDE inhibitor IBMX was administered, suggesting that at least one other PDE plays a dominant role in cAMP degradation in striatonigral MSNs (Polito et al. 2013). Therefore, although PDE2A is expressed similarly in both striatonigral and striatopallidal MSNs, it is likely that this enzyme controls cAMP metabolism preferentially in striatopallidal MSNs.

PDE2A is also regulated by cGMP signals in striatal MSNs (Polito et al. 2013; Lin et al. 2010). In the absence of cGMP, PDE2A activity is low, whereas the binding of cGMP to PDE2A increases its activity and drives cAMP hydrolysis (Martinez et al. 2002). In agreement with this, the NO donor SNAP attenuated forskolin-induced cAMP upregulation and this effect was abolished following PDE2A inhibition (Polito et al. 2013), confirming the role of PDE2A in the inhibitory crosstalk between cGMP and cAMP signaling. Also, the increase in intracellular levels of cAMP induced by DA D1-like receptors on striatonigral MSNs is negatively modulated by cGMP-dependent activation of PDE2A (Polito et al. 2013; Lin et al. 2010). These observations indicate that one of the functions of NO-cGMP signaling is to act as a negative feedback pathway which inhibits further cAMP synthesis following the activation of D1-like receptors on striatonigral MSNs, and potentially, on nNOS interneurons.

**PDE10A:** PDE10A immunoreactivity is abundant in MSNs (Xie et al. 2006) and recent studies suggest that this enzyme is also expressed in striatal interneurons (Leuti et al. 2013). Depending on its phosphorylation at threonine 16 (Thr 16), PDE10A can be tethered to the membrane or expressed in the cytosol (Charych et al. 2010; Kotera et al. 2004). Phosphorylated PDE10A is localized to the cytosol, whereas the non-phosphorylated form of PDE10A is attached to the membrane (Charych et al. 2010). The membrane attachment of PDE10A is controlled by palmitoylation, a posttranslational modification that facilitates tethering of proteins to the membrane and controls subcellular localization. When cAMP levels are high, PDE10A is phosphorylated by PKA at Thr 16 and accumulates in the cytosol to regulate cAMP levels (Charych et al. 2010). When intracellular cAMP levels are low, PDE10A becomes palmitoylated and attaches to membranes (including intracellular transport vesicles) where it can be transported and impact on corticostriatal signal transmission (Charych et al. 2010). The isoforms PDE10A1 and PDE10A2 are expressed in humans and the PDE10A2 and PDE10A3 isoforms are expressed in rodents (Kotera et al. 2004). PDE10A1 and PDE10A3 are enriched in the cytosol whereas the PDE10A2 isoform is enriched in membrane fractions. PDE10A was found to be the major cAMP PDE in lysates of mouse striatum, being responsible

for about 60% of cAMP degrading activity (Russwurm et al. 2015). In synaptosomal membranes, the PDE10A enzyme is part of a large multiprotein complex that contains the scaffold protein AKAP150 (A-kinase anchoring protein 150), PKA, PSD-95 (postsynaptic density protein 95), and NMDA receptors (Russwurm et al. 2015). Due to its ability to associate with membranes in striatal MSNs, it is likely that PDE10A has an important role in the integration and processing of motor information within the basal ganglia (Coskran et al. 2006; Seeger et al. 2003; Xie et al. 2006). Indeed, genetic deletion of the PDE10A enzyme induces hypolocomotion in mice, supporting the idea that PDE10A is critical for the regulation of striatal output and purposeful movement (Siuciak et al. 2006b).

While papaverine has been commonly used to explore the role of PDE10A in the regulation of striatal function, the synthesis of more potent and selective PDE10A inhibitors like TP-10 and MP-10 (developed by scientists at Pfizer) has moved the field forward considerably over the past decade (Schmidt et al. 2008). Early studies demonstrated elevations of cAMP and cGMP in striatal dialysates following papaverive and TP-10 administration (Siuciak et al. 2006a; Schmidt et al. 2008). Inhibition of striatal PDE10A activity also robustly increased the phosphorylation of DARPP-32 at Thr34, GluR1 at Ser845 and CREB (Schmidt et al. 2008; Nishi et al. 2008; Grauer et al. 2009).

The potent effects of PDE10A on basal ganglia output have been attracting considerable interest as selective enzyme inhibitors might prove efficacious for the treatment of numerous diseases associated with striatal dysfunction. Like D1 agonists and D2 antagonists, PDE10A inhibition induces expression of substance P and enkephalin mRNA in striatonigral and striatopallidal MSNs, respectively (Strick et al. 2010). Importantly, these outcomes have been corroborated at the behavioral level (Megens et al. 2014). The effects of PDE10A inhibitors appear to depend on the activation state of striatonigral and striatopallidal MSNs. Therefore, when the activity of direct pathway MSNs is reduced by D1 antagonism resulting in behavioral expression of catalepsy, PDE10A inhibitors potentiate catalepsy by increasing the inhibitory actions of the indirect pathway (acting as a D2-like antagonist) (Megens et al. 2014). In contrast, PDE10A inhibitors (acting as a D1-like agonist) can reverse catalepsy induced by the D2 antagonist haloperidol (Megens et al. 2014), although the opposite effect was observed with lower doses of haloperidol (Siuciak et al. 2006a).

By increasing intracellular levels of cyclic nucleotides, PDE10A inhibitors activate both striatonigral and striatopallidal MSNs, exerting the same function as D1 agonists and D2 antagonists. Several studies have suggested that PDE10A inhibitors activate the striatopallidal MSNs to a greater extent than the striatonigral MSNs (Nishi et al. 2008; Threlfell et al. 2009; Polito et al. 2015; Wilson et al. 2015). In one of the first studies to examine the impact of PDE10A inhibition on striatal MSNs, Nishi et al. (2008) demonstrated that papaverine-induced PDE10A inhibition potentiated D1 receptor signaling and increased DARPP-32 phosphorylation at Thr34 two-fold in striatonigral MSNs. PDE10A inhibition also activated cAMP/PKA signaling in striatopallidal MSNs potentiating adenosine A2A signaling and inhibiting D2 dopaminergic receptor signaling (Nishi et al. 2008). Furthermore, PDE10A

inhibition increased DARPP-32 phosphorylation at Thr34 sixfold in striatopallidal MSNs, suggesting a preferential action of PDE10A inhibitors on the indirect pathway (Nishi et al. 2008). In agreement with these outcomes, electrophysiological data provided by our group demonstrated that the PDE10A inhibitor TP-10 increased cortically-evoked activity in putative striatopallidal MSNs and did not affect cortically-evoked activity in antidromically-identified striatonigral MSNs (Threlfell et al. 2009).

Using c-Fos immunoreactivity as a marker of neuronal activation, recent studies have shown that the response to PDE10A inhibition was higher in the dorsolateral than the dorsomedial striatum (Wilson et al. 2015). This pattern of expression is similar to what is observed with the D2 antagonist haloperidol, whereas the expression of c-Fos in response to the DA D1 receptor agonist SKF82958 occurred preferentially in the dorsomedial areas (Wilson et al. 2015). In contrast, a recent *in situ* hybridization study demonstrated that IEGs were equally expressed in striatonigral and striatopallidal MSNs following PDE10A inhibition (Gentzel et al. 2015). However, while the methodologies used in these studies provide a quantitative measure of the number of neurons that were activated by PDE10A inhibition, they do not determine the level of activation of each individual MSN subtype. To address this issue, a recent study used cytoplasmic and nuclear biosensors in striatal slices to detect PKA-dependent protein phosphorylation and demonstrated that, although forskolin induced similar increments of cAMP in both striatonigral and striatopallidal MSNs, PKA-dependent phosphorylation was higher in the cytoplasm and nucleus of striatopallidal MSNs (Polito et al. 2015). Once activated, PKA can translocate to the nucleus and phosphorylate nuclear proteins. By monitoring the histone H3 phosphorylation (PH3) *in vivo*, Polito et al. (2015) demonstrated that 93% of striatopallidal MSNs in the dorsomedial striatum were immunorreactive for PH3 one hour after administration of the PDE10A inhibitor TP-10, whereas in the dorsolateral striatum both striatonigral and striatopallidal MSNs were positive for PH3 (Polito et al. 2015).

### *10.5.2 cAMP-Specific Phosphodiesterases*

**PDE 4:** The PDE4 family (PDE4A-PDE4D) is made up of cAMP-specific PDEs widely distributed in the CNS (Perez-Torres et al. 2000). PDE4B exhibits immunoreactivity in the dorsal striatum, but to a lesser degree than the nucleus accumbens (Cherry and Davis 1999). Also, the expression of PDE4B is higher in striatopallidal MSNs as compared to striatonigral MSNs (Nishi et al. 2008). PDE4 also regulates DA synthesis and release at striatal dopaminergic terminals, and regulates cAMP-PKA signaling in MSNs (Nishi et al. 2008; West and Galloway 1996). Inhibition of PDE4 with rolipram increased DARPP-32 phosphorylation at Thr-34 in striatopallidal MSNs after administration of an agonist of adenosine A2A receptors, but had no effect on DARPP-32 phosphorylation at Thr-34 in striatonigral MSNs when a DA D1 receptor agonist was administered (Nishi et al. 2008). Therefore, inhibition

of PDE4 with rolipram potentiated cAMP-PKA signaling preferentially in striatopallidal MSNs, suggesting a selective function for this enzyme in the regulation of cAMP signaling in the indirect pathway.

**PDE7:** PDE7A and PDE7B are members of the PDE7 family and are expressed in the striatum. PDE7B mRNA is highly expressed in over 70% of GABAergic cells within the dorsal striatum and nucleus acumbens (Reyes-Irisarri et al. 2005). PDE7A mRNA is moderately expressed in the dorsal striatum but not in the nucleus accumbens (Miro et al. 2001). PDE7B mRNA colocalizes with AC5 in striatonigral and striatopallidal MSNs (de Gortari and Mengod 2010). PDE7B mRNA levels are enhanced after D1 but not D2 agonist administration (Sasaki et al. 2004), suggesting that PDE7B might participate on the regulation of intracellular cAMP tone following DA D1 receptor stimulation in striatonigral MSNs.

### *10.5.3 cGMP-Specific Phosphodiesterase*

**PDE9A:** PDE9A is a key modulator of cGMP levels and therefore regulates neuronal cGMP signaling downstream of multiple signaling pathways (Fisher et al. 1998; Kleiman et al. 2012). The splice variant PDE9A5 localizes exclusively to the cytoplasm, whereas PDE9A1 is confined to the nucleus (Wang et al. 2003). PDE9 mRNA is localized throughout the CNS and has an abundant expression in the caudate-putamen (Van Staveren et al. 2003; Reyes-Irisarri et al. 2007). While PDE9A inhibitors have not been thoroughly explored in terms of striatal function, studies by the Pfizer group have shown that reverse microdialysis of a selective PDE9A inhibitor increases extracellular cGMP levels substantially (Verhoest et al. 2009). Our unpublished data indicate that similar to PDE10A inhibition and cGMP analogues, PDE9A inhibition acts to potentiate corticostriatal transmission (data not shown).

## 10.6 Cyclic Nucleotide Control of Striatal Synaptic Plasticity

Long-term synaptic plasticity occurs in the striatum and is important for the regulation of motor planning and learning and memory processes (Kreitzer and Malenka 2008). Both LTP and LTD can occur at corticostriatal inputs to striatopallidal and striatonigral MSNs. Long-term plasticity in the striatum is bidirectional and LTP can be reversed to baseline levels to reduce synaptic strength and increase the efficiency of information storage by a mechanism termed depotentiation (Calabresi et al. 2007). In striatal networks, depotentiation is thought to be involved in "forgetting" mechanisms in cases where motor information no longer needs to be stored (Huang and Hsu 2001). Interestingly in PD, the lack of depotentiation at corticostriatal synapses might result in abnormal storage of unnecessary motor-related information that will be translated into aberrant motor responses (Picconi et al. 2003, 2008).

High frequency stimulation (HFS) or spike-timing dependent plasticity (STDP) protocols can induce LTD in striatopallidal MSNs. This form of plasticity requires activation of metabotropic glutamate receptors (mGluRs) (Gubellini et al. 2001; Sung et al. 2001), DA D2 receptors (Calabresi et al. 1992c, 1997a) and CB1 receptors (Choi and Lovinger 1997; Gerdeman et al. 2002; Kreitzer and Malenka 2005; Ronesi et al. 2004). The effects of glutamate activation on postsynaptic mGluRs combined with calcium entry through L-type calcium channels stimulate MSNs to release endocannabinoids (eCB). DA activation of D2 receptors contributes to the release of eCB from MSNs, which activates CB1 receptors on the presynaptic glutamate terminals via retrograde signaling leading to reduction of glutamate release and induction of LTD. Therefore, eCB-induced LTD of corticostriatal synapses onto MSNs is initiated postsynaptically, but expressed presynaptically through reduction in neurotransmitter release. In contrast, LTD induced by NO occurs post-synaptically and appears to block eCB-induced LTD of corticostriatal synapses (Rafalovich et al. 2015). How LTD of corticostriatal transmission occurs in striatonigral MSNs is still not completely understood, but a recent study demonstrated a role of acetylcholine actions on $G_i$-coupled M4 muscarinic receptors (Shen et al. 2015) which are expressed in high levels at striatonigral MSNs (Bernard et al. 1992; Hersch et al. 1994). This study suggested that M4 muscarinic receptors promote LTD at corticostriatal synapses onto striatonigral MSNs (Shen et al. 2015) by decreasing intracellular cAMP levels via $G_i$ protein-induced inhibition of AC (Augustin et al. 2014; Kheirbek et al. 2009).

NO has an important role in plasticity and regulates the vesicular GABA transporter (VGAT) and GABA transmission at axon collaterals in striatal MSNs by a mechanism that requires cGMP-induced CREB phosphorylation (Sagi et al. 2014). The NO-cGMP signaling pathway also contributes to LTD induction. For example, increasing levels of cGMP with the PDE inhibitor zaprinast mimics the effect of HFS of corticostriatal fibers and also induces LTD in the striatum (Calabresi et al. 2000). LTD in MSNs can also be pharmacologically induced following stimulation of PKG and is blocked by administration of the sCG inhibitor ODQ and nNOS inhibitors, demonstrating that NO-cGMP signaling is necessary for this type of plasticity (Calabresi et al. 1999). As opposed to LTD induced by eCB signaling, NO-induced LTD occurs post-synaptically and it is not dependent on DA D2 or CB1 receptors, and is not affected by changes in intracellular levels of $Ca^{2+}$ (Rafalovich et al. 2015).

LTP also occurs at corticostriatal synapses (Charpier and Deniau 1997) and requires glutamate activation of NMDA receptors and concurrent increase on intracellular calcium levels (Kerr and Plenz 2002). STDP-induced LTP at striatopallidal MSNs is mediated by adenosine A2A receptor activation and disrupted by it antagonism (Shen et al. 2008). In striatonigral MSNs, activation of DA D1 receptors is necessary for LTP induction (Kerr and Wickens 2001; Centonze et al. 1999).

According to the outcomes observed in the previous studies, the level of intracellular cAMP seems to be a determinant factor for striatal bidirectional plasticity. Under physiological conditions, DA has dual opposite effects on striatonigral and striatopallidal MSNs by acting on D1 (facilitatory) and D2 (inhibitory) DA receptors,

respectively. Due to the fact that D1 receptors are linked to $G_{s/olf}$ protein and D2 receptors are linked to $G_i$ protein, MSNs can respond in opposite ways to dopaminergic stimulation mediated by stimulation or inhibition of AC. In fact, AC control of cAMP levels is essential for long-term plasticity and AC subtype 5 knockout mice exhibit impaired LTD that is not recovered by coactivation of D2 and mGlu receptors (Kheirbek et al. 2009). Also, inhibition of PKA blocks LTD and LTP induced by corticostriatal HFS (Centonze et al. 2003). Recent experiments demonstrated an important role of intracellular cAMP levels in LTD induction in striatopallidal MSNs (Augustin et al. 2014). The authors combined either HFS or LFS of glutamatergic inputs to the striatum with postsynaptic depolarization to mimic the upstate of MSNs, and therefore, activate L-type calcium channels and NMDA receptor function (Carter and Sabatini 2004). These studies demonstrated that HFS of corticostriatal fibers and low levels of cAMP are able to produce LTD at striatopallidal MSNs (Augustin et al. 2014). In contrast, LTP was observed in conditions where LFS was combined with high intracellular cAMP levels (Augustin et al. 2014). These observations are consistent with current models of the pathophysiology of PD: DA depletion would lead to hyperactivity of the indirect pathway and the lack of DA actions onto D2 receptors would increase cAMP levels and block LTD induction. LTD is also blocked by DA receptor antagonists (Calabresi et al. 1992b) and by the genetic ablation of D2 receptors (Calabresi et al. 1997b). In contrast, agonism of D2 receptors with quinpirole (Augustin et al. 2014) or exogenous DA replacement with L-DOPA (Picconi et al. 2003) decrease intracellular cAMP levels and restore LTD in the striatopallidal pathway in a manner with also rescues motor performance (Beeler et al. 2012).

In summary, the nature of GPCR (and the downstream effect on AC and cAMP levels) is a critical determinant factor for the direction of striatal synaptic plasticity. In striatopallidal MSNs stimulation of $G_i$-coupled DA D2 receptors is required for the induction of LTD, whereas $G_{olf}$-coupled adenosine A2A receptors facilitates LTP (Shen et al. 2008). In striatonigral MSNs, LTP is induced by stimulation of $G_{s/olf}$-coupled DA D1 receptors, whereas LTD is dependent on acetylcholine actions on $G_i$-coupled M4 muscarinic receptors (Shen et al. 2015).

## 10.7 Targeting Cyclic Nucleotide Phosphodiesterases for the Treatment of L-DOPA-Induced Dyskinesia in PD

The severity and pattern of DA depletion that occurs in PD can be experimentally replicated in animals by toxins (Lane and Dunnett 2008). The most common PD models are produced by injections of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), or by intracerebral injection of 6-hydroxydopamine (6-OHDA). Administration of L-DOPA to patients or animal models of PD effectively restores purposeful movement, however, with repeated administration, L-DOPA can cause

abnormal involuntary movements called L-DOPA-induced dyskinesias (i.e, LIDs). Studies in parkinsonian rodents have shown that L-DOPA causes dyskinesia only when a severe (>80–90%) lesion of the nigrostriatal system is achieved (Meredith et al. 2008). Substantial research efforts have been conducted to understand the molecular mechanisms that underlie the development and expression of LIDs (Huot et al. 2013). Since DA is required for both forms of long-term plasticity, rodent models exhibiting substantia DA depletion that occurs in PD do not express LTP and LTD at corticostriatal synapses (Calabresi et al. 1992a; Centonze et al. 1999; Picconi et al. 2003; Wang et al. 2006). Chronic L-DOPA administration is able to restore LTP in MSNs of 6-OHDA-lesioned rats, but once dyskinesias are established, depotentiation of LTP is lost (Picconi et al. 2003, 2008). The lack of depotentiation at corticostriatal synapses during LIDs might result in abnormal storage of maladaptive motor-related information that will be translated in aberrant motor responses.

Corticostriatal LTP is also reduced in the parkinsonian striatum (Picconi et al. 2003). DA denervation in rodent models of PD impairs corticostriatal LTP and chronic (but not acute) L-DOPA treatment is able to restore LTP in both dyskinetic and non-dyskinetic rodents (Picconi et al. 2003, 2008). Bidirectional plasticity is observed at corticostriatal synapses in non-dyskinetic animals where a physiological reversal of synaptic strength occurs after LFS (Picconi et al. 2003). Depotentiation is lost in the dyskinetic striatum (Picconi et al. 2003), but the precise subtype of striatal MSN involved in this mechanism remains to be determined. Pioneering studies by Calabresi and colleagues showed no difference in terms of physiological and pharmacological responses between striatopallidal and striatonigral MSNs using double immunohistochemical labeling with biocytin and adenosine A2A or substance P, respectively (Picconi et al. 2011). In contrast, more recent work using brain slices from D1 or D2 bacterial artificial chromosome (BAC) transgenic mice demonstrated that depotentiation in dyskinetic animals is related to changes at the level of the DA D1 receptor signaling pathway (Shen et al. 2015).

Rodent models of LIDs show abnormally low levels of cAMP/cGMP in striatum during peak occurrence of LIDs (Giorgi et al. 2008; Sancesario et al. 2014), suggesting that PDE activity might be increased (or synthesis decreased), resulting in augmented metabolism of cyclic nucleotides. Systemic (Giorgi et al. 2008) or intrastriatal (Picconi et al. 2011) administration of PDE inhibitors (zaprinast or the sildenafil analogue UK-343664) was found to reduce the incidence of LIDs in parkinsonian rats by preventing both the L-DOPA-induced decrease of cyclic nucleotide tone, and the rescuing of striatal LTD. These results suggest that PDE inhibitors could be useful therapeutic agents in the treatment of LIDs due to their ability to restore abnormal glutamatergic transmission.

The pharmacological manipulation of PDE signaling pathways is complex and its relationship with LIDs is not completely understood. The PDE inhibitor used in the previous studies (zaprinast) exhibits relatively poor potency and selectivity across cGMP preferring PDE sub-families and isoforms, and as such, is likely to produce undesirable side effects. In support of this, a study using the *Pitx3*$^{-/-}$ aphakia mouse, a genetic model of PD, demonstrated zaprinast is able to reduce established LIDs, but also reduced motor performance on the rotarod (Solis et al. 2015).

The relationship between striatal NO-cGMP signaling in LIDs is still controversial (Lorenc-Koci et al. 2013; Takuma et al. 2012; Solis et al. 2015; Padovan-Neto et al. 2009, 2015a; Sancesario et al. 2014; Picconi et al. 2011). nNOS inhibitor co-treatment has been shown to prevent the development of LIDs and maintain L-DOPA antiparkinsonian efficacy throughout the chronic treatment period (Padovan-Neto et al. 2009, 2015a; Takuma et al. 2012). Although this result is in contrast with the effects obtained with zaprinast on LIDs (Picconi et al. 2011), it was recently demonstrated (Solis et al. 2015) that decreasing NO/cGMP levels with the selective nNOS inhibitor 7-nitroindazole (7-NI), or boosting NO/cGMP signaling pathway with the NO donor molsidomine or the PDE inhibitor zaprinast, significantly diminished the incidence of AIMs in the *Pitx3*$^{-/-}$ aphakia mouse model of PD. The effects of 7-NI on LIDs do not interfere with the beneficial therapeutic effect of L-DOPA (Padovan-Neto et al. 2009, 2015a; Solis et al. 2015), whereas the antidyskinetic effects of molsidomine and zaprinast occurred at the expense of the antiparkinsonian L-DOPA properties (Solis et al. 2015). Although seemingly paradoxical, these results might occur as a result of a differential rebalancing of the direct (go) and indirect (no-go) pathways: nNOS inhibitors might reduce the over-activation of striatonigral MSNs, whereas the PDE inhibitors might act to disinhibit striatopallidal MSNs which are likely suppressed by L-DOPA-mediated agonism at DA D2 receptors. By re-balancing striatal output within the direct and indirect pathways, both nNOS and PDE inhibitors might be useful pharmacological targets for treating LIDs.

A common view is that LIDs is a consequence of over-activation of DA D1 receptors and over-inhibition of DA D2 receptors on striatonigral and striatopallidal MSNs, respectively (Feyder et al. 2011). The abnormal stimulation of D1 receptors induces post-synaptic expression of molecular markers of LIDs such as the transcription factor FosB/ΔFosB (Andersson et al. 1999; Cenci et al. 1999; Berton et al. 2009; Pavón et al. 2006; Tekumalla et al. 2001), DARPP-32 phosphorylation at Thr 34 (Bateup et al. 2010; Santini et al. 2007, 2010; Picconi et al. 2003; Lebel et al. 2010), PKA-dependent phosphorylation of GluR1 at Ser845 (Santini et al. 2007, 2010) and abnormal levels of phosphorylation of the extracellular signal-regulated kinase 1/2 (ERK 1/2) cascade (Santini et al. 2007, 2010; Gerfen et al. 2002; Pavón et al. 2006; Westin et al. 2007). In agreement with the above studies, antagonism of D1 receptors (Westin et al. 2007; Santini et al. 2009) and genetic deletion of D1 DA receptors (Darmopil et al. 2009) block LIDs in rodent models of PD. In terms of synaptic plasticity, striatonigral MSNs undergo strong LTP as a result of enhanced and abnormal D1 DA receptor stimulation (Picconi et al. 2003; Seifert et al. 2015), and consequently, high intracellular levels of cAMP (Augustin et al. 2014). In support of this, reducing intracellular cAMP tone on striatonigral MSNs by targeting M4 muscarinic receptors with a positive allosteric modulator not only restored depotentiation, but also attenuated LIDs (Shen et al. 2015), confirming the important role of abnormal D1-mediated plasticity in LIDs.

On the other hand, D2 receptor-expressing MSNs are also importantly involved in LID mechanisms. For example, STDP protocols that would normally induce LTP in striatopallidal MSNs, induce LTD in dyskinetic animals (Shen et al. 2015),

contributing to excess inhibition in the indirect pathway. Therefore, knowing that LIDs are characterized by over-stimulation of D1- and over-inhibition of D2-expressing MSNs, PDE10A inhibitors should promote antidyskinetic effects by increasing intracellular cAMP and cGMP levels and restoring LTP in striatopallidal MSNs, releasing this pathway from over-inhibition during LIDs. As discussed before, PDE10A is highly expressed in MSNs and compartmentalized proximal to the plasma membrane of dendritic spines, in position to regulate post-synaptic cyclic nucleotide signaling involved in the integration of glutamatergic and dopaminergic neurotransmission (Xie et al. 2006). Given that other classes of PDE inhibitors exhibit antidyskinetic properties, we recently targeted PDE10A as a possible candidate for the treatment of LIDs. Preliminary studies conducted in our lab demonstrated that chronic (3 weeks) treatment of the PDE10A inhibitor TP-10 together with L-DOPA dose-dependently attenuated LIDs in the 6-OHDA-lesioned rat model of PD, without interfering with rotational or normal behaviors (Figs. 10.2 and 10.3). These observations provide evidence for the first time that robust PDE10A inhibition reduces the incidence and severity of LIDs and indicate that these effects are not due to decreased behavioral activation.

Recent *in vivo* human studies analyzed the availability of PDE10A in PD patients using positron emission tomography molecular imaging with a highly selective PDE10A radioligand (Niccolini et al. 2015). Interestingly, the authors found a loss of PDE10A signaling in the striatum and globus pallidus of PD patients under L-DOPA treatment which was correlated with longer disease duration and more severe motor symptoms (bradykinesia and rigidity), suggesting that compensatory mechanisms might occur to modulate PDE levels in the human basal ganglia. Given the above, future studies will have to clarify how the expression and activity of various PDE subtypes expressed in striatal MSNs changes with disease progression, and how this may impact on pharmacological treatment strategies targeting PDEs for the treatment of LIDs.

PDEs are highly regulated enzymes and participate in many molecular mechanisms responsible for controlling striatal signaling output. Although many groups have provided important behavioral and pharmacological data, little information is available regarding the mechanisms of actions of these enzymes. For example, we now know that PDE10A has a greater impact on striatopallidal MSNs even though the expression of this enzyme is similar in both striatonigral and striatopallidal MSNs. Once we understand the molecular mechanisms that contribute to this effect, better pharmacological approaches will be available to treat neuropsychiatric and movement disorders.

Several PDE isoforms are expressed in the striatum but little is known about how these enzymes interact to produce the appropriate motor output. For example, PDE1B is also expressed within MSNs in the striatum. It seems that this isoform has a preferential modulatory effect on the striatonigral MSNs in contrast to the PDE10A, which has been shown to act preferentially on striatopallidal MSNs. Since striatonigral and striatopallidal MSNs have opposing action on motor control, these two PDEs might act together to control striatal motor output. In this case, targeting multiple PDEs isoforms could be an interesting approach to treat hypokinetic

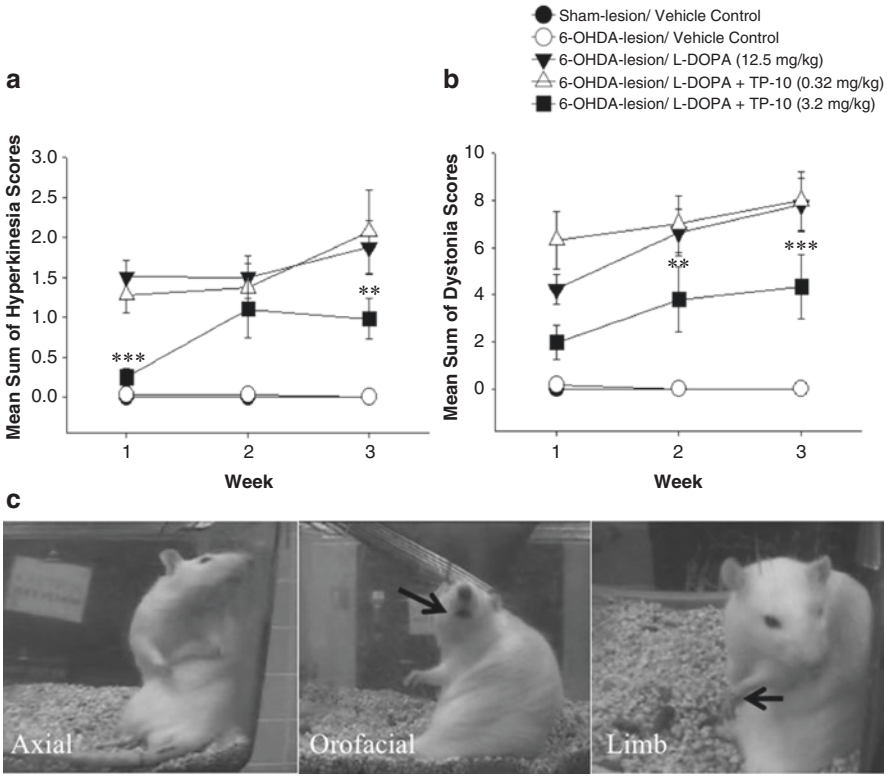


**Fig. 10.2** Effects of the PDE10A inhibitor TP-10 co-administration with L-DOPA on dyskinetic behaviors. (**a**) A significant attenuation of hyperkinetic behaviors was observed in weeks 1 and 3 in L-DOPA plus TP-10 (3.2 mg/kg) treated animals as compared to L-DOPA controls (**p < 0.01 ***p < 0.001). There was no significant difference between groups receiving only L-DOPA therapy and L-DOPA plus TP-10 (0.32 mg/kg). (**b**) A significant attenuation of dystonic behaviors was observed in weeks 2 and 3 in L-DOPA plus TP-10 (3.2 mg/kg) treated animals as compared to L-DOPA controls (**p < 0.01 ***p < 0.001). There was no significant difference between groups receiving only L-DOPA therapy and L-DOPA plus TP-10 (0.32 mg/kg). TP-10 didn't affect rotational behavior and normal behavior such as grooming and exploring (data not shown). Data are derived from n = 8–21 rats per group. (**c**) Still images of dyskinetic behaviors (Maries et al. 2006). (*Left*) Severe axial dystonia. (*Middle*) Mild to moderate orofacial dyskinesia (*black arrow*) and neck dystonia. (*Right*) Left forepaw dyskinesia (*black arrow*)

movement disorders like PD. The combination of PDE1B and PDE10A inhibitors could be used alone or together with low doses of L-DOPA to improve motor function since PDE1B inhibition may preferentially enhance striatonigral MSN activity, whereas PDE10A inhibition may preferentially facilitate striatopallidal MSN activity.

Preclinical tests to access the antidyskinetic effects of the PDE10A inhibitors in non-human parkinsonian primates will be of great value for predicting outcomes in future clinical trials. PDE10A inhibitors are currently being tested for Huntington's

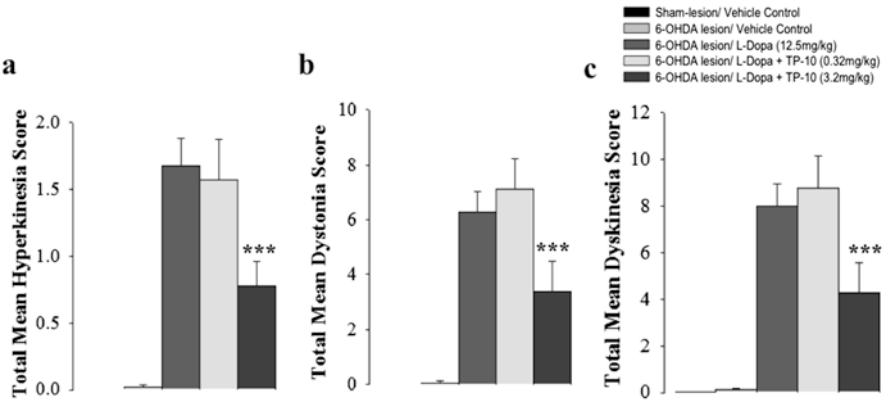


**Fig. 10.3** Summary of the effects of PDE10A inhibitor co-administration with L-DOPA on total combined dyskinetic behaviors. (**a**) A significant decrease in hyperkinetic movements was observed in rats receiving L-DOPA plus TP-10 (3.2 mg/kg) as compared to L-DOPA controls (***$p < 0.001$). No significant difference was observed between rats receiving only L-DOPA and those receiving L-DOPA plus the low dose of TP-10 (0.32 mg/kg). (**b**) A significant decrease in dystonia was observed in rats receiving L-DOPA plus TP-10 (3.2 mg/kg) as compared to L-DOPA controls (***$p < 0.001$). No significant difference was observed between rats receiving only L-DOPA and those receiving L-DOPA plus the low dose of TP-10 (0.32 mg/kg). (**c**) The total dyskinesia score was significantly reduced following co-administration of TP-10 (3.2 mg/kg). Data are derived from N = 8–21 rats per group

disease (HD; clinicaltrials.gov: NCT01806896, NCT02197130, and NCT02342548). These outcomes will be of great interest for LID as well since this hyperkinetic disorder shares some similarities with HD. In both LID and early stages of HD the indirect pathway is hypoactive, whereas the direct pathway is hyperactive. DA produced from L-DOPA is responsible for over-inhibition of striatopallidal MSNs in LID and the selective degeneration of striatopallidal MSNs contributes to the appearance of hyperkinetic movements in early stages of HD. If these clinical trials establish the efficacy of PDE10A inhibitors for treating motor symptoms in HD, a new pharmacological tool will be available to treat this and other hyperkinetic disorders.

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

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# Chapter 11
# Role of Phosphodiesterases in Huntington's Disease

**Francesca R. Fusco and Emanuela Paldino**

**Abstract** Huntington's disease (HD) is an autosomal-dominant rare inherited neurodegenerative disease characterized by a wide variety of symptoms encompassing movement, cognition and behaviour. The cause of the disease is a genetic mutation in the *huntingtin* protein. The mutation leads to an unstable CAG expansion, translated into a polyglutamine domain within the disease protein. Indeed, huntingtin has a CAG/polyglutamine expansion in the range of 6–39 units in normal individuals, whereas it reaches 39–180 units in HD patients. Mutant *huntingtin* interacts with and impairs the function of a number of transcription factors. Indeed, the expression and function of cAMP response element-binding protein (CREB) and the brain-derived neurotrophic factor (BDNF) are severely affected in HD. Drugs targeting CREB loss of function and BDNF decrease have been considered as powerful tools to treat HD. Recently, cyclic nucleotide phosphodiesterase (PDE) inhibitors have been shown to reduce striatal and cortical degeneration in transgenic mouse model of HD. The neuroprotective effect is due to the competency of PDE4, 5 and 10 inhibitors to positively modulate CREB and BDNF protein levels, both in striatum and cortex in HD models. In this chapter, we will summarize the data supporting the use of PDE inhibitors as a therapeutic approach to fight HD, deepening the possible mechanisms of action underlying these effects.

**Keywords** Huntington's Disease • Phosphodiesterase inhibitors • BDNF • striatum

## 11.1 Introduction

Huntington's disease (HD) is an autosomal dominant rare neurodegenerative disorder, characterized by motor disfunction, cognitive decline and psychiatric disturbances. Motor symptoms are dominated by chorea, an involuntary muscle contraction that results from the impairment of the basal ganglia, which is the main target of HD. HD is caused by the mutation of *IT15* gene, which is located on the

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short arm of chromosome 4 and is characterized by a CAG expansion encoding a polyQ repeat at N-terminus of huntingtin protein (Albin and Tagle 1995). The polyQ tract promotes the formation of toxic oligomers and aggregates. In physiological conditions, people have fewer than 36 glutamine repeats in the polyQ region resulting in the production of the cytoplasmatic huntingtin. A sequence of 36 or more CAG repeats results in the production of mutated huntingtin. Generally, the number of CAG repeats is related to the severity of the disease and accounts for about 60% of the variation of the age of the onset of symptoms. In fact, 36–39 repeats result in a reduced penetrance form of the disease with a later onset and slower progression of symptoms. Conversely, a large repeats count determines a full penetrance of HD disease, which might occur even before the age of 20, when it is then referred to a juvenile HD, and this accounts for about 7% of HD carriers.

Huntingtin interacts with over 100 other proteins, and appears to have multiple biological functions. The behavior of the mutated protein is not completely understood, but it is known to be toxic to certain cell types, particularly in the brain, because of the formation of neuronal intranuclear inclusions (NIIs) of mutated huntingtin (Di Figlia et al. 1997). Early damage in HD is most evident in the striatal part of the basal ganglia. The spiny projection neurons, which constitute about 95% of the striatum, degenerate massively in HD (Auer et al. 1984; Smith et al. 1984; Kalimo et al. 1985). Interestingly, a similar marked loss of the striatal projection neurons occurs in experimental cerebral ischemia (Meade et al. 2000). Signs of neurodegeneration are observed also in the cortex, thalamus, and globus pallidus (in the later stages of the disease). Cortical pathology also occurs, contributing to the overall dramatic brain atrophy in the late stages of the disease (Hong et al. 2012, Unschuld et al. 2012, Gray et al. 2013, Samadi et al. 2013 Moreover, signs of cortical dysfunction are often observed before a neuropathological signs are apparent.

One of the mechanisms underlying the vulnerability of striatum in HD is explained by the fact that these neurons do not synthesize sufficient amounts of BDNF. BDNF is very important for survival of mature neurons in the striatum (Zuccato and Cattaneo 2007). Striatal BDNF depends on the cortex for its synthesis and release, as it is synthesized by cortical neurons and released in the striatum by cortico-striatal anterograde transport (Zuccato et al. 2003). This microtubule-based transport depends on huntingtin and is altered in HD. Low levels of BDNF mRNA have been reported in the rat striatum (Baquet et al. 2004).

CREB is a transcription factor, and its function is impaired by mutated huntingtin (Altar et al. 1997; Steffan et al. 2000; Sugars et al. 2004). This supports the hypothesis that inhibition of cAMP response element-mediated gene transcription contributes to HD. In fact, cAMP levels are decreased in cerebrospinal fluid of HD patients and transcription of CREB-regulated genes is reduced in the R6⁄2 transgenic mouse model of HD (Luthi-Carter et al. 2000; Nucifora et al. 2001; Wyttenbach et al. 2001).

Huntingtin modulates the expression of neuron-restrictive silencer factor (NRSF)-controlled neuronal genes, including *BDNF* gene (Zuccato et al. 2003). Therefore, wild-type huntingtin directly stimulates production of BDNF, whereas mutant huntingtin inhibits it. In fact, BDNF is decreased in the brain of HD patients

and in mice transgenic for mutant huntingtin (Ferrer et al. 2000; Duan et al. 2003; Zhang et al. 2003). Overexpression of BDNF showed to be neuroprotective in the R6/1 mouse model of HD (Gharami et al. 2008; Yuxiang et al. 2010). However, mice overexpressing BDNF display higher susceptibility to seizure to kainic acid *in vivo* and hyper-excitability in CA3 region of the hippocampus and entorhinal cortex *in vitro*, because of the effects of BDNF on epileptogenic regions, such as the entorhinal cortex and hippocampus (Papaleo et al. 2011). Moreover, overexpression of BDNF in experimental animals leads to increased anxiety-like behavior and deficits in working memory (Bimonte et al. 2003). Thus, both excess and insufficient BDNF can be detrimental, and such issues have to be addressed before BDNF is used to treat HD patients. BDNF knockout mice have not only an earlier age of onset, but also more severe motor symptoms. Thus, a specific involvement of BDNF was demonstrated in the pathophysiology of the disease in several ways.

## 11.2 Cyclic Nucleotides Phosphodiesterases

The cyclic nucleotide phosphodiesterases (PDEs) are a group of enzymes that selectively catalyze the hydrolysis of the 3′ cyclic phosphate bonds in the second messenger molecules of adenosine and/or guanosine 3,5′ cyclic monophosphate (cAMP and cGMP). They can regulate the localization, duration, and amplitude of cyclic nucleotide signaling within subcellular domains. cAMP and cGMP are second messengers responsible of the transduction of several extracellular signals, including hormones and neurotransmitters. Cyclic nucleotides are formed from ATP and GTP by the catalytic reactions of adenylyl cyclase (AC) and guanylyl cyclase (GC). These enzymes are activated when agonists bind to their appropriate G-protein coupled receptors (GPCR) and stimulate the heterotrimeric G-protein Gs (Lefkowitz 2004) or following activation of a diffusible second messanger, such as nitric oxide (NO) (Corbin et al. 2000; Das et al. 2005) or via an intracellular signal, such as calcium($Ca^{2+2+}$)/calmodulin (Goraya and Cooper 2005). The synthesized cAMP diffuses throughout the cell to sites where it can bind to and activate its target enzymes represented by cAMP- and cGMP-dependent protein kinases, such as protein kinase A (PKA) and protein kinase G (PKG). These kinases act phosphorylating substrates such as ion channels, transcription factors and contractile proteins that regulate key cellular functions. cAMP and cGMP signaling responses are compartmentalized, and this compartmentalization allows spatially distinct pools of PKA and PKG to be differentially activated. This idea was confirmed by observations of cAMP signaling in live cells by FRET, that showed that the accumulation of this second messenger occurs in localized cAMP pools (Houslay 1995, 1998). Such microdomains are created by physical interactions between different components of signaling cascades and structural elements of the cell (Houslay and Milligan 1997). Signal termination mechanisms are essential for cellular homeostasis in order to modulate fluctuations of cAMP within these compartments. Such critical process is catalyzed by cAMP/cGMP hydrolyzing enzymes known as cyclic nucleotide PDEs. In fact,

the basis of cyclic nucleotide gradients is concerted by the activity and localization of both cyclases, which generate cAMP or cGMP, and PDEs, which degrade them. In particular, sequestration and anchoring of PDEs to distinct sites is the principal mechanism to create cyclic nucleotide gradients allowing selective actions (Houslay and Milligan 1997; Houslay and Adams 2003). The basis of this compartmentalization is that various PKA isoforms are anchored to different specific intracellular sites by proteins called A-kinase anchoring proteins (AKAPs) (Rubin 1994). It is postulated that AKAPs, with their distinct pattern of intracellular distribution, allow discrete PKA populations to control the gradients of cAMP in the cell and to modify localized target proteins. In this way, AKAPs sequester PKA to distinct subcellular locations, and position specific enzymes to respond to changes in local cAMP concentrations (Sanderson and Dell'Acqua 2011). However, the predominant regulatory event is the hydrolysis of cAMP by phosphodiesterases. High PDE activity reduces cellular cAMP levels and thus decreases the ability of anchored PKA to become active, whereas reduced PDE activity will favor PKA activation (Bauman and Scott 2002). Inhibition of PDE activity in the brain can, thus, lead to increased intracellular cAMP and/or cGMP levels, thereby modulating neuronal function. Twenty-one genes encode for the superfamily of PDEs, which is subdivided into 11 families according to structural and functional properties (Bender and Beavo 2006). Each PDE family has several different isoforms and splice variants (Beavo et al. 1994); they differ in their three-dimensional structure, mode of regulation, intracellular localization, cellular expression, pharmacological properties and sensitivity to inhibitors. PDEs activity are localized in the cytosol and in a number of membrane, nuclear and cytoskeletal structures (Hardingham and Bading 1998; Houslay 2001). Individual isozymes modulate distinct regulatory pathways in the cell, and on the basis of substrate specificity can be divided into three groups: cAMP-selective hydrolases (PDE 4, 7 and 8), cGMP-selective hydrolases (PDE 5, 6, and 9), and dual (cAMP and cGMP) hydrolases (PDE 1, 2, 3, 10, and 11).

## *11.2.1 PDEs in the Brain*

### 11.2.1.1 Regional Distribution of PDEs

Several PDEs are expressed in neurons, each playing different roles in cAMP and cyclic GMP (cGMP) signaling. *In situ* hybridization and immunohistochemistry demonstrated that the PDE1A isoform is expressed especially in cerebral cortex, striatum and pyramidal cells of the hippocampus (Polli and Kincaid 1994). PDE1B isoform is also expressed in several brain areas such as striatum, nucleus accumbens, dentate gyrus of hippocampus, medial thalamic nuclei, and brainstem (Menniti et al. 2006). Mice lacking PDE1B exhibit increased DARPP-32 phosphorylation at Thr34, thus indicating that PDE1B normally down-regulates cAMP/PKA signaling in striatal neurons (Reed et al. 2002). PDE2A expression is highest in the brain, where the enzyme is typically localized in the cortex, hippocampus and striatum

(Repaske et al. 1993). PDE3A is relatively highly expressed in platelets, as well as in vascular smooth muscle, cardiac myocytes, adipose tissue, liver, and in several cardiovascular tissues (Shakur et al. 2001). PDE4 family is the most widely studied of the PDEs. There are four genes that encode different PDE4 enzymes, of which PDE4A, PDE4B and PDE4D, but not PDE4C, are expressed in the CNS with high concentrations in the cortex, hippocampus, area postrema and striatum (Cherry and Davis 1999).

In the rodent brain, PDE5A mRNA was studied in the Purkinje cells of the cerebellum, in the pyramidal cells of CA1, CA2 and CA3, as well as in the dentate gyrus of the hippocampus (Van Staveren et al. 2003). PDE6 was initially thought to be exclusively distributed to the retina, however, PDE6B mRNA expression was also described in mouse hippocampus (Jarnaess and Tasken 2007). The PDE7 family is composed of two genes coding for high-affinity, rolipram-insensitive, cAMP-specific enzymes PDE7A and PDE7B. While the distribution of these enzymes at the protein level has not been reported, high mRNA concentrations of both PDE7A and PDE7B are expressed in rat brain and in numerous peripheral tissues. Peak concentrations of PDE7 mRNA are found in the olfactory bulb and tubercle, the hippocampus, particularly in the granule cells of the dentate gyrus, and several brainstem nuclei as well as in cerebellum and several thalamic nuclei (Andreeva et al. 2001, Van Staveren et al. 2004).

The expression of mRNA of PDE9A in the rodent brain was described in the Purkinje cells and granule cells of the cerebellum, striatum, olfactory bulb, tubercle as well as CA1 and dentate gyrus of the hippocampus (Fujishige et al. 1999; Sasaki et al. 2002). In the human brain, PDE9 mRNA expression has been reported in the insula and in the visual cortex as well as in the CA1, CA2 and CA3 subfields and dentate gyrus of the hippocampus (Loughney et al. 1999). According to immunohistochemistry and *in situ* hybridization studies, PDE10A is particularly expressed in the brain with the highest levels in both the dorsal and ventral striatum (caudate nucleus, nucleus accumbens, and olfactory tubercle) and, to a lesser extent, in the cerebellum, thalamus, hippocampus, and spinal cord (Seeger et al. 2003, Hebb et al. 2004, Reyes-Irisarri et al. 2005, Reyes-Irisarri et al. 2007). The presence of mRNA transcript PDE10A in the caudate region of the basal ganglia suggests a role in modulating striato nigral and striato pallidal pathways (Coskran et al. 2006).

### *11.2.2 Cellular and Subcellular PDEs Distribution*

Originally, PDE10A immunoreactivity was described only in medium spiny neurons (Xie et al. 2006). In contrast, studies reported that PDE10A was not expressed in striatal interneurons. However, recent studies demonstrated that the striatal interneurons (parvalbuminergic, calretininergic and somatostatinergic) share a common pattern of PDE10A immunoreactivity (Leuti et al. 2013). Indeed, all these interneurons have a nuclear localization of PDE10A, with little or no perikaryal immunoreactivity. Immunoreactivity for PDE10A was observed in moderate

amounts in the nuclei of all striatal interneurons except for cholinergic ones. The observation of PDE10A exclusively in the neuronal nuclei of interneurons was intriguing, as it sheds light on the idea that cyclic nucleotide signaling is highly compartmentalized within cells, and that PDEs exert distinct physiological functions within the cell (Sample and Yang 2012; Van Staveren et al. 2002, 2003).

Each PDE might have differential localization within single neuronal types. This different cellular compartmentalization could be important to control distinct physiological processes and signaling pathways; an example is the differential distribution of PDE2A and PDE10A in hippocampal pyramidal neurons. Indeed, the nuclear localization of PDE10A in interneurons is possibly explained by the observation that cyclic nucleotides and protein kinase A have been described in the nuclei of brain cells (Van Staveren et al. 2003). By contrast, PDE2A is excluded from the soma but densely distributed throughout the neuronal processes (Hepp et al. 2007).

### *11.2.3 Functions of PDEs in Relation to their Distribution*

PDE1B and PDE10A, as well as PDE2A, can metabolize both cAMP and cGMP, while PDE10A is membrane-bound in the vast majority of neurons, and PDE1B is contained only in a soluble intracellular compartment. Moreover, membrane-bound PDE2A is specifically enriched in lipid rafts associated with high concentrations of adenylyl cyclase V/VI and PKA. Because of their distinct subcellular distribution in the medium spiny neurons, they play a different role in regulating the excitability of medium spiny neurons (Siuciak et al. 2008; DiPilato et al. 2004). Moreover, PDEs, because of their ability to modulate cAMP/PKA signaling, can control the dopaminergic signaling in the striatum, where dopamine plays a key role in the regulation of motor and cognitive functions. Moreover, cAMP/protein kinase A (PKA) signaling cascade is essential for dopamine transmission (Zhu et al. 2004; Siuciak et al., 2006a; Siuciak et al. 2006b). Dopamine can have distinct effects in striatonigral or striatopallidal neurons. In fact, by acting on D1 receptors, dopamine stimulates cAMP/PKA signaling via active G protein-mediated activation of adenylyl cyclase, whereas by acting on D2 receptors, it inhibits cAMP/PKA signaling via inactive G protein-mediated inactivation of adenylyl cyclase (Seino and Shibasaki 2005). PDE10A and PDE4 are differently expressed in neuronal subtypes in the striatum, and such discrete cellular localization confers distinct roles in dopaminergic neurotransmission. Striatal PDE10A is localized proximally to the plasma membrane of postsynaptic sites in medium spiny neurons dendritic spines (Stoof and Kebabian 1981; Kotera et al. 2004). This particular localization allows PDE10A to regulate post-synaptic cyclic nucleotide signaling, which is involved in the integration of glutamatergic and dopaminergic neurotransmission. PDE10A is also highly expressed in medium spiny neurons axons/terminals in the SNr and external globus pallidus.

In particular, PDE10A regulates cAMP/PKA signaling (Sano et al. 2008) as well as gene expression (Nishi et al. 2008) in both direct and indirect pathway neurons. In neurons of the direct pathway, PDE10A inhibition by papaverine upregulates

cAMP/PKA signaling, thus leading to the potentiation of dopamine D1 receptor signaling by phosphorylation of cAMP-dependent substrates, including CREB and extracellular receptor kinase (ERK). PDE10A inhibition by papaverine is also able to upregulate cAMP/PKA signaling, in neurons of the indirect pathway, by potentiating adenosine A2A receptor signaling and inhibiting dopamine D2 receptor signaling simultaneously (Strick et al. 2010). Thus, PDE10A inhibition effectively counteracts dopamine D2 receptor signaling in striatopallidal neurons and potentiates D1 receptor signaling in striatonigral neurons, mainly via cAMP-mediated effects (Threlfell et al. 2009; Padovan-Neto et al. 2015). Because the inhibition of conditioned avoidance response has been used as a measure of antipsychotic activity of many drugs (Grauer et al. 2009), PDE10A inhibitors have been suggested as therapeutic agents for schizophrenia. Indeed, the PDE10A inhibitor papaverine counteracts dopamine D2 receptor signaling and potentiates dopamine D1 receptor signaling, so that the pharmacological profile of papaverine resembles that of atypical antipsychotics (Siuciak et al. 2006a). This observation supports the concept that PDE10A inhibition is beneficial for symptoms and cognitive deficits of psychosis.

On the other hand, PDE4B regulates cAMP/PKA signaling at striatal dopaminergic terminals, and inhibition of PDE4 by rolipram upregulates TH phosphorylation and dopamine synthesis, leading to an increase in dopaminergic tone (Menniti et al. 2007).

The level of expression level of PDE4B is higher in striatopallidal neurons than in striatonigral neurons where PDE4 inhibition selectively potentiates cAMP/PKA signaling. Rolipram treatment increases phosphorylation of Thr34 DARPP-32 in response to an adenosine A2A receptor agonist, but has no effect on phosphorylation mediated by a dopamine D1 receptor agonist (Menniti et al. 2007).

## 11.3 Role of PDE in the PKA/CREB/BDNF Pathway

PDEs degrade cyclic nucleotides, which makes them responsible for neuronal cAMP/cGMP content regulation. PDE4 has been reported to play a major role in this mechanism, and PDE4 specific-inhibitors, such as rolipram, exhibit antidepressant effects (Krebs and Beavo 1979) by increasing the levels of activated CREB (Itoh et al. 2004). CREB is a transcription factor required for the survival of adult CNS neurons, and it is known to mediate nuclear calcium-regulated gene transcription following a variety of extracellular and intracellular signals, such as neuronal cell membrane depolarization (Hosoi et al. 2003). It has been largely established that CREB plays a key role in proliferation, growth, survival and differentiation of all types cells.

CREB is phosphorylated and activated by cAMP-dependent protein kinase, PKA. CREB phosphorylation on its $Ser^{133}$ site, which togheter with CREB-binding protein (CBP), bind he $Ca^{2+}$ and cyclic AMP response elements ($Ca^{2+}$ CREs) on the promoter region of many target DNAs, leading their transcription (Hardingham and Bading 1998). Many genes are regulated by phosphorylated CREB-binding protein (pCREB) among the striatal neurons, under both physiological and pathological

conditions, such as neuropeptides (Konradi et al. 1994) and immediate early genes (Vallejo 1994; Andersson et al. 2001). The pCREB-regulated inducible gene expression is thought to contribute to transcription-dependent adaptive changes in neural plasticity related to memory, especially to long-term mental illnesses derived from dysfunctional striatal neuronal activities (Kobierski et al. 1999). Consequently, it facilitates the transcription of a large number of genes playing an important role in memory, especially in long-term memory formation following new protein synthesis (Nestler and Aghajanian 1997). One of the CREB target genes is BDNF, suggesting a protective role of pCREB in several neurodegenerative diseases (Guzowski and McGaugh 1997).

In HD, the activity of CREB is impaired by mutated huntingtin (Steffan et al. 2000; Luthi-Carter et al. 2000). In fact, it has been postulated that inhibition of CRE-mediated gene transcription contributes to HD pathology. The inhibition of PDE activity in the brain can lead to increased intracellular cAMP/cGMP levels, thereby modulating the neuronal function. Interestingly is that PDE10A inhibition induced by the specific inhibitor TP-10 (Pfizer) results in robust increase in cAMP and in CREB phosphorylation in the striatum. Indeed, augmenting cAMP signaling through PDE4 inhibition is associated with the consolidation and retention of long-term memory. Moreover, there has also been a longstanding interest in the use of PDE4 inhibitor Rolipram for the treatment of depression (Schmidt et al. 2008). Recent studies also indicate that PDE4 inhibitors could provide a new approach to the treatment of psychosis (Siuciak et al. 2007; Halene and Siegel 2008; Wieschollek and Manahan-Vaughan 2012). Furthermore, it was shown that pCREB is differently modulated in the different neuronal populations of the striatum according to their unique vulnerability to HD in the rat Quinolinic Acid excitotoxic model, in which the striatal spiny neurons die in a way that resembles HD. In particular, levels of pCREB decrease progressively in projection neurons, parvalbumin (PARV) and calretinin (CALR) interneurons, whereas they remain stable in cholinergic and somatostatin interneurons (Giampà et al. 2007). Thus, it has been speculated that the ability of cholinergic interneurons to maintain their levels of CREB after excitotoxic lesions is one of the factors determining their protection in Huntington's disease (Fusco et al. 2003).

Furthermore, a decreased transcription of CREB-regulated genes was observed in HD transgenic animals. Decreased cAMP in cerebrospinal fluid of HD patients was observed, and CBP was found in the nucleus of 100% of wild- type cells and only in 18% of HD mutant cells (Gines et al. 2003).

CREB-regulated gene expression has been associated with neuronal survival and neuronal plasticity. Changes in PDE expression and subsequent cyclic nucleotide signaling can modulate neuroprotection via CREB (Jancic et al. 2009). Moreover, BDNF activates the MAPK signaling pathway, which is also an important gene product of CREB-mediated transcription, and is up-regulated by cyclic nucleotide level elevation. The expression of PDEs in several neurodegenerative disorders where neuronal survival and plasticity are impaired merits attention. In fact, changes in PDE expression, cyclic nucleotides and their downstream target genes have been reported in various neurodegenerative disorders, including Alzheimer's disease,

Parkinson's disease, HD (Sancesario et al. 2004). In Alzheimer's disease (AD), the most prominent symptom is the progressive decline in cognitive functions, mainly memory. A chronic, progressive loss of neurons leading to atrophy in mainly temporal and parietal lobes underlies AD cognitive symptoms. The neuropathological hallmarks of AD, namely amyloid β (Aβ) plaques and neurofibrillary tangles, are critical in the disease process. Interestingly, cAMP and cGMP are both affected in AD, and cAMP is thought to play a role in the etiology of neurofibrillary tangles via tau phosphorylation (Shi et al. 2011). Aβ plaques may display a detrimental effect on LTP, via the inhibition of both the cAMP/PKA/CREB and cGMP/PKG/CREB pathway, in addition AC, GC and pCREB levels are reduced in the temporal lobe of AD patients (Vitolo et al. 2002; Puzzo et al. 2005; Hanger et al. 2009). In AD, an increase in the expression of PDE4A, PDE4B, and PDE7A are observed in early stages of AD, while, in the most advanced clinical stages, an increase in PDE8B expression is observed in the brain regions associated with memory, such as the enthorhinal cortex (Walsh et al. 2002). An involvement of PDEs in Parkinson's disease (PD) was demonstrated by the observation that decreased BDNF expression determines loss of dopaminergic neurons in the substantia nigra. Moreover, an impairment in cyclic nucleotide signaling mechanisms has been reported in human PD and in experimental models (Nishino et al. 1993). Phosphodiesterase 7 (PDE7), a cAMP hydrolyzing enzyme, is highly expressed in striatal and nigral neurons. Significant upregulation in neuroinflammatory events have been implicated in dopaminergic neuronal loss in PD. Microglial activation has been demonstrated in SNpc and striatum of postmortem PD brains, as well as in experimental models of PD (Cicchetti et al. 2002; McGeer et al. 2003; Orr et al. 2005). The degeneration of dopaminergic terminals from the substantia nigra pars compacta, along with the decrease in dopamine levels in the striatum, accounts for the motor and cognitive deficits in Parkinson's disease (Calabresi et al. 1996, 2006; Wichmann and DeLong 2003). Levodopa (L-dopa) is a substitutive pharmacological compound directed towards restoring physiological concentration of dopamine in the striatum, and it represents the most effective therapeutic approach for Parkinson's disease (Picconi et al. 2003; Olanow et al. 2006). However, chronic exposure to L-dopa induces movement behavior fluctuations and dyskinesia in most patients with Parkinson's disease. The effects of dopamine loss on cGMP levels are controversial. Indeed, it has been recently observed that striatal cGMP signaling decreases at the peak of L-dopa-induced dyskinesia in Parkinson's disease rat model (Giorgi et al. 2008). On the other hand, an increase in both activity and protein level of GC in striatum after MPTP injection was observed. Moreover, in an experimental rat model of PD, PDE10A mRNA levels were diminished in striatal neurons 10 weeks after 6-hydroxydopamine (6-OHDA) midbrain lesions (Sagi et al. 2014; Giorgi et al. 2011; Tseng et al. 2011; Chalimoniuk and Langfort 2007; Chalimoniuk et al. 2004; Chalimoniuk and Stepien 2004). Lesions of the nigro-striatal dopaminergic projections with 6-OHDA, rather surprisingly, induce an increase in cAMP levels, as demonstrated by increased basal adenylate cyclase activity in dopamine-denervated rat striatum (Hossain and Weiner 1993; Tenn and Niles 1997). Conversely, cGMP levels decrease in response to dopamine loss. Such down-regulation of cGMP is

associated with decreased nitric oxide synthase expression and activity, probably leading to a decrease in the nitric oxide-guanylate cyclase pathway. Furthermore, loss of dopamine increases (PDE1B)1B levels, suggesting that the modulation of second messenger system in dopamine-denervated rat striata may be affected by changes in synthesis as well as catabolism (Tenn and Niles 1997).

## 11.4 PDEs in Huntington's Disease

Intracellular cAMP and cGMP concentrations depend on the rate of their synthesis from ATP and GTP by AC and GC, respectively, the rate of efflux from the cell, and the rate of degradation. PDEs hydrolyze cAMP and cGMP limiting both the duration and amplitude of the cyclic nucleotide signal (Conti and Jin 1999; Francis et al. 2000; Van Staveren et al. 2001). PDE1B levels were reduced in 12-week old R6/2 mice model of HD. Interestingly, PDE4 distribution is mainly observed in the cortex, which was not altered in a major way in HD.

The most interesting PDE in HD is PDE10A, because of its particular distribution in the striatum, which represents the main target of the disease. PDE10A is highly expressed in regions of the brain that are innervated by dopaminergic neurons such as the striatum, nucleus accumbens and olfactory tubercle (Soderling et al. 1999). Moreover, it is highly expressed in GABAergic spiny projection neurons with localization to the membrane of dendrites and dendritic spines. A decrease in the protein levels of the striatum-enriched PDE10A has been found to precede the actual impairment of motor functions in R6/1 and R6/2 HD mice. It is also been described that PDE10A expression levels are reduced in the postmortem brain of HD patients (Hu et al. 2004). Because cyclic nucleotides are important for intracellular signaling, these changes may contribute to changes in cell function that cause motor, cognitive or psychiatric disturbances observed in HD patients.

However, it has been shown that PDE inhibition has a beneficial role in HD animal models, leading to an apparent conflict between the decreased PDEs levels associated with HD and the beneficial effect of PDE inhibitors in a R6/2 mouse model of HD (Giampà et al. 2010). To address this issue, PDE10A protein expression levels in the R6/2 mice was recently investigated, with particular attention to the different neuronal subpopulation of the striatum. The results showed a dramatic increase in PDE10A in medium spiny neurons of R6/2 transgenic HD mice compared to their wild type littermates (Leuti et al. 2013). Conversely, in the striatal cholinergic interneurons, PDE10A levels were lower, and were not significantly modified by disease progression. In the other subsets of striatal interneurons (parvalbuminergic, somatostatinergic, and calretininergic interneurons), PDE10A immunoreactivity was higher in the R6/2 compared to the wild-type mice. However, densitometric studies of the whole striatum showed that PDE10A immunoreactivity was lower in the R6/2 compared to the wild-type mice. Moreover, it was shown that PDE10A increases in the perikarya of projection neurons, but is reduced in the whole striatum of the R6/2 mice. This suggests that, in HD, mutant huntingtin protein disrupts PDE10A synthesis and trafficking, resulting

in PDE10A accumulation in the perikarya of spiny projection neurons, which are vulnerable to the disease, thereby decreasing cAMP and cGMP locally. (Leuti et al. 2013). Therefore, even if levels of PDE10A are lower in the striatum *in toto*, the enzyme might be too abundant in the somata of medium spiny neurons where it downregulates cyclic nucleotides signaling, which is detrimental for cell life. In that study, we observed that cholinergic interneurons, resistant to HD degeneration, have a moderate amount of PDE10A in the early stages, both in the R6/2 and in the wild types (Fusco et al. 1999). A previous study, showed that striatal cholinergic interneurons contain higher amounts of BDNF, compared to the more vulnerable medium spiny neurons and that cholinergic interneurons are more enriched with phosphorylated CREB (Fusco et al. 2003). Therefore, it is possible that the low levels of PDE10A found in cholinergic interneurons are related to their selective resistance to HD neurodegeneration.

PDE10A immunoreactivity was observed in moderate amounts in the nuclei of all striatal interneurons except for cholinergic ones, and its levels were higher in the R6/2 than in the wild type mice (Leuti et al. 2013). As mentioned above, nuclear localization in interneurons, although unexpected, can be explained by the observation that cyclic nucleotides and PKA have been described in the nuclei of brain cells (Van Staveren et al. 2002).

### *11.4.1 PDEs Inhibition Effects in Huntington Disease*

Phosphorylated CREB is differently expressed in several neuronal subpopulations of the striatum, both in control animals and in the quinolinic acid model model of HD. Different levels of activated CREB were described to be associated with the individual vulnerability to excitotoxic lesions (Giampà et al. 2006). In particular, it was suggested that higher levels of CREB could account for the selective resistance of selected neuronal populations (Lee et al. 2004).

In light of this, drugs targeting CREB loss of function could be considered as a powerful therapeutic for the treatment of neurodegenerative disorders such as HD. The PDE4 inhibitor, rolipram, increases CREB phosphorylation. In fact, our early studies showed that rolipram was able to exert a neuroprotective effect and to increase significantly the levels of activated CREB in the striatal spiny neurons, in the QA excitotoxic model of HD (De March et al. 2006). The beneficial effect observed following rolipram treatment were also amenable to the maintenance of BDNF protein expression levels. BDNF is, in fact, synthetized in the cortex and anterogradely transported to the striatum. Thus, the increased CREB phosphorylation exerted by rolipram in the QA model was likely responsible for the neuroprotection through an increase in cAMP levels.

In a later study, it was shown that rolipram is able to increase survival and ameliorate clinical signs in the R6/2 mouse model of HD (De March et al. 2008). In that study, PDE4 inhibition through rolipram was shown to exert a neuroprotective role by increasing both phosphorylated CREB and BDNF in the striatum. Rolipram prevented CREB binding protein sequestration into striatal neuronal intranuclear

inclusions, thus sparing parvalbuminergic interneurons of R6/2 mice, and rescuing motor coordination and motor activity deficits (Giampà et al. 2009). Moreover, an increase in ERK phosphorylation was reported in the medium spiny neurons of R6/2 mice after rolipram tratment. ERK phosphorilation have considerable importance if we consider that extracellular signal-regulated protein kinases activation pattern is altered in HD (Fusco et al. 2012).

Another possible target for neuroprotection in HD is PDE5, selective for cGMP. PDE5 is found in several brain regions including the cortex, hippocampus, and basal ganglia (Marte et al. 2008; Puerta et al. 2009). In a recent study, Puerta and coworkers have shown that PDE5 inhibitors sildenafil and vardenafil were able to ameliorate neurological symptoms, reduce striatal projection neurons loss and increase pCREB levels in the 3-nitroproprionic intoxication model of HD in rats (Puerta et al. 2010). Noteworthy, it was shown that mRNA and protein BDNF levels were significantly elevated in sildenafil treated rat cortex, which accounted significantly for their neuroprotective effects (Wang et al. 2014).

These results provided strong theoretical support for targeting cyclic nucleotides and CREB signaling through PDE inhibition. A PDE10 inhibitor (TP10, Pfizer) was administered to the QA rat surgical model of HD (Giampà et al. 2009). Chronic administration of TP10 was effective in reducing the QA lesion area by 52%, sparing medium spiny neurons, and in increasing CREB levels in the surviving striatal neurons. Interestingly, TP10 treatment also had beneficial effects on cortical neurons. In fact, decreased retrograde cortical neuron loss and increased levels of phosphorylated CREB and BDNF were observed, although the effect of TP10 on cortical levels of BDNF was moderate and only limited to the early time point. Because PDE10A is mostly expressed in striatal medium spiny neurons, it is possible that these effects on the survival of cortical neurons may be indirect. Thus, sparing of striatal neurons by TP10 might have translated into a higher level of activity in the cortical neurons, which in turn would account for the increase in phosphorylated CREB and BDNF (Giampà et al. 2009). Following these results, PDE10A inhibition was further investigated by administering to the R6/2 mouse model of HD (Giampà et al. 2010). Predictably, TP10 was able to rescue striatal neuropathology in terms of neuronal loss, NIIs formation, microglial reaction. Also, TP-10 treatment was associated with a significant increase in phosphorylated CREB and BDNF in cortex and striatum. The increase of cAMP signaling that was recorded in medium spiny neurons and resulted from PDE10A inhibition could be explained by a number of downstream mechanisms. First, PDE10A inhibition in wild-type mouse brain causes a robust increase of CREB phosphorylation downstream of cAMP, which is accompanied by a significant increase in BDNF levels in striatum of R6/2 mice following TP-10 administration. CREB-mediated transcription and BDNF levels both may contribute to the significant amelioration of striatal pathology resulting from treatment of the R6/2 mice with PDE10A inhibitor TP-10. Moreover, PDE10A inhibition also had a beneficial effect on cortical pathology in the R6/2 mice. TP-10 treatment counteracted the decrease in cortical neuron counts by 40%. Amelioration of striatal pathology by PDE10A inhibition might maintain corticostriatal synaptic connections, which may reduce cortical neuron pathology by preventing retrograde

degeneration. However, it is also conceivable that there is a direct effect of TP-10 treatment on cortical CREB phosphorylation and BDNF synthesis resulting from inhibition of the nuclear/perinuclear PDE10A present in the cortex (Fusco and Giampà 2015).

Chronic inhibition of PDE10A stimulates the up-regulation of mRNAs encoding genes such as PDE1C, prodynorphin, synaptotagmin10, and diacylglycerol O-acyltransferase. Moreover, it produces down-regulation of mRNAs encoding choline acetyltransferase and Kv1.6, which suggests that long-term suppression of PDE10A is associated with altered striatal excitability (Fusco and Giampà 2015). These results support the hypothesis that PDE inhibitors could be considered as a valid therapeutic approach for HD. However, more studies regarding the effects of PDEs and their inhibitors on patients health are required to promote clinical trials for neurodegenerative diseases.

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

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# Part IV
# PDEs and Psychiatric Disorders

# Chapter 12
# The Role of Phosphodiesterase-2 in Psychiatric and Neurodegenerative Disorders

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**Abstract** Cyclic nucleotide PDEs are a super-family of enzymes responsible for regulating intracellular levels of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Through their catalysis, PDEs are able to exert tight regulation over these important intracellular signaling cascades. Previously, PDEs have been implicated in learning and memory, as well as in mood disorders, such as anxiety and depression. PDE2 is of special interest due to its high level of expression in the forebrain, specifically in the isocortex, entorhinal cortex, striatum, hippocampus, amygdala, and medial habenula. Many of these brain regions are considered participants of the limbic system, which is known as the emotional regulatory center of the brain, and is important for modulating emotion and long-term memory. Therefore, PDE2s coincidental expression in these areas suggests an important role for PDE2 in these behaviors, and researchers

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are continuing to uncover the complex connections. It was shown that PDE2 inhibitors have pro-cognitive effects in tests of memory, including the object recognition test. PDE2 inhibitors are also protective against cognitive deficits in various models of cognitive impairment. Additionally, PDE2 inhibitors are protective against many different forms of stress-induced anxiety-like and depression-like behaviors. Currently, there is a great need for novel therapeutics for the treatment of mood and cognitive disorders, especially anxiety and depression, and other neurodegenerative diseases, such as Alzheimer's disease, and PDE2 is emerging as a viable target for future drug development for many of these diseases.

**Keywords** PDE2 • Memory and cognition • Stress • HPA axis • Depression • Anxiety • Pain

## 12.1 History of PDE2

The 3′5-cyclic-guanosine monophosphate (cGMP)-stimulated cyclic nucleotide phosphodiesterase (PDE) that hydrolyzes cAMP was first identified in a rat liver by Beavo, Hardman and Sutherland in 1971 (Beavo et al. 1971). They noted that, while cGMP tended to inhibit PDE activity in bovine heart, it was able to stimulate PDE in rat liver, indicating a likely novel subtype. Similar activity from this novel enzyme was subsequently found in numerous other tissues, including bovine adrenal and heart tissue (Martins et al. 1982), and was eventually given the name PDE2. PDE2 was later recognized as a dual specificity enzyme, capable of hydrolyzing both cGMP and cAMP. This ability to modulate activity in response to cGMP allows the enzyme to mediate the 'cross-talk' between the cGMP and cAMP signaling cascades.

PDE2 was cloned and sequenced in 1986 (Chen et al. 1986; Sass et al. 1986). Three different splice variants of PDE2 are encoded by a single gene (PDE2A) and differ only in their N-terminal, which determines intracellular localization to the membrane (PDE2A2 or PDE2A3) or soluble fraction (PDE2A1). PDE2A2 was originally cloned from rat brain cDNA (Repaske et al. 1992; Yang et al. 1994), while PDE2A1 and 2A3 were cloned from bovine heart cDNA (Sonnenburg et al. 1991; Tanaka et al. 1991). An identical PDE2A3 variant from bovine was also cloned from human brain tissue (Rosman et al. 1997). Researchers have yet to find any 2 of the splice variants co-expressed in one of these systems. A similar cAMP-stimulated PDE2, is found in the parasite *Trypanosoma brucei,* but has yet to show significant cGMP activity (Laxman et al. 2005; Rascón et al. 2002).

Though PDE2 activity has been demonstrated in a variety of tissues over the last three decades, the lack of a brain penetrant PDE2-selective inhibitor has limited progress in fully understanding the role of PDE2 in biological systems and signaling. Nevertheless, behavior work that has been carried out over the last 20 years has shown PDE2 as a promising target for drug development for the potential treatment of a variety of cognitive disorders (Table 12.1). Though no PDE2 inhibitors have

**Table 12.1** Overview of effects of PDE2 inhibitors in the CNS

| Task | | Model (species) | PDE2 inhibitors used | Results | Reference |
|---|---|---|---|---|---|
| Memory and cognition | Social and object recognition; T-maze | Unimpaired (rats and mice); MK-801 induced memory impairment (mice) | Bay 60-7550 | Memory consolidation improved in unimpaired animals; MK-801 induced memory deficits were reversed | Boess et al. (2004) |
| | Object recognition | Unimpaired (rat) | Bay 60-7550 | Memory consolidation improved | Rutten et al. (2007a, b) |
| | Object recognition | Age related memory impairment (rat) | Bay 60-7550 | Memory acquisition and/or consolidation improved | Domek-Łopacińska and Strosznajder (2008) |
| | Object recognition | Acute tryptophan depletion induced memory deficits (rats) | Bay 60-7550 | Memory acquisition impairment was reversed | van Donkelaar et al. (2008) |
| | Object location | Unimpaired (rats) | Bay 60-7550 | Memory consolidation improved | Rutten et al. (2009) |
| | Object location; Y-maze | AD model (APPswe/PS1dE9 mice) | Bay 60-7550 | Chronic treatment reversed memory deficits in AD mice | Sierksma et al. (2013) |
| | Object recognition | Scopolamine or MK-801 induced memory impairment (rats) | Bay 60-7550 | Memory acquisition impairment was reversed | Reneerkens et al. (2013) |
| | Object recognition | Unimpaired (rats) | Bay 60-7550 | Memory consolidation improved | Bollen et al. (2014) |
| | Object recognition | Unimpaired (mice) | Bay 60-7550, ND7001 | Memory acquisition, consolidation and recall improved | Lueptow et al. (2016) |
| | Morris water maze; object recognition; object location | Chronic stress induced memory impairment (mice) | Bay 60-7550 | Chronic treatment reversed memory deficits induced by chronic stress | Xu et al. (2015) |

(continued)

**Table 12.1** (continued)

| Task | | Model (species) | PDE2 inhibitors used | Results | Reference |
|---|---|---|---|---|---|
| Schizophrenia-related cognition | Object recognition; ED/ID; P20-N40 auditory gating | PCP induced memory deficits (rats); DBA/2 mouse model with symptoms related to schizophrenia | Lu AF64280 | PCP-induced or genetic executive functional deficits were reversed | Redrobe et al. (2014) |
| | ED/ID | PCP induced memory deficits (rats) | Bay 60-7550 | Executive functional deficits were reversed | Rodefer et al. (2012) |
| Anxiety disorder | Elevated plus maze; open field; hold-board | BSO (oxidative stress) induced behavioral deficits (mice) | Bay 60-7550 | Anxiolytic-like effects | Masood et al. (2008) |
| | Elevated plus maze; (?) | Unimpaired or acute restraint stress induced behavioral deficits (mice) | Bay 60-7550; ND7001 | Anxiolytic-like effects | Masood et al. (2009) |
| | Elevated plus maze; marble burying | Chronic stress induced behavioral deficits (mice) | Bay 60-7550 | Anxiolytic-like effects | Ding et al. (2014) |
| Depressive disorder | Tail suspension; novelty suppressed feeding | Chronic stress induced behavioral deficits (mice) | Bay 60-7550 | Antidepressant-like effects | Xu et al. (2013) |
| | Tail suspension; forced swim | Chronic stress induced behavioral deficits (mice) | Bay 60-7550 | Antidepressant-like effects | Ding et al. (2014) |
| Inflammatory pain | Measurement of body weight shift | Medical meniscal transection (MMT) model of osteoarthritis pain (mice) | Compound 22 | Alleviation of inflammatory pain | Plummer et al. (2013b) |

completely made it through clinical trials so far, interest in PDE2 as a drug target has been growing in recent years. Numerous novel PDE2 inhibitors are currently in development.

## 12.2 Biology of PDE2

PDE2 is a homodimer, with each monomer being approximately 105 kDa (Gesellchen and Zaccolo 2011; Martinez 2006). X-ray structural studies have revealed that the N-terminal consists of two GAF (cGMP-binding PDE, Anabaena adenylyl cyclases, Eschericihia coli FhlAs) domains, GAF-A and GAF-B. PDE2 is activated upon binding of cGMP to the GAF-B domain, which induces positive cooperativity for the hydrolysis of both cAMP and cGMP. cAMP has a 20-fold lower affinity for the GAF-B binding site, and is not shown to induce hydrolysis. Endogenous substrate cAMP/cGMP binds to the catalytic pocket that consists of a purine binding site, where the hydrolysis reaction takes place. Notably, there is invariant conserved residue, Gln859 in PDE2A, forming hydrogen bonds with the purine ring of the substrate at the apex of the purine-binding site. The free rotation of Gln859 in PDE2 makes it possible to form hydrogen bonds with the exocyclic amino group of cAMP and the exocyclic carbonyl oxygen of cGMP, which determined the selectivity for both cAMP and cGMP (C.-R. Yang et al. 2012). However, the rate of cAMP hydrolysis is increased nearly 30-fold in the presence of low, micromolar concentrations of cGMP, and once activated, PDE2 is able to hydrolyze both cAMP and cGMP with a similar Km value (30 μM and 10 μM, respectively) (Gesellchen and Zaccolo 2011; Martinez 2006).

Expression of PDE2 in the central nervous system (CNS) is fairly restricted to forebrain regions, and very limited in mid or hind brain regions. *In situ* hybridization of PDE2A in the rat brain showed high levels of PDE2 in the gray matter forebrain, the pyramidal cell layer, and to a lesser extent the granule cell layer, of the hippocampus, and amygdala, but not in the substantia nigra or other mesencephalic or diencephalic brain regions (Stephenson et al. 2012) (Fig. 12.1). Immunohistochemistry shows similar expression patterns (Stephenson et al. 2012; Diane T Stephenson et al. 2009). Furthermore, human, primate, dog and mouse cortex share a similar protein expression pattern in cortex with rat (Diane T Stephenson et al. 2009; Sadhu et al. 1999). In human, PDE2 mRNA expression was observed with comparable levels in healthy adults, patients with Alzheimer's disease and age matched controls (Lakics et al. 2010; Reyes-Irisarri et al. 2007). For most of the brain regions that have PDE2A immunoreactivity, PDE2A expression is mainly found in the neuropil rather than cell bodies, with a notable exception of the medial habenula (Stephenson et al. 2009). Therefore, PDE2 has a complex biochemical regulation over a variety of different physiological processes and functions in the brain. However, to date PDE2 inhibitors have only served primarily as research tools, rather than being used in clinical studies. Again, a unique property of PDE2 is its ability to mediate negative "cross talk" between the cGMP and cAMP pathways, because of the cooperative kinetics in hydrolysis of cAMP and cGMP by this enzyme.

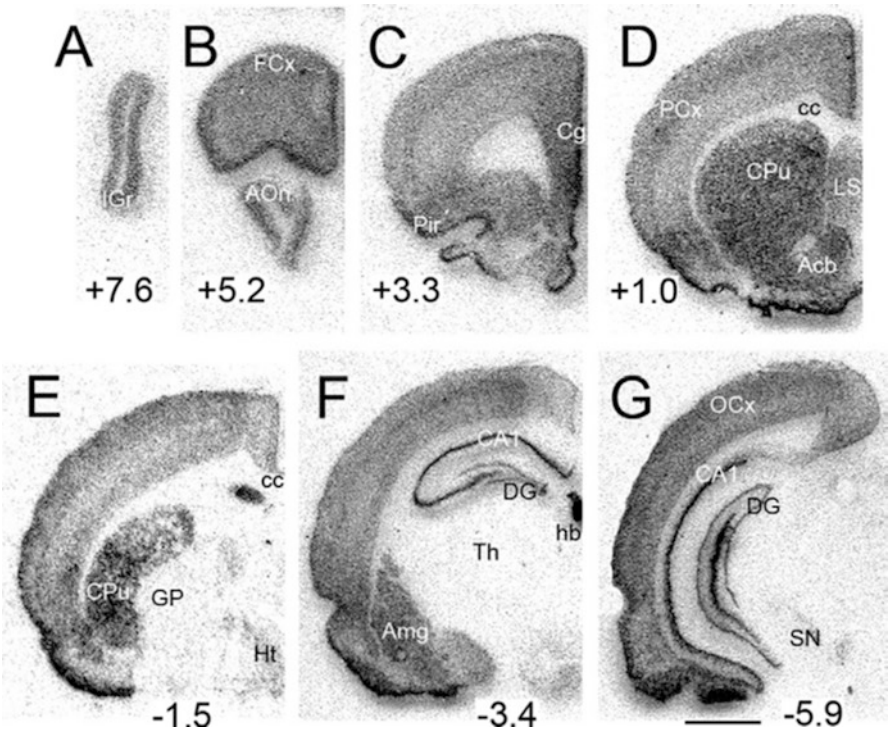


**Fig. 12.1** Distribution of PDE2A mRNA in coronal sections of rat brain, determined by in situ hybridization. Hybridization signal is black. Numbers at bottom of panels are distance from bregma, according to the atlas of Paxinos and Watson (1997). Message is high in cortical regions, striatum, and the habenula. *A* cerebral aqueduct, *ac* anterior commissure, *Acb* n. accumbens, *ACo* anterior cortical amygdaloid n, *Amg* amygdaloid complex, *AOn* anterior olfactory n., *BLA* basolateral amygdaloid n, *CB* cerebellum, *CC* central canal, *cc* corpus callosum, *Cg* cingulate cortex, *Ce* central amygdaloid n, *CP* caudate-putamen, *CPu* caudate n. and putamen, *dc* dorsal columns, *dcs* dorsal corticospinal tract, *DG* dentate gyrus, *DR* dorsal raphe, *FCx* frontal cortex, *GC* granule cell layer, *GP* globus pallidus, *GPe*, external part of globus pallidus, *hb* habenula, *Ht* hypothalamus, *IC* inferior colliculus, *IGr* internal granule cell layer of olfactory bulb, *In* interpeduncular nucleus, *LL* lateral lemniscus, *LS* lateral septal n., *M* molecular layer, *MR* median raphe, *MVe* medial vestibular nucleus, *OCx* occipital cortex, *Opt* optic tract, *PC* Purkinje cell layer, *PCx* parietal cortex, *Pir* piriform cortex, *Pl* plexiform layer of olfactory bulb, *Py* pyramidal cell layer, *RF* rhinal fissure, *SG* substantia gelatinosa, *SL* stratum lucidum, *SN* substantia nigra, *SNc* substantia nigra pars compacta, *SNr* substantia nigra pars reticulata, *SO* stratum oriens, *SR* stratum radiatum, *Th* thalamus, *3v* 3rd ventricle, *VH* ventral horn. Scale bar in G = 2.5 mm. (Reprinted by permission from Macmillan Publishers Ltd.: Neuroscience, Stephenson et al. (2012) copyright 2012)

*In vivo* analysis of the role of PDE2 in rodent behavior was only first started in 2002, when Suvarna and O'Donnell began investigating the role of PDE2 in cAMP/cGMP hydrolysis in the central nervous system using rat primary cerebral cortical and hippocampal cultures, a mostly unexplored area at the time in regards to PDE2 function. Using this neuronal preparation, they were able to demonstrate the differential role of PDE2 and PDE4 in cGMP and cAMP hydrolysis, respectively (Suvarna and O'Donnell 2002). While PDE2 is a dual-hydrolysis enzyme,

capable of degrading both cAMP and cGMP, they showed that NMDA-mediated increases in cAMP were enhanced by the PDE4 inhibitor rolipram, while NMDA-mediated increases in cGMP were blocked by PDE2 inhibitor EHNA. Subsequent research has continued to focus on the role of PDE2 in CNS and behavior, and is reviewed below.

## 12.3 Current PDE2 Inhibitors

Though PDE2 was identified almost 50 years ago, it wasn't until the early 2000s and the development of a selective inhibitor that researchers were confidently able to begin to analyze the role of PDE2 in mammalian systems. Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), a potent adenosine deaminase inhibitor, was the first compound discovered to have PDE2 isozyme selective properties, with an $IC_{50}$ of ~0.8 uM for human PDE2A (Méry et al. 1995; Podzuweit et al. 1995). Unfortunately, because EHNA is also a potent adenosine deaminase inhibitor, studies require additional controls and careful interpretation of any findings. A major advancement came with the synthesis of Bay 60-7550 by Bayer in 2002 (Niewohner et al. 2003). Bay 60-7550 has an $IC_{50}$ value of 2.0 ± 0.7 nM when used on purified PDE2 from bovine heart, and an $IC_{50}$ of 4.7 ± 1.0 nM when used on human recombinant PDE2 (Boess et al. 2004). Additionally, Bay 60-7550 has 50-fold selectivity for PDE2 over PDE1, and 100-fold or more selectivity over other PDEs (Boess et al. 2004; Buijnsters et al. 2014; Gomez and Breitenbucher 2013; Heine et al. 2013; Martinez 2006; Masood et al. 2009). And despite having solubility and pharmacokinetic issues, Bay 60-7550 has opened the door for uncovering the role of PDE2 in mood and cognitive disorder that will be reviewed here.

One published study measured the amount of Bay 60-7550 in the rat brain following oral administration. Brain and plasma exposure of 1 mg/kg Bay 60-7550 was only detectable 30 min after drug administration (about 0.045 ng/g). Recent data from our lab suggests that brain exposure to Bay 60-7550 might be higher than that using a modified LC/MS method (data not published). However, even if minimal amounts of Bay 60-7550 are able to cross the blood brain barrier, it can still be taken into consideration that a low brain concentration of the compound would be sufficient to produce the cognitive enhancement. This is probably due to the rich expression of PDE2 in brain regions responsible for the object recognition memory, and the fact that Bay 60-7550 could affect both cyclic nucleotide (cAMP and cGMP) signal cascades, thereby causing biological responses because of signal amplification in the cascades. To support these hypotheses, a second, potent PDE2 inhibitor would be required in similar studies. Moreover, it is also possible that an active metabolite instead of Bay 60-7550 itself is responsible for the memory enhancing effects.

Though Bay 60-7550 is the primary PDE2 inhibitor used in the literature, ND7001 was synthesized around the same time (Abarghaz et al. 2005). It has an $IC_{50}$ of about 50 nM for human PDE2, with high selectivity over other PDEs (Gomez

and Breitenbucher 2013). Both Bay 60-7550 and ND7001 increase levels of cGMP in primary neuronal cultures; however, unlike Bay 60-7550, ND7001 is unable to significantly increase levels of cAMP (Masood et al. 2009). In France in 2005, Neuro3d (now Evotec AG) initiated Phase I clinical trials with ND7001 for the treatment of anxiety and depression. The results of these trials are unknown, and no further development has since been reported. As of 2010, the clinical trial appears to have been discontinued. Wyeth also published series of PDE2/10 dual inhibitors around that time, which exhibited slightly higher potency for PDE2, improved memory and cognition, and reduce anxiety behaviors after oral dosage to rodents (Gomez and Breitenbucher 2013). Based on modification of these structures, Boehringer Ingelheim International Gmbh published series of patents application disclosing two compounds with similar SAR, high selectivity for PDE2a over PDE10 (either 15- or 250-fold), and reported pro-cognitive, antidepressant, anxiolytic, and anti-convulsant effects in *in vivo* models in rodents. Janssen and Lundbeck also published a number of patent applications related to triazolopyrazine analogs, which could possess about 25-fold selectivity on PDE2, even though behavioral-related data were reported. Further down the road, Pfizer published a novel series of pyrazolopyrimidine PDE2 inhibitors, among which PF-05180999 ($IC_{50}$ = 0.001 μM, 2000-fold selective over PDE10) is now available from Sigma (CAS Number 1394033-54-5), and exhibited free brain/plasma ratio of 0.5 in rat (Gomez and Breitenbucher 2013). Pharmacological evaluation of the compound showed that it attenuated the ketamine-induced disruption of working memory, as well as scopolamine-induced spatial learning and memory. Between 2011 and 2012, the compound was reported to be well tolerated at doses between 0.1 and 60 mg in a phase I clinical trial aimed at determining the safety, tolerability, and pharmacokinetics after 14 days of treatment in healthy subjects. However, a similar phase I study planning to use twice daily doses over 14 days was withdrawn prior to enrollment due to safety concerns. For the purpose of evaluating the potential benefit from PDE2 inhibition in the central nervous system, most of the studies reviewed here is based on use of Bay 60-7550.

## 12.4 PDE2 in Cognitive Function

### *12.4.1 Basic Concepts of Memory and Cognition*

There are four main stages involved in learning and memory: acquisition, consolidation, storage and retrieval (García-Osta et al. 2012). Memory can further be divided into short-term memory (STM) and long-term memory (LTM). STM, also known as working memory, is able to maintain several items simultaneously for brief periods of time. Working memory provides temporary storage and manipulation of information for the brain and plays an important role in on-demand human cognition. Formation of LTM, on the other hand, may be based on the successful

processing of working memory. LTM can be divided into three categories based on the type of information: explicit memory (declarative memory), implicit memory (procedural memory), and autobiographical memory. The role of PDE2 in these memory processes will be discussed in this section.

### *12.4.2 Evidence for Involvement of PDE2 in Cognitive Function*

The expression of PDE2 in the CNS is primarily in limbic and cortical brain regions, including the hippocampus, all of which have been implicated in learning and memory (Gesellchen and Zaccolo 2011; Martinez 2006; Stephenson et al. 2009; Van Staveren et al. 2003). In fact, research has shown PDE2 inhibition is linked to enhance learning and memory in numerous cognitive tasks in rodents (Boess et al. 2004; Bollen et al. 2014; de Vente et al. 2006; Domek-Łopacińska and Strosznajder 2008; Lueptow et al. 2016; Redrobe et al. 2014; Reneerkens et al. 2013; Rodefer et al. 2012; Rutten et al. 2007a, b, 2009; Sierksma et al. 2013; van Donkelaar et al. 2008; Xu et al. 2015). Additionally, the underlying signaling involved in mediated memory enhancement following PDE2 inhibition appears to be primarily the nitric oxide synthase (NOS)/cGMP/protein kinase G (PKG) pathway, over the cAMP/protein kinase A (PKA) pathway (Bollen et al. 2014; Lueptow et al. 2016; Rutten et al. 2007a, b). Though, as will be discussed below, there is likely more to the story, including differential, time sensitive elevation of cAMP vs cGMP (Bollen et al. 2014; Rutten et al. 2007a, b).

In 2004, Boess and colleagues first reported the significant memory enhancing effects of Bay 60-7550 in the object recognition test (ORT) and social recognition test (SRT) (Boess et al. 2004). In the ORT, mice and rats were exposed to 2 identical objects. Immediately after training, they were injected with vehicle or Bay 60-7550. Twenty-four hours later the rodents were returned to apparatus and exposed to one of the familiar objects and one novel object. Mice treated with Bay 60-7550 showed significantly enhanced memory (Fig. 12.2). Similarly, an adult rat and juvenile rat were allowed to interact in the SRT. Immediately after the interaction, they were injected with vehicle or Bay 60-7550. Twenty-four hours later, they were reintroduced, and those treated with Bay 60-7550 showed significant decreases in time exploring the juvenile rats, indicated enhanced memory (Fig. 12.2).

In addition to behavior changes observed following Bay 60-7550 administration, these researchers have also demonstrated some of the *in vitro* mechanisms of Bay 60-7550 and PDE2. Incubation of Bay 60-7550 in primary cerebral cortical neurons from both mice and rats resulted in a dose-dependent increase in both cAMP and cGMP, though the changes of cGMP levels were much more dramatic than those of cAMP. This is perhaps due to the presence of several other cAMP metabolizing PDEs in the incubation systems. The results were consistent with the observation that the PDE2/adenosine deaminase inhibitor EHNA enhanced the NMDA receptor-

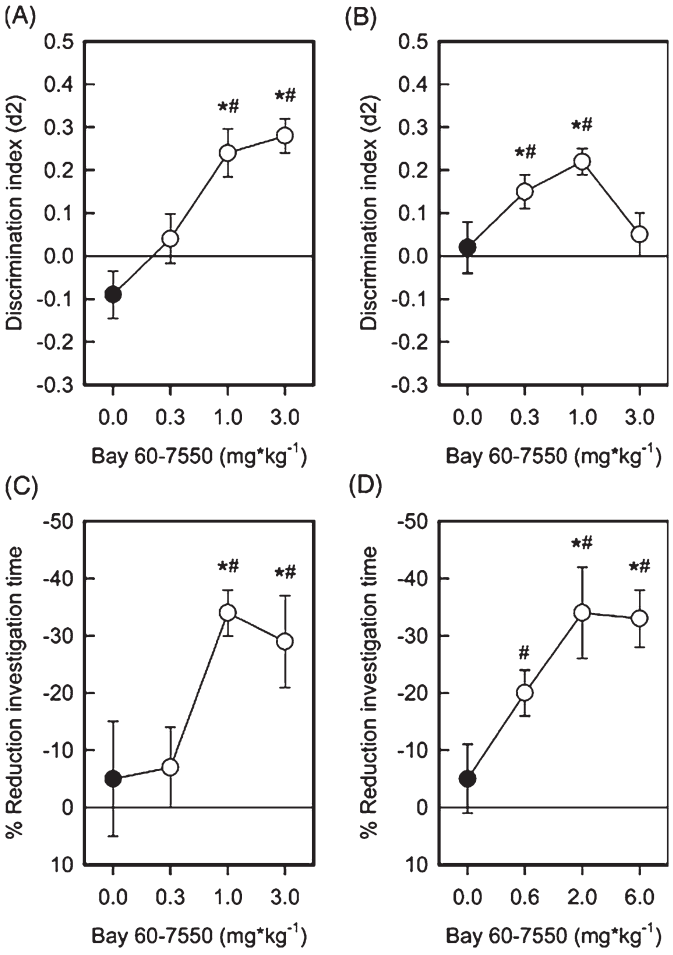


**Fig. 12.2** Effects of Bay 60-7550 on object recognition and social memory. Bay 60-7550 improved object recognition performance in rats (A), and mice (B), measured as an increase in the discrimination index d2 [d2 = exploration time new object/exploration time familiar object)/(total exploration time)]. Independent groups of rats were tested (12 rats were used for each treatment group). All mice (n = 24) were tested in all treatment conditions using a within sub- jects design with three or more days between treatments. Bay 60-7550 improved social memory in rats (C and D) measured as a reduction of the time a juvenile is investigated by an adult rat during the second encounter. Independent groups of rats were tested (10 rats were used for each treatment group).? $p < 0:05$ different from vehicle control (Fischer's LSD post hoc comparison). #$p < 0:05$ different from zero (one-sample t-statistics). (Reprinted by permission from Macmillan Publishers Ltd.: Neuropharmacology, (Boess et al. 2004) copyright 2004)

induced elevation of cGMP (but not cAMP) levels in rat cortical and hippocampal neurons in primary culture (Suvarna and O'Donnell 2002).

Bay 60-7550 also significantly increased long-term potentiation (LTP) in the CA1 region but did not affect basal synaptic transmission, suggesting a use-dependent enhancement of synaptic function. Based on these findings, the effect on

synaptic efficacy may be due to a PKG-mediated increase in neurotransmitter release. It is also possible that postsynaptic changes due to PKG-mediated phosphorylation of ion channels or other elements of signal transduction pathways, may also contribute to the changes in the strength of the postsynaptic response during LTP. Nevertheless, there is a clear role of NO/cGMP signal transduction in synaptic plasticity, and PDE2 is an important element of this pathway. Moreover, since both cAMP and cGMP are known to induce vasodilation, which could contribute to improved cognitive functions, Rutten et al. (2009) examined this possibility by measuring the effects of PDE inhibitors on local cerebral blood flow and glucose utilization in rats. It was shown that the cGMP hydrolyzing PDE5 decreased the ratio between blood flow and glucose utilization, indicative of general oligaemia, whereas the cAMP hydrolyzing PDE4 increased this ratio, indicative of general hyperemia. Both oligaemic and hyperemic conditions do not explain memory enhancement, suggesting that the memory enhancement induced by PDE inhibitors are not likely due to cerebrovascular effects.

### *12.4.3 Time-Sensitive Improvement of Memory Induced by PDE2 Inhibition*

Decades of memory research have suggested that memory enhancement as a result of increases in either cyclic nucleotide (cAMP or cGMP) is time-dependent. cGMP and cAMP have been proposed to participate in the early and late stages of LTP, respectively. Accordingly, cGMP-regulated processes in the hippocampus play an important role in the early stages of memory consolidation and cAMP signaling pathways are involved in the later consolidation mechanism.. It was shown that a post-training, intra-hippocampal infusion of 8 Br-cGMP (1.25 micrograms per side), a membrane-permeable analogue of cGMP, enhanced retention test performance when given immediately (0 min) after training, but not when given 180 min after training. Conversely, intra-hippocampal infusion of the same dose of 8 Br-cAMP facilitated memory consolidation when given 180 min, but not 0 min, after training. Moreover, rats submitted to an inhibitory avoidance task showed a significant increase in the amount of cGMP in the hippocampus at 0 and 30 min after training, and in the amount of cAMP at 30 and 180 min after training. By injecting the protein synthesis inhibitor anisomycin or the PKA inhibitor Rp-cAMPs at various time windows after training, it was discovered that contextual memory has either one or two brief consolidation periods requiring both synthesis of new proteins, and activation of PKA. Of these, weak training results in two time periods of sensitivity to inhibitors of protein synthesis and PKA, whereas stronger training exhibits only one. Inhibition of PDE2 may lead to increases in either cAMP, cGMP, or both. Therefore, use of a brain penetrant PDE2 inhibitor may not only affect different phases of memory formation and recall but also provide a better understanding of the underlying pathways that are involved in PDE2 inhibitor-induced changes in memory. Unfortunately, different research groups tend to use individual protocols

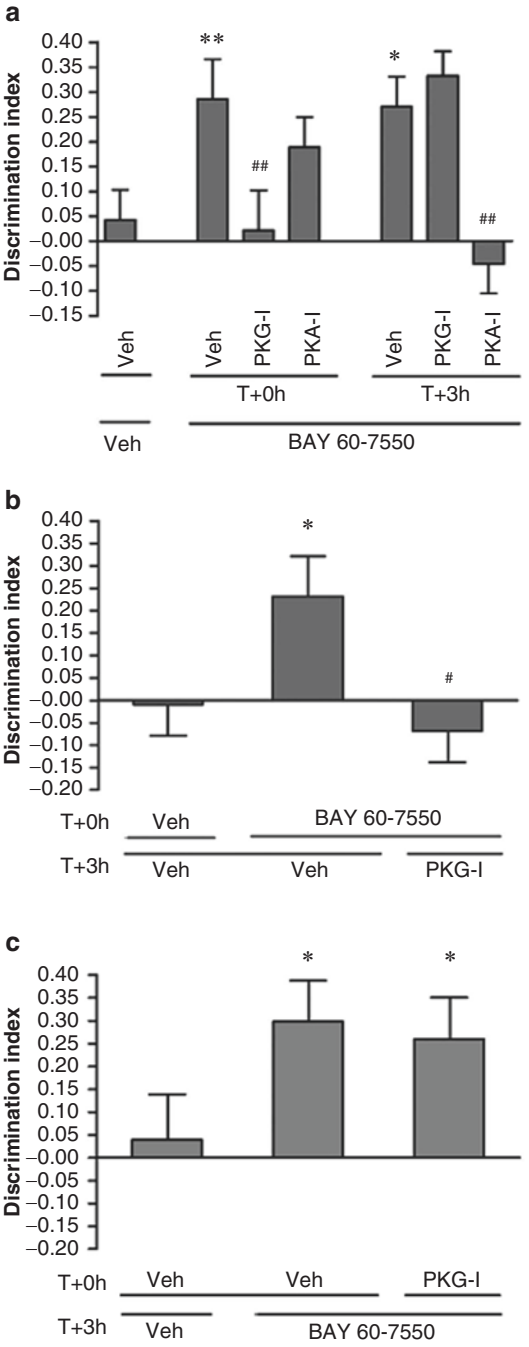


**Fig. 12.3** Role of cAMP vs cGMP in PDE2 inhibition. (**a**) Bay 60-7550 3 mg/kg, p.o., n = 26/19/18/19/18/16/19 on discrimination performance (discrimination index; means ± SEM) in a 24-h-delay object recognition task. PDE inhibition was administered immediately after

with different training and testing parameters, as well as different apparatuses, so observed time-dependent changes may be due to these protocol differences, rather than replicable changes in cAMP and cGMP (Bourtchouladze et al. 1998).

Studies by Rutten et al. have shown that Bay 60-7550 enhanced memory in the ORT to be time-sensitive as well. When administered immediately after training or 3 h. after training, Bay 60-7550 significantly enhanced memory in the ORT in rats (Fig. 12.3a). However, when given 1 or 6 h. after training, Bay 60-7550 did not affect memory in the ORT, suggesting PDE2 plays a role in specific early and later consolidation processes (Rutten et al. 2007a, b). In this same test, treatment with vardenafil, which is a PDE5 (a cGMP selective PDE) inhibitor immediately after training, but not 1 or 3 h after training resulted in enhanced memory, suggesting the efficacy of Bay 60-7550 during early consolidation is due to cGMP Alternatively, when rolipram, which is a PDE4 (cAMP selective PDE) inhibitor was given 3 h after training, there was a significant enhancement of memory, but not immediately after training or 1 h. after training, which suggests he memory enhancing effects of Bay 60-7550 when administered 3 h after training is due to enhacement of cAMP. Since Bay 60-7550 has a rather short elimination half-life of approximately 45 min after p.o. injection, it suggests that time windows for influencing early consolidation processes that are possibly via cGMP, and late consolidation processes that are possibly through cAMP, are specific and narrow. The results from the experiment with Bay 60-7550 support the notion of a specific and time-critical role of cGMP and cAMP in memory consolidation, which is also supported by data from the PDE4 inhibitor rolipram and the PDE5 inhibitor vardenafil (Rutten et al. 2007a, b).

Time-course analysis in mice resulted in a slightly different effect. Lueptow et al. showed that Bay 60-7550 administration 30 min prior to training, immediately after training, or 30 min prior to recall resulting in significantly enhanced object recognition memory, with no effect when administered 1 or 3 h. after training (Lueptow et al. 2016). These differences may be due to methodological disparities between the research groups, including between-subjects vs within-subjects design, different training schedules, as well as different apparatuses. Despite the species difference, housing protocols also vary. In studies with rats, subjects were singly housed, which

**Fig. 12.3** (continued) (T + 0 h) or 3 h (T + 3 h) after the first trial. When PDE inhibition combined with saline intra-hippocampal injections (veh) yielded significant improvement of discrimination, we subsequently combined PDE treatment with inhibitors of PKG (PKG-I; RP-8-Br-cGMPS 1 mg/side) or PKA (PKA-I; RP-8-Br-cAMPS 1 mg/side). (**b**) Bay 60-7550 3 mg/kg, n = 28/12/13) in combination with PKA inhibition (PKA-I; RP-8-Br-cAMPS; i.h.; 1 mg/side) 3 h (T + 3 h) after the first trial on discrimination performance (discrimination index; means ± SEM) in a 24-h-delay object recognition task. (**c**) PDE2 inhibition—Bay 60-7550 3 mg/kg, n¼9/10/11) administered 3 h after learning in combination with PKG inhibition (PKG-I; RP-8-Br-cGMPS; i.h.; 1 mg/side) immediately after the first trial on discrimination performance (discrimination index; meansþSEM) in a 24-h-delay object recognition task. Asterisks indicate significant differences (*p < 0.05, **p < 0.01) of PDE inhibition treatment from vehicle condition. Hashes indicate a significant reversal of PDE-induced memory improvement ((#)p < 0.056, #p < 0.05, ##p < 0.01). (Reprinted by permission from Macmillan Publishers Ltd.: Neuropsychopharmacology, (Bollen et al. 2014) copyright 2014)

may cause a type of stress that is related to social isolation. Social isolation in rats has been shown to result in a hyperactive state with increased novelty seeking and increases in exploration time during the ORT, which complicates the interpretation of both behavioral and biochemical outcomes following PDE2 inhibition in these rats. Numerous behavioral, neurochemical, and neurophysiological changes, as well as differential responses to psychotropic drugs are indeed associated with social isolation, which in turn can be used as a model for various psychiatric disorders, including schizophrenia (Bianchi et al. 2006; Douglas et al. 2003; Thorsell et al. 2006). In another sense, social isolation also leads to increased levels of anxiety-like behavior and results in altered hippocampal organization and hypothalamic–pituitary–adrenal axis function (Bianchi et al. 2006; Weiss et al. 2004). These changes are important to note because PDE2 inhibition is protective against stress-induced anxiety- and depressive-like behavior, as well as stress-induced deficits in learning and memory, further complicating the interpretation of results in socially isolated rats (Ding et al. 2014; Masood et al. 2009; Masood et al. 2008; Xu et al. 2013, 2015). Also, different route of drug administrations may also contribute to the discrepancies from rat and mouse studies. When Bay 60-7550 was given through oral gavage like most of the rat studies did, an additional gut absorption phase on top of the distribution to the brain needs to be taken into consideration.

Probing a little deeper, Bollen and colleagues have shown that PKG, but not PKA inhibition prior to Bay 60-7550 administration, which was given immediately after training, blocks memory enhancement in the ORT (Bollen et al. 2014). Additionally, PKA but not PKG inhibition prior to Bay 60-7550 administration, which was given 3 h. after training, blocks memory enhancement in the ORT (Fig. 12.3b). These findings again suggest that cGMP enhancement is critical for early consolidation mechanisms, while cAMP enhancement is critical for later consolidation mechanisms (Bollen et al. 2014). Additionally, administration of Bay 60-7550 immediately after training plus a PKA, but not PKG, inhibitor 3 h. after training, blocks memory enhancement in the ORT, suggesting that early cGMP activity requires later cAMP activity (Bollen et al. 2014) (Fig. 12.3c). This highlights the possibility that differences in the window for Bay 60-7550 memory enhancement between mice and rats mentioned previously may be due to a shift in the critical window for cGMP and/or cAMP enhancement. This time-course specific enhancement of cAMP vs cGMP demonstrates the time-dependent importance of both cyclic nucleotides in memory consolidation processes. Additionally, the differential regulation of cAMP/cGMP emphasizes the importance in furthering our understanding of the role of PDE2 at these different time points.

## 12.5 PDE2 in Cognitive Dysfunction

Memory impairments and cognitive decline are a significant health problem around the world, especially with aged population and individuals with neurodegenerative conditions such as Alzheimer's, depression, and schizophrenia. Extensive research

has been conducted to determine the fundamental biological mechanisms underlying not only learning and memory, but also cognitive dysfunction. Nevertheless, there is still a significant medical need for new drugs which delay or reverse these cognitive deficits. To date, most commercially available drugs are not able to delay or reverse cognitive deficits, but only relieve some symptoms during the mild to moderate stages of disease progression. These drugs are targeted to one of the major neurotransmitter systems, include the acetylcholine, serotonin, histamine, glutamate, and dopamine systems. While these classes of drugs are able to ameliorate some of the cognitive deficiencies temporarily, because they target large transmitter systems, they do come with some adverse side effects An alternate approach is to target downstream signaling cascades, which trigger the long term transcriptional and structural response to neuronal activity that underlies the formation of long term memories. PDE2 serves as an important potential biological target for therapeutic intervention in a variety of disorders, due to its important role in terminating transcriptional cascades triggered by cAMP and cGMP.

As mentioned earlier, research using ORT and similar memory tests has shown PDE2 inhibition enhances learning and memory in both mice and rats. In terms of translational possibilities, it is also important to analyze PDE2 in more pathological systems. To that end, the PDE2 inhibitor Bay 60-7550 has shown to be protective against a number of different memory-disrupting conditions. One approach is to disrupt ORT memory with pharmacologic agents, among which the NMDA receptor antagonist MK-801, as well as the anticholinergic agent scopolamine are two most commonly used ones. Scopolamine is a widely used anti-cholinergic agent to create a memory deficit model. It is often used to study the effects of drug administration before the learning trial (Klinkenberg and Blokland 2010). This has shown to impair memory in several behavioral tests including ORT. It was demonstrated that the cAMP-selective PDE4 inhibitor rolipram reversed a scopolamine-induced memory deficit in the ORT and other behavioral tests in rodents (Egawa et al. 1997; Rutten et al. 2006; Zhang et al. 2000). MK-801 is another commonly used agent to induce memory deficit in preclinical cognition research. It is an N-methyl-D-aspartic acid (NMDA) antagonist which impairs memory function and attention processes and is therefore assumed to be more affiliated to cognitive deficits related to schizophrenia (van der Staay et al. 2008). Treatment with Bay 60-7550 30 min before training reversed the memory impairment induced by both scopolamine and MK-801 (Reneerkens et al. 2013). With a 24 h. inter-trial interval, the discrimination index of control animals has been reported close to 0, therefore a 1 h. interval between T1 and T2 was used in this study in order to create a differentiated baseline between the normal animals and the scopolamine treated animals. Similarly, T-maze continuous alternation task is a measurement of predominantly spatial working memory. Boess et al. (Boess et al. 2004) demonstrated that administration of 3 mg/kg Bay 60-7550 (p.o.) 30 min before the test session reversed a MK-801-induced working memory deficit in the T-maze in mice, while vehicle-treated mice had an alternation rate higher than the chance level, mice injected with MK-801 (0.04 mg/kg) 30 min before the test had a lower continuous alternation rate.

Another method for disrupting memory involves depletion of tryptophan (ATD, acute tryptophan depletion), which results in depletion of serotonin in the brain and ultimately temporary deficits in cognition. ATD is frequently used to induce short-term deficit in brain 5-HT synthesis through manipulation of the essential amino acid tryptophan (TRP), the dietary 5-HT precursor. 5-HT along with its receptors have been found to be critically important for learning and memory. Through depletion of TRP from ingestion, less peripheral TRP is available to enter the brain for synthesis into 5-HT, thereby creating a so-called "serotonergic deficit model" and has been widely used to interfere with short-term memory in the rat object recognition task (Jans et al. 2007, 2010; Jans and Blokland 2008; Lieben et al. 2004; Olivier et al. 2009; Rutten et al. 2007a, b; van Donkelaar et al. 2008). It has been shown that only moderate peripheral TRP depletion or a single administration of a TRP-free nutritional mixture is required to generate the memory deficit model. Following serotonin depletion, 3 mg/kg Bay 60-7550 restored memory in the ORT with a 2 h. inter-trial interval (Eva L van Donkelaar et al. 2008). However, these behavioral changes induced by inhibition of PDE2 were not accompanied by altered levels of 5-HT or its precursor 5-HIAA (van Donkelaar et al. 2013), suggesting that mechanisms other than direct regulation of 5-HT neurotransmitter is involved in the process.

Memory loss can also result from unwanted daily stress. In learning and memory studies using rodents, two commonly used types of stress include the chronic unpredictable stress (CUS) and oxidative stress. CUS involves daily, randomly administered mild stressors, including wet cage bedding, short-term food/water deprivation, overnight lights on, etc., that, over time, result in increases in anxiety and depression, as well as decreases in cognitive abilities. In response to these repeated stressors, the brain undergoes a complex array of cellular and molecular changes that lead to maladaptive remodeling, which are correlated with the observed changes in behavior. Therefore, while much of stress research has focused on interventions for anxiety and depression, attenuating or reversing the deleterious effects of stress on cognitive processes is also important. PDE2 inhibition appears to be a promising avenue for such an intervention. Xu et al. found that treatment with Bay 60-7550 during the administration of CUS prevents cognitive impairment in ORT, object location test (OLT), and Morris Water Maze (MWM). Furthermore, the protective effects of PDE2 inhibition were shown to be mediated via the NO/cGMP/PKG pathway (Xu et al. 2015).

Oxidative stress, on the other hand, is often characterized by increased reactive oxygen species (ROS) generation and decreased antioxidant capacity, i.e. an unbalanced oxidant-antioxidant status in brain. Specifically, the brain consumes large amounts of oxygen, and when damaged, cellular respiration and enzymatic activity increase, resulting in increased ROS, which interact with fatty acids and proteins. The brain has an abundance of polyunsaturated fatty acids, but relatively less of antioxidant agents compared to other organs (Cheng et al. 2010; Mehta et al. 2012; Shelat et al. 2008). This results in damage to cell membranes, interruption of membrane-bound proteins, and altered cellular permeability, i.e. a pro-oxidative state. If such cellular stress is not effectively removed, ROS may cause oxidative cell injury, protein damage, and lipid peroxidation, and energy failure that finally

causes altered neuronal function and overall brain activity, leading to onset of neurodegenerative disease, such as cognitive deficits in AD, Parkinson's disease, as well as symptoms observed following traumatic brain injury.

The link between PDE2 and oxidative stress has been demonstrated in several in vitro studies (Drobna et al. 2012; Masood et al. 2008). In the mouse hippocampal cell line HT-22, oxidative stress results in an increase in the expression of PDE2. In endothelial cells, PDE2 promotes activation of NADPH oxidase-dependent ROS production and subsequent endothelial proliferation and angiogenesis. In yeast, PDE2 hydrolyzes cAMP, prevents PKA activation, and decreases the cellular stress responses. Recent *in vivo* data from our lab (not published) suggests that the memory enhancing effects of Bay 60-7550 may be through regulation of several subunits of the NADPH oxidase enzyme, which is a critical component responsible for a number of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis.

Besides the pharmacologically induced memory deficit models, a more clinically relevant model in terms of pathology is the triple transgenic mouse line that results in Alzheimer's disease (AD) -like state. AD is one of the most prevalent neurodegenerative diseases, characterized by memory loss, personality changes, psychiatric symptoms, and severe cognitive dysfunction. It is the most frequent form of dementia found in the elderly. In the early stages of AD, there are more manageable symptoms such as impaired short-term memory; however, in later stages of the disease, individuals have almost complete loss of their cognitive abilities and are unable to function on their own, putting a great burden on friends and family members (Taylor et al. 2013).

Currently, there are only a few FDA approved drugs to treat AD symptoms, such as cholinesterase inhibitors (ChEIs) (tacrine, donepezil, rivastigmine and galantamine) and N-methyl-D-aspartate (NMDA) receptor modulators (memantine) (Nguyen and Woo 2003). However, while these drugs may temporarily ameliorate short term memory loss during earlier stages of the disease, no drugs are able to slow or reverse the course of AD (Guan et al. 2011). Therefore, researchers are interested in identifying effective drug targets that may either slow the progression of the disease in order to allow the AD patients to remain socially active and productive or to completely halt, and potentially reverse, the disease course.

The neuropathology of AD involves the overproduction and accumulation of the peptide amyloid β (Aβ), as well as neurofibrillary tangle formation leading to synaptic dysfunction. The first and most severely affected brain regions are those involved in learning, memory and other higher cognitive function (Eckert et al. 2013). Abnormal cleavage of Aβ results insufficient clearance and subsequent accumulation. Research shows that Aβ oligomerization decreases the phosphorylation of the transcription factor CREB and impairs LTP. This reduction in CREB phosphorylation and change in LTP is likely dependent on several kinases, such as PKA and PKG, which are downstream of cyclic nucleotide activation (Y. Chen and Cai 2003; Sanderson and Sher 2013). Coincidentally, reduction in the activity of specific PDEs have shown to enhance memory performance in different animal models of AD (Vitolo et al. 2002). PDE inhibitors that elevate concentrations of cAMP and/or

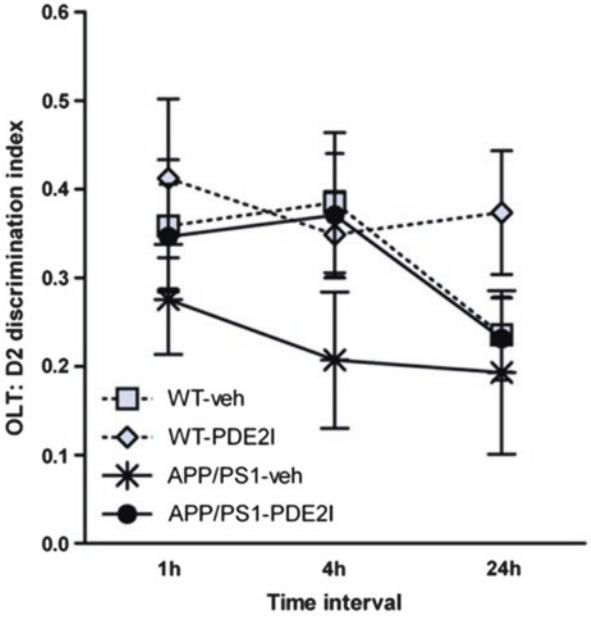


**Fig. 12.4** Performance in the object location task (OLT) over 3 intervals (1 h, 4 h, 24 h). Values indicate mean and S.E.M per group. Although all animals were able to signifi- cantly distinguish between the novel and the familiar location at the 1 h and 4 h interval, i.e. $D2 > 0$, $p < 0.05$, it is interesting to note that the APPswe/PS1dE9-PDE2I group has almost the same absolute D2 scores as theWT-veh group in all intervals. At the 24 h interval APPswe/PS1dE9-veh group is not significantly able to distinguish between the novel and the familiar location, while the other groups are. (Reprinted by permission from Macmillan Publishers Ltd.: Neuropharmacology, (Sierksma et al. 2013) copyright 2012)

cGMP result in stimulation of the cAMP/PKA/CREB and cGMP/PKG/CREB pathways, respectively, to enhance synaptic transmission through increasing CREB phosphorylation and BDNF transcription. The effect of PDE2 inhibition in improving memory in the APPswe/PS1dE9 mouse model of AD has been demonstrated by Sierksma et al. (2013). Chronic treatment of the APPswe/PS1dE9 mice with Bay 60-7550 significantly improved their performance in the object location test with 1, 4 or 24 h. inter-trial intervals, and Y-maze test. Behaviors related to depression or anxiety were not affected by such treatment. Interestingly, Bay 60-7550 treatment did no alter Aβ amyloid plaque load, phosphorylation of CREB, BDNF or synaptic density in those animals (Figs. 12.4), suggesting that the memory enhancing effects of PDE2 inhibition in this AD model may not be through canonical signaling transduction.

Other types of memory deficits include those associated with aging or neuropsychiatric disorders. Studies have shown that aged brain contains a lower cGMP concentration and altered nitric oxide synthase activity (Domek-Łopacińska and

Strosznajder 2008; Liu et al. 2003). NO is an important signaling molecule in the brain that is responsible for stimulation of the soluble isoform of guanylyl cyclase (sGC), which synthesizes cGMP. During physiological aging, cognitive performance of the brain decreases, and this is often correlated with decreases of cGMP in the brain. Accordingly, inhibition of PDE2 can not only improve memory function in young adult rats, but also in 12 and 24 month-old rats (Domek-Łopacińska and Strosznajder 2008). All age groups benefited from Bay 60-7550 administration when given during memory acquisition or consolidation. Moreover, nNOS activity in all age groups also increased in response to Bay 60-7550 treatment, and blockade of nNOS activity by its selective inhibitor NAAN prevented the memory enhancing effects of Bay 60-7550. Since phosphorylation of NOS by PKA or PKG diminishes the catalytic activity of NOS rather than reinforcing it (Dinerman et al. 1994), the activity of cyclic nucleotide gated (CNG) calcium channels following elevated cGMP concentrations may be responsible for the increased activity of NOS. Interestingly, only Bay 60-7550 improved the object recognition memory in the 24 month-old rats, suggesting that the activity of PDE2 over other PDEs, specifically PDE5, is the most influential at that time point. Overall, these data suggest that inhibition of PDE2 increases cGMP levels not only by suppression of cyclic nucleotide degradation, but also by further stimulation of cyclic nucleotide synthesis through the NOS/NO/sGC/cGMP pathway.

## 12.6 PDE2 and Neural Plasticity

Synaptic plasticity is a critically important property of neurons that is thought to underlie learning and memory. Neuroplasticity involves both functional plasticity (regulation of synaptic transmission) and structural plasticity (such as adult neurogenesis) (Lopez et al. 2009; Mohs 2008). In the context of memory and cognition, function neuroplasticity emphasizes the role of LTP, which is a long-lasting increase in the efficacy of synaptic transmission that has been proposed as a cellular substrate underlying mammalian learning and memory (Mehta et al. 2012). Various studies have also shown that LTP and memory formation are dependent on cellular cascades stimulated by an increase of intracellular cAMP/cGMP with the subsequent activation of PKA/PKG (Domek-Łopacińska and Strosznajder 2010). A growing body of evidences indicates that cAMP/PKA/CREB and the cGMP/PKG/CREB pathways are involved in LTP and long-term memory formation (Eckert et al. 2013; Vitolo et al. 2002).

As previously discussed, cAMP is a vital intracellular second messenger involved in signaling pathways that are initiated via neurotransmitters, hormones, and many other molecules. Protein kinase A (PKA) is considered to be the main downstream kinase activated by cAMP. Phosphorylated/activated PKA leads to phosphorylation/activation of CREB and increased production of BDNF. Accumulating data regarding CREB-dependent learning and memory in mice, rats, and drosophila all indicate that CREB activation by phosphorylation at the serine residue 133 is implicated in

plasticity of synapses necessary for the maintenance of LTP and formation of long-term memory (Ermak and Davies 2002; Titus et al. 2013). Moreover, the critical genes known to be involved in neuroplasticity, such as BDNF, are also known to be affected by CREB activation. In both AD and in Huntington's disease models, the PKA inhibitor H-89 can block the activation of CREB as well as memory improvement induced by rolipram (Sanderson and Sher 2013). The dominant-negative CREB mutation as well as CREB isoform deletions can affect memory formation and long-term synaptic changes, as well as alter nerve growth and regeneration in mice (Kishida and Klann 2007). Due to the large involvement of cAMP in such processes, it is reasonable to speculate that synaptic plasticity can be modulated by manipulation of the activity of PDE2 as well (Mseeh et al. 2000). Indeed, numerous studies show that cellular cAMP levels are affected by concentrations of $Ca^{2+}$ indirectly via manipulation of the adenylyl cyclase and PDE activity (Ota et al. 2008; Reneerkens et al. 2013; Rutten et al. 2007a, b).

Similarly, cGMP is an important second messenger generated by two varieties of guanalyl cyclases (GCs). One is cytosolic soluble GC (sGCs) activated by nitric oxide (NO) and the other is membrane-bound particulate GC (pGCs) activated by natriuretic peptides. In the central nervous, the NO-sensitive sGC isoform is the major enzyme responsible for cGMP synthesis. Numerous researchers have shown that the NO/sGC/cGMP/PKG signal transduction pathway plays a significant role in coordinating presynaptic and postsynaptic alterations that underlie long-term synaptic plasticity and memory formation (Maes et al. 1993; Puzzo et al. 2005). NO is a highly reactive radical produced by nitric oxide synthase (NOS), which can be either neuronal (nNOS), endothelial (eNOS) or inducible (iNOS). Several studies reveal that nNOS and eNOS are abundant in brain areas critical for learning and memory such as the hippocampus, relying on the $Ca^{2+}$/calmodulin enzymes for activation, whereas inducible NO synthase contains tightly bound calmodulin and is permanently activated (Bonkale et al. 1995; Francis et al. 2010; Puzzo et al. 2005). Neuronal NOS is the most relevant enzyme in terms of cGMP activation, as it is strategically positioned near NMDAR to generate NO involved in the cGMP signaling in hippocampus (Tsai and Kass 2009). In vitro research has shown that pharmacological manipulation of NO signaling in the lateral amygdala using either a NOS inhibitor or a membrane-impermeable scavenger of NO impaired memory consolidation in auditory fear conditioning in mice (Fischmeister et al. 2005). Moreover, in AD, cognitive deficits are accompanied by the formation of beta-amyloid (Aβ) plaques, which deactivates the NO/cGMP/PKG pathway and decrease the phosphorylation of CREB during hippocampal synaptic plasticity (Knight and Yan 2012). Accumulating evidence indicates that both the NO donor DEA/NO and cGMP analogs counteract the Aβ-induced impairment in CREB phosphorylation and LTP. Appropriately, deletion of the gene encoding NO synthase 2 (NOS2) results in worsening of the AD phenotype in APP mouse model (Rybin et al. 2000). According to Bonkale et al., the concentration of sGC in AD patients is less than normal (Buxton and Brunton 1983). Additionally, NO-dependent LTP in rat hippocampal and amygdala slices was inhibited by the sGC inhibitor, but enhanced by the sGC activator (Houslay and Milligan 1997). Together, the data suggest that the

NO-mediated modulation of synaptic plasticity is a key step in learning and cognition and that Alzheimer's disease may involve an sGC-cGMP-dependent mechanism. The final concentration of cGMP in cells depends on the balance of not only its synthesis via sGC but also the degradation by PDEs. There is substantial evidence indicating that cGMP-specific PDEs are involved in memory and synaptic plasticity (Mokni et al. 2010). In fact, current research suggests that the primary mechanism of PDE2 inhibitors is mediated through NO-cGMP signaling.

Organotypic slice co-cultures of the nigrostriatal dopaminergic system in combination with biocytin tracing and tyrosine hydroxylase labeling has been used to examine the neural outgrowth induced by PDE2 inhibitors (Heine et al. 2013). The quantification shows a significant increase of tyrosine hydroxylase-positive fiber density in the border region of the co-cultures induced by PDE2 inhibitors BAY60-7550 and ND7001, as well as significantly increased expression of nerve growth factor. This is supported by prominent PDE2A immunoreactivity found near the striatum in the isocortex, hippocampus, and basal ganglia, as well as a high expression in the substantia nigra and ventral tegmental area on cell bodies and terminals. Moreover, the growth-promoting effect of the PDE2 inhibitors was compared with the stimulating capacity of NGF, which is known to promote neuronal survival and elicit neuronal fiber outgrowth. The action of NGF, as well as other neurotrophic factors such as BDNF and GDNF, is mainly through a cAMP-independent signaling pathway involving Ras, PKC, and extracellular signal-regulated protein kinase. It has been shown that both cAMP and cGMP induces neuronal differentiation in PC12 cells (Laasberg et al. 1988; Ng et al. 2009). As a critical enzyme that modulate the concentration of both cAMP and cGMP, PDE2 is surely involved in survival, repair and remodeling of the central nervous system both during development and after injury. Moreover, the cellular and subcellular localization of PDE2 might determine its role in modulating neuronal plasticity. PDE2 staining has been found in the hippocampal CA1 area, especially the stratum oriens, while little expression was observed in somatic regions or proximal dendrites of pyramidal neurons. In CA3, highest staining was observed in the subiculum, the hilus and the area where the mossy fiber terminates. These data suggested that PDE2 might be highly expressed in axons and axon terminals (Stephenson et al. 2012). Moreover, PDE2 staining in CA3 did not overlap with the dendritic marker MAP2 (Stephenson et al. 2012), while in hippocampal cultures PDE2 is partially co-localized with the presynaptic marker synaptophysin (Russwurm et al. 2009), suggesting that PDE2 may modulate LTP at a presynaptic location. However, whether PDE2 inhibitors modulate a component of LTP that is expressed pre-synaptically or postsynaptically has not been directly addressed.

The increase of use-dependent enhancement of synaptic function (LTP) in hippocampal CA1 region induced by Bay 60-7550 is mainly through regulation of cGMP. In the hippocampus, cGMP levels were increased in the perforant pathway, which is next to varicose fibers throughout the hippocampus. The main cGMP increase might take place post-synaptically, followed by a PKG-mediated increase in neurotransmitter release, or phosphorylation of ion channels in the signal trans-

duction pathway, which are characteristic of induced changes in strength of the postsynaptic response during LTP (Lynch 2004). In the study by Boess et al., it was demonstrated that sub-maximal LTP in the CA1 region induced by a weak tetanic stimulation of the Schaffer-collateral pathway was strengthened by 10 or 100 nM of Bay 60-7550 (Boess et al. 2004). Treatment with Bay 60-7550 starting 20 min before the weak tetanic stimulation caused a significantly larger potentiation of the field excitatory postsynaptic potential (fEPSP) slope than that observed in control slices stimulated in a similar fashion. Higher concentrations of Bay 60-7550 induced a longer-lasting strengthening of LTP observable up to 40 min after stimulation. The lowest concentration of Bay 60-7550 tested (1 nM), did not change the potentiation of the fEPSP slope compared to control.

Inhibition of PDE2 by Bay 60-7550 may also reverse the neuronal morphological changes in hippocampal CA1 region induced by chronic stress (Xu et al. 2015). In the stressed animals, the neuronal atrophy was characterized by reduced number of dendritic apical branching points, shortening of the basal and apical dendrites, and the spine density. However, the morphologic changes within the CA1 neurons seen during chronic unpredictable stress were not apparent in mice treated with 3 mg/kg Bay 60-7550. The CA1 neurons are sensitive to stress, even though less well characterized morphologically compared with that of CA3. Restoration of neuronal plasticity, reflected by increased dendritic branches and neuronal sprouting in the CA1, very well correlated with the behavioral changes related to memory and cognition in the same study. Moreover, the neuroprotective effects of Bay 60-7550 were blocked by the NMDA receptor inhibitor MK-801, the calcium calmodulin kinase II (CaMKII) inhibitor myr-AIP, and the PKG inhibitor KT5823, suggesting a role of NMDAR-CaMKII-nNOS-cGMP pathway on dendritic remodeling during stress. Alteration of neuroplasticity affected by stress hormones may also be reflected by change of expression of plasticity-related proteins. The effects of Bay 60-7550 on Egr-1 and BDNF, both related to hippocampal memory, mirror those found with neuronal morphology, further suggesting that function of PDE2 may be involved with both presynaptic and postsynaptic mechanism. Reactive oxygen species have also been shown to impair LTP, learning and memory, and biochemical signal transduction cascades that are believed underlying LTP and memory formation (Du et al. 2013). Tau et al. showed that impairments in hippocampal synaptic plasticity and memory in AD mice can be alleviated by decreasing mitochondrial ROS (Diebold et al. 2009). Additional research has also shown that the age-related LTP impairments in hippocampal area CA1 or the DG are in part due to the increase in ROS levels (Diebold et al. 2009; Ermak and Davies 2002; Mehta et al. 2012; Rutten et al. 2007a, b). It is thought that the destructive properties are mediated through abnormalities in mitochondria and increased NADPH oxidase subunit expression (Du et al. 2013; Ota et al. 2008; Reneerkens et al. 2013; Zhang et al. 2012). While ROS serve an appropriate biological function in regulating synaptic plasticity-related signaling molecules, including NMDA receptors, $Ca^{2+}$ and $K^{+}$ channels, CaMKII, ERK, and CREB, excessive ROS can be extremely destructive (Duszczyk et al. 2012). On the other hand, Aβ-induced inactivation of PKA/CREB signaling can be blunted by SOD/catalase or pharmacological scaven-

gers of ROS, by increasing phosphorylation of PKA at C subunit Thr 197, which functions to eliminate pro-oxidant that could otherwise promote the generation of excessive ROS (Calabrese et al. 2007). Studies from the O'Donnell group demonstrate that the PDE2 inhibitor Bay 60-7550 mediates decreases in oxidative stress, namely ROS expression and NADPH oxidase subunits p47 phox and gp91 phox in cultured neurons (Puzzo and Sapienza 2008). Thus, we conjecture that PDE2 inhibitors increase intracellular cAMP and enhance antioxidant capacity, which would disturb Aβ-induced inactivation of PKA/CREB signaling, and promote the synaptic plasticity.

## 12.7 PDE2 in Psychiatric Illnesses

Emotional processing takes place in many of the same brain regions responsible for learning and memory. Therefore, it would not be surprising that PDE2 also plays a role in mood disorders and other psychiatric illnesses. While the etiology and pathogenesis of many of these disorders is unknown, current research suggests inhibition of PDE2 may ameliorate some of the symptoms of a variety of mood and psychiatric illness. For example, PDE2 has proven to be protective against stress-induced anxiety and depression in mice (Ding et al. 2014; Masood et al. 2008, 2009; Xu et al. 2013, 2015). The potential role of PDE2 in various psychiatric disorders is discussed below.

### *12.7.1 PDE2 and Schitzophrenia*

Neuropsychological testing of individuals with schizophrenia reveals significant cognitive deficits in attention, working memory, and executive functioning. Administration of phencyclidine (PCP) has been reported to induce positive, negative symptoms as well as cognitive impairments that are characteristic of schizophrenia (Rodefer et al. 2012). Subchronic administration of rats with PCP induces behavioral inhibition based on new or changing information about conditioned stimulus-reward associations, spatial memory, and latent inhibition, and causes neurochemical changes in rats resembling those in patients with schizophrenia. Selective atypical antipsychotics have been shown to attenuate these symptoms. However, since the process involves multiple neurotransmitter systems, there is still lack of efficacy in current treatments. Targeting intracellular second-messenger systems that affect multiple neurotransmitter systems might provide a feasible alternative. Extradimensional-intradimensional (ED/ID) task is a rodent cognitive that is similar to those used in humans and non-human primates for assessing executive function. It is sensitive to the effects of lesions, natural aging, and pharmacological manipulations. Rodefer et al. (Rodefer et al. 2012) demonstrated that sub-chronic treatment with PCP produced a selective impairment on ED shift

(EDS) performance without significant impairment on any other discrimination problem when compared to saline-treated control animals. 3 mg/kg Bay 60-7550 attenuated this cognitive deficit in EDS performance. Similarly, a newer PDE2 inhibitor Lu AF64280 attenuated sub-chronic PCP-induced deficits in novel object exploration in rats, and blocked early postnatal PCP-induced deficits in the ED/ID shift task in rats and attenuated spontaneous auditory gating deficits in DBA/2 mice (Redrobe et al. 2014). As mentioned previously, Bay 60-7550 can improve performance in rodent object recognition tasks, which is mainly dependent on the proper functioning of hippocampus and amygdala. These data suggested that inhibition of PDE2 might also contribute to cognitive enhancement through frontal mechanism.

### *12.7.2 PDE2 and Depression*

Major depressive disorder (MDD) is currently the most common mood disorder in the United States, and is characterized by a pervasive and persistent negative mood, loss of interest in daily activities, and anhedonia. The fundamental mechanism of action of most antidepressants involves the enhancement of levels of monoamines (serotonin, norepinephrine and/or dopamine) at the synaptic cleft. This may result in modulation of the hypothalamus-pituitary-adrenal (HPA) axis and other major neurotransmitter systems, as well as increased neuroplasticity and LTP (Andrew Alt et al. 2006; Hebb and Robertson 2007; O'Donnell and Zhang 2004). While the basic mechanism of action for these drugs is simple, the downstream effects of these drugs may be numerous.

Antidepressants can be broken down into a few categories based on their specific targets. Monoamine oxidase inhibitors (MAOIs), which inhibit the degradation of monoamines, were the first compounds discovered to have antidepressant properties. The next major classes of antidepressants are collectively known as the reuptake inhibitors, which block the reuptake of neurotransmitters such as serotonin, norepinephrine, and/or dopamine. While having different drug targets, the biochemical outcome for both classes of antidepressants is the enhancement of synaptic monoamine levels. However, despite the variety of currently available therapeutics, only about one third of patients respond to their first treatment and up to one third do not respond at all and are considered treatment resistant (Aan het Rot et al. 2009). Other limitations include delayed treatment response, relapse, and intolerable side effects for some patients. Research has indicated that the improvement of depressive symptoms may instead be due to changes in the intracellular messaging systems, such as differential transcription, translation and expression of downstream receptors (Zhang et al. 2002, 2006). As a result, instead of focusing on alterations in neurotransmitter levels, current research is focusing on intracellular signaling cascades, of which the cyclic nucleotide second messenger system has become a primary interest.

More recently PDEs have stepped into the spotlight, due to their role in "turning off" the cyclic nucleotides, mainly cAMP and cGMP. Both cAMP and cGMP regulate respective downstream protein kinases, which activate transcription factors, ultimately controlling the expression of specific genes that have been shown to be involved in the expression of depressive symptoms or symptom relief (Burgin et al. 2010; Zeller et al. 1984; Zhang et al. 2002). Inhibition of PDE4 has shown robust antidepressant-like effects in rodent behavior testing (Benes et al. 2001; Lakics et al. 2010; Shaywitz and Greenberg 1999), especially the prototypic inhibitor rolipram that entered phase II clinical trials (Dwivedi and Pandey 2008). Unfortunately, due to its lack of PDE4 subtype specificity, rolipram resulted in unpleasant side effects such as nausea and emesis, which hindered it from reaching the market (Shaywitz and Greenberg 1999). Researchers are currently exploring the utility of more selective PDE4 subtype inhibitors that do not appear to cause emesis (Yamada et al. 2003).

Meanwhile, with the continued development of novel, more selective PDE inhibitors, researchers have been able to investigate the potential antidepressant-like properties of other PDEs. Among these, PDE2 has received particular interest, not only because of its ability to hydrolyze both cyclic nucleotides, but also because of its high levels expression in regions of the brain known to be involved with depression (Dwivedi and Pandey 2008).

As mentioned previously, the cAMP pathway is one of the major secondary messaging systems activated by numerous neurotransmitter families and is responsible for relaying ligand-receptor signals within cells, such as initiation of signaling to the nucleus for alterations in gene transcription. In the context of depression, it is thought that, following elevation of neurotransmitter levels by typical antidepressants, cAMP signaling leads to increased transcription of genes for various receptors and neurotrophic factors that enhance and stabilize neuronal outgrowth over time (O'Donnell and Zhang 2004). The slow development of clinical relief may be related to gene transcription, indicating the clinical response may be partially dependent upon the neuronal changes that happen via the cAMP signaling system specifically (H.-T. Zhang et al. 2002). For example, increased cAMP signaling activation of cAMP-dependent protein kinase A (PKA) leads to phosphorylation of a serine residue (S133) on the transcription factor cAMP response element binding protein (CREB), which, in the presence of the transcriptional co-activator CREB binding protein (CBP), then stimulates the transcription of cAMP responsive genes (Conti and Blendy 2004; Dash et al. 1991; Nestler et al. 2002; Sheng et al. 1991; Sun et al. 1994). Genes that are targeted via this pathway have been shown to be involved in several aspects of neuronal functioning, including the development of central nervous system, excitation of nerve cells, long-term synaptic plasticity and survival of neuronal cells (Alt et al. 2005; Saarelainen et al. 2003). Coincidentally, CREB mRNA levels and phosphorylated/activated CREB (pCREB) are reduced in postmortem brain samples of depressed patients (Conti et al. 2002). Antidepressants, on the other hand, are known to induce pCREB and CREB mRNA expression in the hippocampus, which is believed to be partially responsible for change of behaviors (Menniti et al. 2006). In addition, kinases that are activated by intracellular calcium,

including the calcium/calmodulin-dependent kinases (CaMKII & CaMKIV), have been reported to activate or inhibit CREB transactivation depending on the serine residues that are phosphorylated (Boess et al. 2004; Xu et al. 2013). While CaMKII phosphorylates and activates S133 on CREB, it has a second phosphorylation site at S142, which is thought to block transcriptional activation, whereas CaMKIV only phosphorylates CREB at S133, and is therefore a more potent activator of CREB (Malenka and Nicoll 1999). One major transcriptional outcome of CREB activation is thought to be the production of brain-derived neurotrophic factor (BDNF) (A Alt et al. 2005; Menniti et al. 2006). Indeed, in CREB knockout mice, antidepressants were not able to increase BDNF levels (Sheng et al. 1991). Moreover, there is a reciprocal interaction between CREB and BDNF that results in a positive feedback loop. This amplification mechanism is potentially critical for the trophic effects of antidepressants, i.e., pCREB activates BDNF production, BDNF induces phosphorylation/activation of CREB, pCREB activates BDNF production, etc. (Conti and Blendy 2004; Duman et al. 1997). Despite these findings, CREB-deficient mice show a normal behavioral response to antidepressants, indicating that the antidepressant effects are not achieved solely through the activation of CREB (Hayashi et al. 1999).

One alternative mechanism for the enhancement of the cAMP pathway includes inhibition of PDE2, which has dual substrate affinity for both cAMP and cGMP. Research has shown that the PDE2 inhibitor Bay 60-7550 is able to increase cAMP concentrations in the presence of forskolin, an activator of adenylyl cyclase, which is responsible for the synthesis of cAMP (Raison et al. 2006). In a chronic stress-induced depressive mouse model, Bay 60-7550 appears to have neuroprotective effects behaviorally (Ding et al. 2014). While the main behavioral effects seem to be conferred through cGMP/PKG signaling (discussed below), Bay 60-7550-induced increases in cAMP levels that led to changes in phosphorylation of ERKs, members of the mitogen-activated protein kinase (MAPK) family. These kinases mitigate cellular stress by interacting with nuclear targets involved in neuronal survival and plasticity, including CBP that acts as a transcriptional coactivator with several other transcription factors and components of the RNA polymerase II, as well as the neurotrophic factor BDNF. Therefore, inhibition of PDE2 appears to be a valuable drug target for enhancing cAMP signaling.

Other potential pathways affected by increases in cAMP levels include the α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor-induced signaling cascades belong to the family of ionotropic glutamate receptors. These receptors are expressed ubiquitously throughout the central nervous system and mediate the majority of rapid excitatory neurotransmission. They are not only important in regulating postsynaptic depolarization and neuronal firing, but they are also involved in a variety of other cellular responses, including the recruitment of voltage-gated ion channels and NMDA receptor activity, and the development and expression of long-term synaptic plasticity (Essayan 2001). There is also an indication that AMPA receptors are involved in the induction of neurotrophic factors including BDNF. Specifically, when stimulated by cAMP, PKA phosphorylates the GLUA1 subunit of AMPA receptors at Serine831/845 residues, activating the recep-

tor and leading to an induction of BDNF expression through both the $Ca^{2+}$-dependent and $Ca^{2+}$-independent pathway. The former is through increased AMPA receptor conductance for $Ca^{2+}$, which leads to activation of $Ca^{2+}$/calmodulin-dependent protein kinase (CaMK)/CREB/BDNF signal transduction pathways (Gustafsson and Brunton 2002; Seybold et al. 2005). The latter is through a mitogen-activated protein kinase (MAPK) pathway mediated by Lyn, a member of the Src family of protein tyrosine kinases, which is physically associated with AMPA receptor subunits and can be activated upon AMPA receptor activation (Feil et al. 2005). While PDE2 has not been directly linked to the AMPA receptor-mediated signaling cascades, the role of these molecular pathways in the action of current or novel antidepressants has been established, and inhibition of PDE2 may be an indirect contributor via the increase in cAMP. Therefore, future research into these potential PDE2-cAMP-AMPA receptor connections is worthwhile, especially for the development of novel PDE2 inhibitors that may influence activation of these transduction pathways.

More recently, cytokines have been implicated in the pathogenesis of depression, and, coincidentally, cAMP is known to interact with cytokines. Data indicate that immune activation, as reflected by increased plasma and CSF concentrations of different cytokines including IL-1, IL-2, IL-6 and TNF-α, is associated with depression (Domek-Łopacińska and Strosznajder 2005). The mechanism of cytokine effects on depressive behavior are believed to be related in part to their ability to desensitize glucocorticoid receptors (GR) leading to glucocorticoid resistance (Lein et al. 2007). Stress exposure, a well-known inducer for depression, has been shown to activate inflammatory signaling pathways and induce glucocorticoid resistance in neuroendocrine and immune tissues in mice (Gur et al. 2007). To date, there are no direct evidence suggesting that PDE2 inhibition alleviates depressive symptoms via cytokine regulation and decreased neuro-inflammation processes. Nevertheless, it has been implicated that PDE2 is involved in certain immune responses (Corbin et al. 2000), including neuro-inflammatory processes in which cytokines such as TNF-α has been shown to induce a dramatic increase in PDE2 levels (X. Liu et al. 2013). Reciprocally, a PDE2 inhibitor EHNA is able to restore the cAMP accumulation that is previously reduced by IL-1β treatment (Snyder et al. 1999).

The cGMP signaling pathway is another intracellular nucleotide cascade that has also been implicated in various neuropsychiatric disorders. While the cGMP cascade has some overlap with cAMP signaling, the system has received far less attention in terms of its relation to MDD and antidepressant activities. The major downstream effector of cGMP is the protein kinase G (PKG), which belongs to a family of serine/threonine kinases. There are two major subtypes of PKGs, based upon their subcellular localization. PKGI, the cytosolic form, is mainly distributed in mammalian brain regions including Purkinje cells of the cerebellum, the hypothalamus, hippocampus, olfactory bulb and amygdala, most of which have high concentrations of PDE2 expression (RALL and SUTHERLAND 1958). PKGII, the membrane bound form of PKG, is also widely expressed throughout the rodent brain, including the cerebral cortex, cerebellum, brainstem, thalamus, septum, amygdala and olfactory bulb (Fujishige et al. 1999; L. Wang et al. 2005). Interestingly, the palmitoylation of certain splice variants of PDE2 primarily anchors

the enzyme to the membrane, where it functions as a component in the signaling complexes assembled in lipid rafts. This compartmentalization of PKGII and PDE2 in specific cellular microdomains provides an excellent example of the way in which the large PDE family exerts such precise control over cyclic nucleotide signaling, and offers a cellular explanation for the specific role of PDE2 in mood disorders (American Psychiatric Association Task Force On DSM-IV 2000). One function of the activated PKGs is to phosphorylate and activate PDEs that are located in these distinct macromolecular complexes, which in turn decrease the levels of cGMP, forming a negative feedback loop (Crisp et al. 2005). In cell culture, Bay 60-7550 was able to reverse corticosterone-induced neurotoxicity in different types of neuronal cells. In both cortical and hippocampal neurons, Bay 60-7550 was also found to potently increase the cGMP concentrations in the presence of guanalyl cyclase activators (Maes et al. 1993). In a mouse model of chronic stress-induced depression, Bay 60-7550 was able to reverse the behavioral abnormalities in mice, which were accompanied by significant increases in cGMP levels. These effects were blocked by administration of the PKG inhibitor KT5823, but not the PKA inhibitor H89, indicating that the antidepressant-like effects of Bay 60-7550 on behavior were mediated largely through cGMP/PKG (Bierhaus et al. 2003). Additional evidence has implicated involvement of cGMP-related pathways in neurogenesis, which is a critical marker for alleviation of depressive disorder. In an *in-vitro* study using neurospheres, activation of cGMP was shown to stimulate neurogenesis through the PI3-K/Akt/GSK-3 (phosphoinositide 3-kinase/protein kinase B (aka AKT)/glycogen synthase kinase 3) pathway (Beer et al. 1972). And Xu et al. (2015) has demonstrated that Bay 60-7550 was able to reverse the impairment of neuronal morphology in hippocampal CA1 and CA3 induced by chronic stress.

### *12.7.3 PDE2 and Anxiety Disorders*

Anxiety disorders are a class of mood disorder characterized by excessive rumination, worrying, uneasiness and fear about future uncertainties either based on real or imagined events (Barton et al. 2004). Current psychiatric diagnostic criteria recognize a wide variety of anxiety disorders, including generalized anxiety disorder, phobic disorder, and panic disorder. Recent surveys have found that as many as 18% of Americans and 14% of Europeans may be diagnosed to have one or more of the above symptoms, indicating a great need for improved treatment options (Wang et al. 2013). Both psychological and pharmacological therapies are recognized to be effective management strategies, with a combination approach often being most successful. While benzodiazepines are frequently used to treat anxiety for the past few decades, more recent findings suggest that both selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are a safer, more tolerable first-line therapy for most of the anxiety disorders (Arranz et al. 2007), particularly for those that suffer comorbidity with depression. Selective serotonergic agents are especially preferred for certain subtypes of anxiety, such as

obsessive-compulsive disorder. Other antidepressants, such as tricyclic antidepressants (TCAs) or monoamine oxidase inhibitors (MAOIs), are generally reserved for second- and third-line strategies due to tolerability issues (Arranz et al. 2007). Anticonvulsants and atypical antipsychotics have been shown to have adjunctive beneficial effects as adjunctive treatments with antidepressants in cases of treatment resistance, while azapirones have been used effectively for generalized anxiety disorders, especially for panic disorder (Bouayed et al. 2007). Despite notable advances, many patients with anxiety disorders have a low tolerance to certain classes of drugs or fail to adequately respond to other pharmacological treatments. Researchers have focused on combined pharmacologic-psychosocial strategies and new drug targets to address treatment resistance and tolerability issues in order to increase overall success rate of remission for anxiety disorders.

Cyclic nucleotide signaling has been implicated in numerous cellular processes known to be important for proper functioning of regions of the brain specifically involved in anxiety disorders. Currently anxiolytics appear to ameliorate behavioral symptoms through either direct or indirect manipulation of the cyclic nucleotide pathways. For example: (i) potent anxiety-reducing agents are also potent inhibitors of brain PDE activities; (ii) dibutyryl cyclic adenosine monophosphate, a cell permeable cAMP analog, has the ability to reduce anxiety; (iii) the methylxanthines, semi-products of purine degradation that have great selectivity for PDEs, show significant anxiety-reducing effects (Kuloglu et al. 2002; Puzzo and Sapienza 2008).

Stress and anxiety are often closely related. Glucocorticoids and adrenal steroid hormones (i.e., corticosterone in rodents) are released in response to physical and psychological stressors, and their dysregulation is often implicated in anxiety disorders. Secretion of these hormones is the final step in the neuroendocrine cascade, known as the hypothalamic-pituitary-adrenal (HPA) axis, which is triggered following stimulation of the hypothalamus and pituitary gland. Elevated corticosterone in rodents after stress is the hallmark of the feed-forward activation of the HPA axis, which is also observed in clinically anxious patients (Gur et al. 2007; Masood et al. 2009). PDE2 is highly expressed in the rat adrenal cortex and pituitary gland (Masood et al. 2008; Raison et al. 2006). It may function as a regulator of the release of these stress hormones, specifically, aldosterone in the adrenal cortices and glucocorticoids from the pituitary gland (Masood et al. 2009). Reduction of PDE2 activity through pharmacological inhibition of this enzyme may intervene in certain stress-induced anxiety disorders via increases in intracellular cGMP concentrations (Abel and Lattal 2001). Investigation into the underlying processes involved in the protective effect of PDE2 inhibition from stress-induced changes in anxiety and depression have implicated a few different systems. Cell culture work using primary hippocampal neuronal cultures showed that corticosterone increases PDE2 expression in a dose-dependent manner, and in HT-22 cells, Bay 60-7550 was protective against corticosterone-induced cell death (Xu et al. 2013). In the same cell line, corticosterone application resulted in decreased cAMP and cGMP levels. Bay 60-7550 restored cGMP, but not cAMP levels. Additionally, a PKG inhibitor blocked the protective effects of Bay 60-7550 in the HT-22 cells, suggesting this mechanism is via the cGMP/PKG pathway (Xu et al. 2013). While CUS signifi-

cantly increases serum corticosterone levels in stressed mice, treatment with Bay 60-7550 was able to reverse these effects and return corticosterone levels to baseline. Potential anxiolytic-like effects of Bay 60-7550 was demonstrated using the elevated plus maze and marble burying test with the CUS mice (Ding et al. 2014). As a continuation of previous studies, it was further shown that pretreatment with Bay 60-7550 every day before stress effectively prevented the decrease of Cu/Zn superoxide dismutase (SOD) that could have been induced by CUS, supporting the notion that inhibition of PDE2 could protect the neurons from oxidative stress (Masood et al. 2008). Moreover, CUS-induced oxidative damage to the brain is often characterized by neuronal apoptosis, a programmed cell death that is critically controlled by the balance between pro- and anti-apoptotic proteins. Within the Bcl-2 family, Bcl-2 and Bax belong to the pro-survival and pro-apoptotic subfamily, respectively. Bax promotes the release of apoptogenic factors such as cytochrome c, which in turn stimulates initiator caspases, leading to the activation of effector caspases such as Caspase 3, a main executor of the apoptotic process. Hippocampus and amygdala in the study both expressed higher levels of Bax and Caspase 3 after CUS, while a clear regional specificity Bcl-2 expression were seen in the two brain regions (Ding et al. 2014). These changes indicative of neuronal apoptosis in the hippocampus and amygdala were prevented by pretreatment with Bay 60-7550, suggesting another mechanism of action involved with the anxiolytic effects of PDE2 inhibition. Another stress model that has been used to provide direct evidence

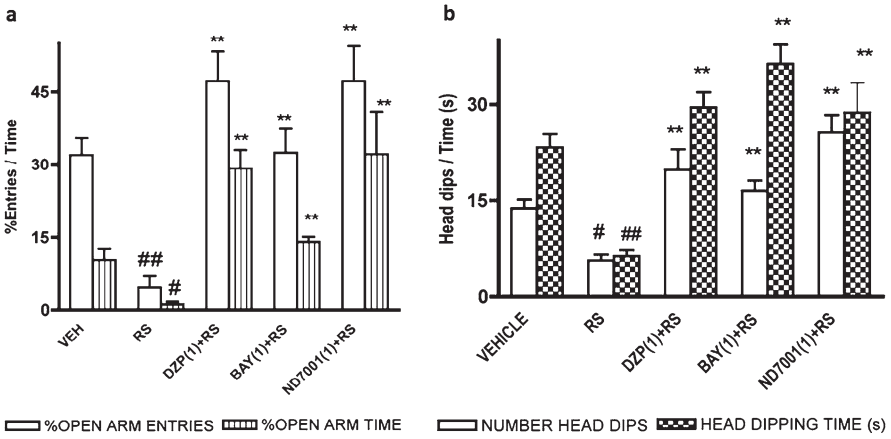


**Fig. 12.5** Effects of the PDE2 inhibitors Bay 60-7550 (BAY) and ND7001 on behavior in the (**a**) elevated plus-maze and (**b**) hole board test in stressed mice. Increases in percentages open-arm entries and time indicate an anxiolytic effect, whereas decreases in these measures indicate an anxiogenic effect. Increases in head-dips and time of head-dipping indicate an anxiolytic effect, whereas decreases in these measures indicate an anxiogenic effect. Values are expressed as means ± S.E.M. (n = 6–8). #, $p < 0.05$ and ##, $p < 0.01$ compared with vehicle (VEH). ** $p < 0.01$ compared with restraint stress (RS). *DZP* diazepam. Doses shown parenthetically are milligrams per kilogram intraperitoneally. (Reprinted by permission from Macmillan Publishers Ltd.:,(Masood et al. 2009) copyright 2012)

for the benefit of PDE2 inhibition in anxiety disorder is the restraint stress (Masood et al. 2009). Increased cGMP signaling, either by administration of the PDE2 inhibitors Bay 60-7550 (0.5, 1, and 3 mg/kg) or ND7001 (1 mg/kg), or the NO donor detanonoate (0.5 mg/kg), antagonized the anxiogenic effects of restraint stress on behavior in the elevated plus-maze, hole-board, and open-field tests. These effects were antagonized by the GC inhibitor ODQ (20 mg/kg). By contrast, the NOS inhibitor L-NAME (50 mg/kg), which reduces cGMP signaling, produced anxiogenic effects similar to restraint stress, suggesting a critical role for NO/GC/cGMP signaling (Fig. 12.5).

Increasing evidence has also implicated signs of increased reactive oxygen generation and decreased antioxidant capacity in anxiety disorder (Kang et al. 2013; Rolls et al. 2013). The findings of Kuloglu et al. show that patients with anxiety disorders have higher activity levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase, an indication of high oxidative stresses, as well as higher lipid peroxidation activity compared to healthy controls (Reierson et al. 2011). Therefore, oxidative metabolism manipulation is considered a plausible pathway for the regulation of anxiety. Masood and colleagues first uncovered a direct role for the protective effects of PDE2 inhibition against oxidative stress-induced changes in behavior. Using the pharmacologic agent and inducer of oxidative stress, L-buthionine-(S,R)-sulfoximine (BSO), they induced an anxiety-like phenotype in mice. They were then able to pretreat with PDE2 inhibitor Bay 60-7550 and block the BSO-induced anxiety-like behavior (Masood et al. 2008).

Despite accumulating evidence for the anxiolytic-like effects of PDE2 inhibitors, further investigation is still required. Future research should focus on exploring the precise trafficking and subcellular localization of PDE2 splice variants, specifically in the context of potential signaling partners, in order to gain a better understanding of the crosstalk between PDE2, cyclic nucleotides, and other PDEs. In addition, researchers must continue to investigate the exact underlying working mechanisms of selective PDE2 inhibitors by using central administration paradigms, blood flow measurements, and parallel behavioral experiments. Taken together, PDE2 inhibitors offer a promising target for mood disorders.

## 12.8 PDE2 and Pain

Inflammatory pain is a condition of hypersensitivity in nociception that is accompanied by inflammation. One of the cardinal features of inflammation is allodynia, or a state in which normally innocuous stimuli produce pain. Chronic pain due to inflammation is often difficult to manage and pharmacologic treatment options are quite limited. Therefore, pain management has significant unmet medical need and is currently affecting a large population of patients suffering from arthritis, pancreatitis, colitis, cystitis, and other autoimmune diseases. Existing therapeutics such as NSAIDs are limited in scope, while more powerful analgesics, such as opiates, have a high rate of tolerance, addiction, and withdrawal effects, as well as narrow

therapeutic window at high doses. Therefore, it is important to identify new medicines with novel mechanisms of action for effective treatment of patients with chronic pain (Tramèr et al. 1998).

The involvement of PDE2 in the production and modulation of pain has been summarized by Plummer et al. (2013b): (1) PDE2 is highly expressed in tissues responsible for nociception, i.e., brain, spinal cord, dorsal root ganglia (DRG), as well as non-pain related tissues including adrenal cortex, heart, and platelets; (2) The non-hydrolyzable cGMP analog, 8-bromo-cGMP, when delivered intrathecal, reduces nociceptive behavior in rats at low doses in formalin-induced inflammatory pain, but causes hyperalgesia when injected at higher doses (Tegeder et al. 2002); (3) PDE2 mRNA expression is increased after formalin injection; (4) PDE2 inhibitor with higher potency (oxindole, Bay 60-7550) have effect on nociceptive behavior. In their study, series of structural modification were carried out on PDE4 inhibitors to optimize residual PDE2 activity while minimizing PDE4 activity at the same time. Modified compounds exhibited a cGMP-like binding mode to the enzyme, high selectivity to PDE2 (Plummer et al. 2013b). Among these, compound 22, pyrazolodiazepinone, was greater than 1000-fold selective for PDE2 (Plummer et al. 2013a), and had significant analgesic activity in a rat model of osteoarthritis pain.

## 12.9 Conclusions and Future Studies

PDE2 clearly plays an important role in both mood and cognitive functions. The research summarized above demonstrates the pro-cognitive effects of PDE2 inhibition, as well as the potential of PDE2 as a target for treatment of cognitive impairment. However, while researchers have identified critical periods when PDE2 inhibition exerts its effects, those appear to differ between mice and rats. While the reasons for this are currently unknown, replication and further elucidation of the time-course and its underlying mechanisms are essential for any hope of successful translation to human use. Additionally, PDE2 inhibitors have shown to be protective against stress-induced changes in anxiety, depression, and cognition, on a behavioral and cellular level. The potential applications for this are far reaching, especially when considering the potential use in individuals exposed high-stress/high-risk environments, such as military in combat situations. However, for such application, further investigation needs to be done, specifically with models of post-traumatic stress disorder.

While our knowledge of PDE2 in mood and cognitive disorders has greatly advanced over the past 10 years, our overall understanding of PDE2 in the CNS remains in its infancy. Researchers must not only continue to uncover the role of PDE2 in rodent behavior, but also the underlying cellular and molecular pathways responsible for mediating those changes. Nonetheless, research has confirmed the potential of PDE2 and/or members of its signaling pathway as therapeutic targets for drug discovery for the treatment of mood and cognitive disorders.

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

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# Chapter 13
# Phosphodiesterase 1: A Unique Drug Target for Degenerative Diseases and Cognitive Dysfunction

**Lawrence P. Wennogle, Helen Hoxie, Youyi Peng, and Joseph P. Hendrick**

**Abstract** The focus of this chapter is on the cyclic nucleotide phosphodiesterase 1 (PDE1) family. PDE1 is one member of the 11 PDE families (PDE 1–11). It is the only phosphodiesterase family that is calcium/calmodulin activated. As a result, whereas other families of PDEs 2–11 play a dominant role controlling basal levels of cyclic nucleotides, PDE1 is involved when intra-cellular calcium levels are elevated and, thus, has an "on demand" or activity-dependent involvement in the control of cyclic nucleotides in excitatory cells including neurons, cardiomyocytes and smooth muscle. As a Class 1 phosphodiesterase, PDE1 hydrolyzes the 3′ bond of 3′-5′-cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Here, we review evidence for this family of enzymes as drug targets for development of therapies aimed to address disorders of the central nervous system (CNS) and of degenerative diseases. The chapter includes sections on the potential for cognitive enhancement in mental disorders, as well as a review of PDE1 enzyme structure, enzymology, tissue distribution, genomics, inhibitors, pharmacology, clinical trials, and therapeutic indications. Information is taken from public databases. A number of excellent reviews of the phosphodiesterase family have been written as well as reviews of the PDE1 family. References cited here are not comprehensive, rather pointing to major reviews and key publications.

**Keywords** Phosphodiesterase 1 • PDE1A • PDE1B • PDE1C • Cyclic nucleotide • Calcium-Calmodulin Stimulation • Phosphorylation • GM-CSF • Gene knockout • Smooth muscle cells • CNS Disease • Cognitive dysfunction • Parkinson's disease • Heart failure • Schizophrenia • Vinpocetine • ITI-214 • Dopamine D1 Receptor • Neuroprotective • NOR Model

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## 13.1 Introduction and Focus

The focus of this chapter is on the cyclic nucleotide phosphodiesterase 1 (PDE1) family. PDE1 is one member of the 11 PDE families (PDE 1–11). It is the only phosphodiesterase family that is calcium/calmodulin activated. As a result, whereas other families of PDEs 2–11 play a dominant role to control basal levels of cyclic nucleotides, PDE1 is involved when intra-cellular calcium levels are elevated and, thus, has an "on demand" or activity-dependent involvement in the control of cyclic nucleotides in excitatory cells including neurons, cardiomyocytes and smooth muscle. As a Class 1 phosphodiesterase, PDE1 hydrolyzes the 3′ bond of 3′-5′-cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Here, we review evidence for this family of enzymes as drug targets for development of therapies aimed to address disorders of the central nervous system (CNS) and of degenerative diseases. The chapter includes sections on the potential for cognitive enhancement in mental disorders, as well as a review of PDE1 enzyme structure, enzymology, tissue distribution, genomics, inhibitors, pharmacology, clinical trials, and therapeutic indications. Information is taken from public databases. A number of excellent reviews of the phosphodiesterase family have been written (Bender and Beavo 2006a) as well as reviews of the PDE1 family (Goraya and Cooper 2005). References cited here are not comprehensive, rather pointing to major reviews and key publications.

## 13.2 Structure

The PDE1 family of enzymes includes three genes, PDE1A, PDE1B and PDE1C, with the following human gene names:

PDE1A: NCBI Gene NP_001245241.1;
PDE1B: NCBI Gene NP_000915.1;
PDE1C: NCBI Gene NP_001177987.1.

PDE1 enzymes are globular, mainly cytosolic proteins. As is the rule in the PDE superfamily, PDE1 enzymes exist as dimers of identical subunit enzymes. The human PDE1B enzyme is depicted in Fig. 13.1 as a stick diagram showing a 536 amino acid protein with an N-terminal regulatory domain (aa 1–197) containing two calmodulin binding sites, a catalytic domain (aa 198–496) containing an H-loop, a dimerization domain, two helix and a M-loop, and a C-terminal extension. The role of the C-terminal is not well understood. Sites of phosphorylation of PDE1 on the N-terminal have been proposed (Kakkar et al. 1999), and may influence calmodulin binding affinity (Sharma et al. 2006), but in general the role of phosphorylation of PDE1 is poorly understood (Beltman et al. 1993). Details of the N-terminal domain including calmodulin binding domains have been proposed (Sonnenburg et al. 1995).

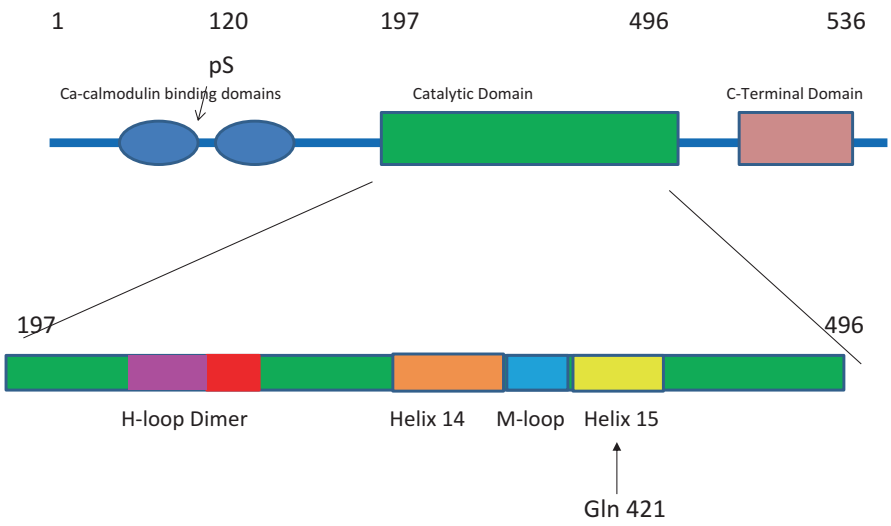



**Fig. 13.1** Stick diagram of the secondary structural details of hPDE1B

Several crystal structures of the catalytic core of PDE1 enzymes have been published (Card et al. 2005), including structures with bound inhibitors (Humphrey et al. 2014). An ITI-214 inhibitor-bound crystal structure from our work was recently published (Li et al. 2016). Docking of a PDE1 inhibitor, taken from the patent literature, to a model enzyme catalytic core structure we developed is shown in Fig.13.2. Inhibitors to this class of enzymes are generally competitive for the cyclic nucleotide binding site. Since the publication of the first PDE catalytic domain crystal structure of PDE4, the cyclic nucleotide binding site of this PDE family has been well detailed. This site includes domains of a hydrophobic pocket, a region referred to as a "lid region", a metal binding site and a core pocket. For PDE1 enzymes the active site also includes the Gln421 "switch" (number from hPDE1B isoform) (Zhang et al. 2004). This Gln421amino acid accommodates both cAMP and cGMP, hence referred to as a switch in the case of the PDE1 family, allowing for enzyme activity towards both cyclic nucleotides.

PDE1 isoforms are highly conserved across species (Fig. 13.3). This high degree of amino acid sequence conservation is greater than the sequence conservation seen between human isoforms of PDE1 A, B and C, indicating a fundamental important role of each distinct isoform. Similarly, the sequences of human PDE1A, B, and C are highly homologous (Fig. 13.4). Sequence homology/identity between PDE1 A, B and C can be used to predict potential selectivity of inhibitors. The ~85% homology between these three isoform enzymes at the level of the catalytic domain supports the prediction that inhibitors selective to one PDE1 isoform would be difficult to discover. On the other hand, PDE1 enzymes are quite distinct from other PDE families (2–11) as shown in Fig. 13.5, and PDE1 inhibitors that are highly selective for the PDE1 class over all other classes are now available. This will be discussed

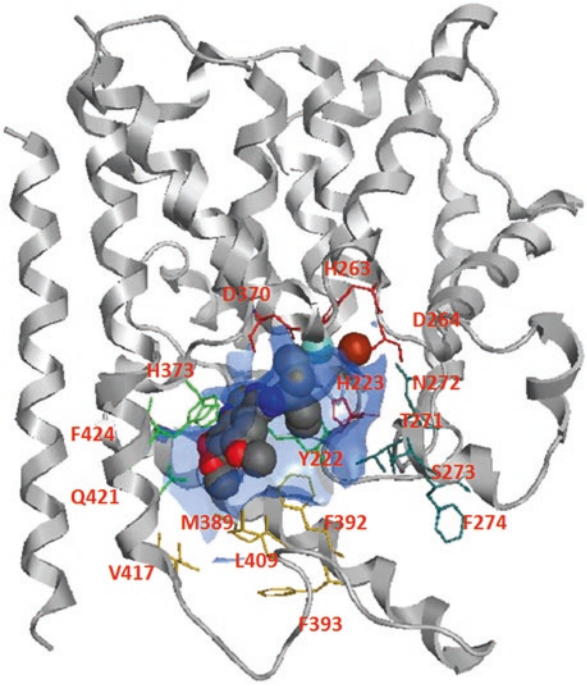
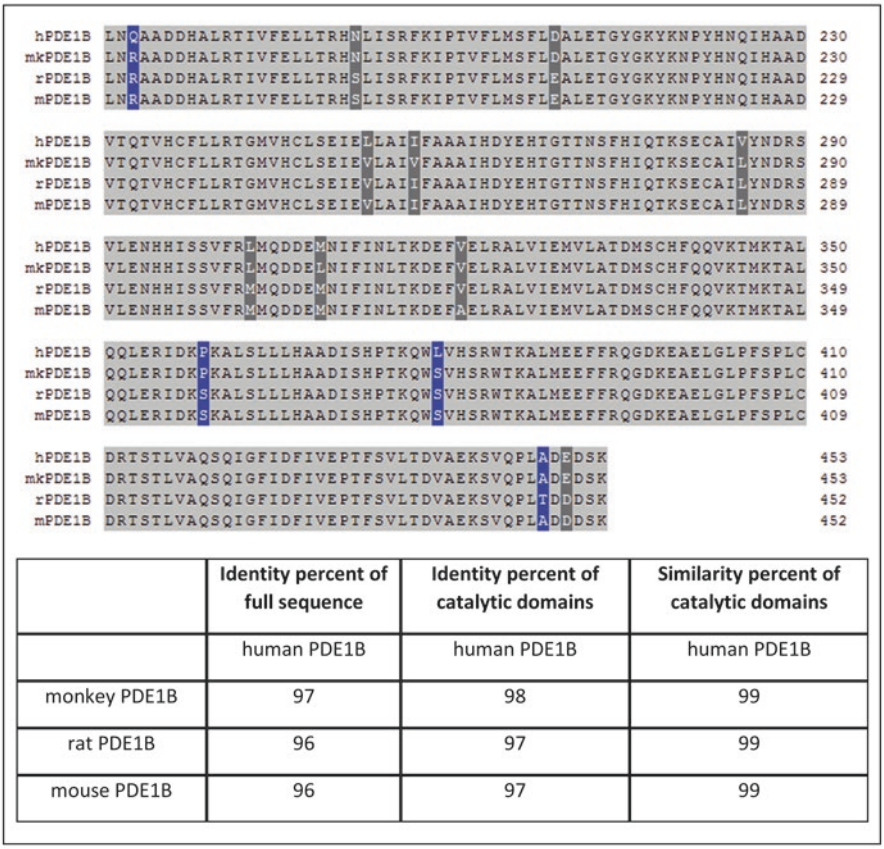



**Fig. 13.2** Structure of human PDE1B and inhibitor PF-04471141 (PDB ID: 4NPV). PDE1B is shown in gray ribbon and PF-044711141 is shown in a space-filled model and colored by atom-type. Amino acids participating in the binding of inhibitor are shown and labeled. Zinc and Magnesium ions are shown in cyan and red balls. The molecular surface of the binding pocket is shown in light blue. The core pocket is in green; the lid region is in dark cyan; the metal binding pocket is in red, and the hydrophobic pocket is in yellow

later. With the most recent class of inhibitors being described below, the agents are sufficiently specific to be suitable for target validation studies.

The possibility has been raised of a calcium-activated protease called calpain to cleave PDE1 enzymes between the calmodulin domains and the catalytic domain (Kakkar et al. 1999). This cleavage, if it occurs, would unleash the enzyme from control by calcium and calmodulin and would be likely to further contribute to the progression of degenerative diseases (Sharma et al. 2006). In Fig. 13.6, the cleavage site for PDE1B is depicted as between amino acid (aa) 126 and 127 of the human enzyme (Sharma et al. 2006).

PDE1 N-terminal calmodulin binding domain structure has not been resolved in high resolution, but structural information is included in Fig. 13.6. A conserved potential phosphorylation site just C-terminal from the second calmodulin domain at Threonine 148 of hPDE1B is present. Calcium-calmodulin activated kinase II (CaMKII) is responsible for phosphorylation of PDE1B, while PKA is able to phosphorylate PDE1A and C (see Table 13.1) (Sharma et al. 2006; Florio et al. 1994; Heredia et al. 2003). Phosphorylation results in a decreased affinity of the enzyme



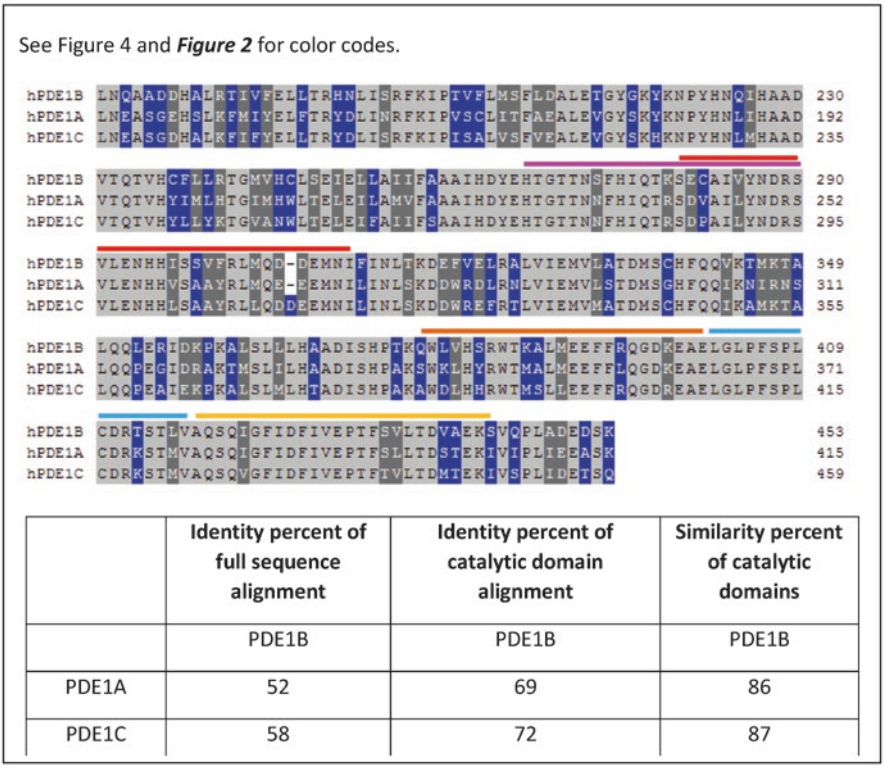

| | Identity percent of full sequence | Identity percent of catalytic domains | Similarity percent of catalytic domains |
|---|---|---|---|
| | human PDE1B | human PDE1B | human PDE1B |
| monkey PDE1B | 97 | 98 | 99 |
| rat PDE1B | 96 | 97 | 99 |
| mouse PDE1B | 96 | 97 | 99 |

**Fig. 13.3** Alignment of the amino acid sequence of the catalytic domain of PDE1B across species. Sequence alignment of the catalytic domains of PDE1Bs from different species. *h* human, *mk* rhesus monkey, *r* rat, *m* mouse. *Blue* non-conserved substitution, *Dark Grey* conserved substitution, and *Light Grey* identical amino acids. Alignment of sequences was performed with the CLUSTAL W multiple sequence alignment program. Definitions for similarity and identity are given in a manuscript by Higgins et al. (1996)

for calmodulin (Florio et al. 1994). In the first calmodulin binding domain there is a basic nine amino acid insert in PDE1C versus 1A and 1B (Fig. 13.6 bottom). The implication of this insertion is not understood.

## 13.3 Enzymology

As discussed above, the basic architecture of cyclic nucleotide phosphodiesterases includes N-terminal regulatory domains attached to C-terminal catalytic domains. Among the upstream regulatory motifs in different (non-PDE1) PDE families are




| | Identity percent of full sequence alignment | Identity percent of catalytic domain alignment | Similarity percent of catalytic domains |
|---|---|---|---|
| | PDE1B | PDE1B | PDE1B |
| PDE1A | 52 | 69 | 86 |
| PDE1C | 58 | 72 | 87 |

**Fig. 13.4** Sequence alignment of the catalytic core of the three human PDE1 isoforms. The color bars above the sequences indicate different motifs as shown in Fig. 13.1. See Fig. 13.3 and Fig. 13.1 for color codes

| | 1A | 1C | 2A | 3A | 4B | 4D | 5A | 6A | 7A | 8A | 9A | 10A | 11A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PDE1B | 69 | 72 | 25 | 36 | 39 | 40 | 24 | 25 | 31 | 32 | 28 | 20 | 28 |
| PDE1B | 52 | 58 | 20 | 26 | 24 | 25 | 15 | 18 | 21 | 18 | 18 | 12 | 17 |

**Fig. 13.5** Sequence identity comparison among PDE families. *Top* row is for the sequence identity as a percent of catalytic domain amino acids. *Bottom* row is for the sequence identity as a percent of full length alignments

nucleotide-binding (GAF) domains, ligand binding PAS domains, and UCRs (for Upstream Conserved Region). There are no such domains in the PDE1 family. Instead, unique to the PDE1 family, are Calcium-Calmodulin ($Ca^{2+}$/CAM) binding motifs (Bender and Beavo 2006a). Regulation in the PDE1 family occurs via tandem upstream calcium-calmodulin binding motifs (Bender and Beavo 2006a). All three PDE1 enzymes catalyze the hydrolysis of both cAMP and cGMP cyclic

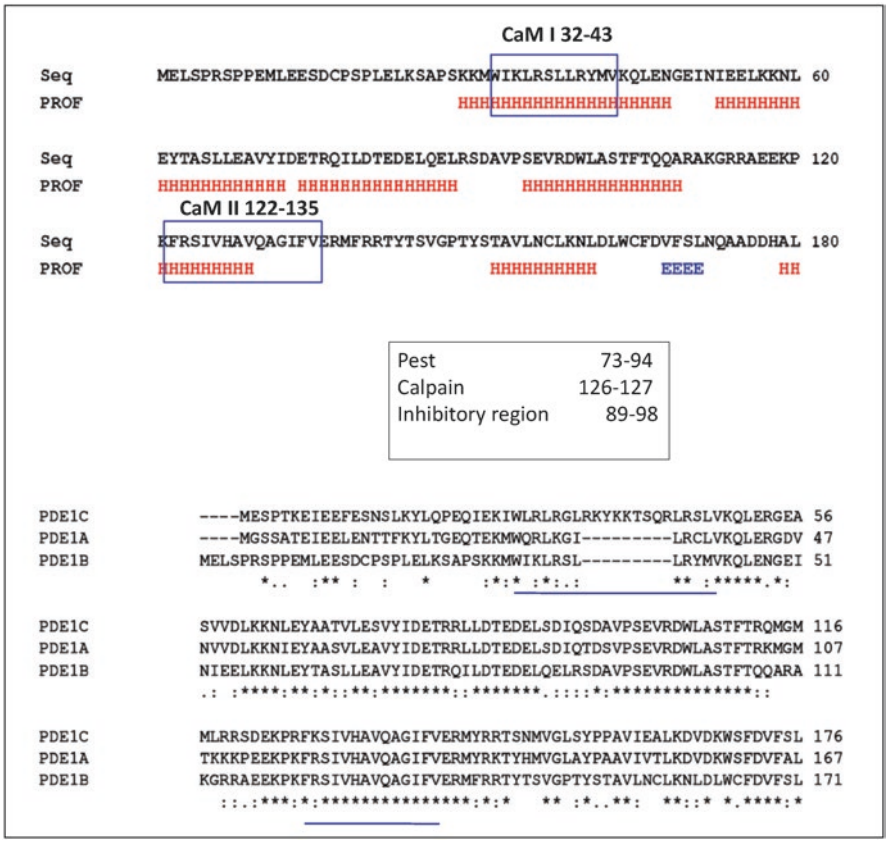



**Fig. 13.6** Diagram of the N-terminal regulatory region of hPDE1B. *Top*, diagram of N-terminal region of hPDE1B. Data is derived from information derived by Sonnenburg et al. (1995). *Bottom*, sequence alignment of hPDE1B N-terminal domains. * identical amino acids, : homologous amino acids, . similar amino acid

nucleotides, though their relative affinities for cAMP and cGMP differs (Bender and Beavo 2006a; Sharma et al. 2006). PDE1A and PDE1B are relatively cGMP-specific, with a higher $K_m$ (weaker affinity) for cAMP than cGMP, while PDE1C hydrolyzes both nucleotides with similar affinity (Table 13.2).

Early purification of the PDE1 isoforms was performed from bovine brain (63 Kd and 60 Kd isoforms—referred to as PDE1B1 and PDE1A2 (Sharma et al. 2006)), heart and lung tissues (Sharma and Kalra 1994). This work revealed enzymes of similar $V_{max}$ activity for cGMP from all tissues. A 63 Kd enzyme from bovine brain was reported to have significantly lower $V_{max}$ for cAMP versus cGMP. Heart enzyme, was activated by significantly lower calcium concentrations in the presence of maximal calmodulin concentration. However, the PDE1 isoform identity of this heart PDE1 was not clearly determined. The issue of the identity of PDE enzyme forms will be discussed further below, under Tissue Distribution.

**Table 13.1** Protein—protein interactions of the PDE1 family members as identified in the Pathway Studio Knowledgebase from Elsevier. The protein-protein interactions are annotated by type and contain descriptions that are pulled directly from the public literature by the Knowledgebase software.

| Relation | Type | Description | TextRef | # of references | Effect | Source | Mechanism |
|---|---|---|---|---|---|---|---|
| Calmodulin → CaM-dependent cyclic nucleotide PDE | DirectRegulation | All members of the PDE1 family can be stimulated by Ca(2+) in the presence of calmodulin in vitro. Some PDE1 isozymes have similar kinetic and immunological properties but are differentially regulated by Ca2+ and calmodulin | info:pmid/15988057#abs:2, info:pmid/16786160#abs:4, info:pmid/2561985#abs:1, info:pmid/19741056#abs:3, info:pmid/8569735#abs:4, info:pmid/2543685#abs:2, info:pmid/15599707#abs:8, info:pmid/2286933#abs:8, info:pmid/24222327#abs:4, info:pmid/6088765#abs:3 | 100 | Positive | | Direct interaction |
| Calmodulin → PDE1A | DirectRegulation | Furthermore, all calmodulin isoforms were able to activate bovine calcium/calmodulin-dependent phosphodiesterase. Results obtained with electrophoresis in native conditions indicated that calmodulin is tightly bound to PDE1A. The phosphorylation of calmodulin-dependent phosphodiesterase is blocked by Ca2+ and calmodulin and reversed by the calmodulin-dependent phosphatase | info:pmid/10531384#abs:5, info:pmid/12135876#abs:9, info:pmid/16592937#abs:3, info:pmid/2840101#abs:5, info:pmid/8569735#abs:2, info:pmid/7982300#abs:5, info:pmid/2536706#abs:4, info:pmid/6122610#abs:4, info:pmid/8166665#abs:4, info:pmid/7739760#abs:3 | 22 | Positive | | Direct interaction |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| PKA → PDE1A | ProtModification | The bovine heart calmodulin-dependent phosphodiesterase can be phosphorylated by cAMP-dependent protein kinase, resulting in a decrease in the enzyme's affinity for calmodulin. The cAMP-dependent protein kinase was found to catalyze the phosphorylation of the purified cardiac calmodulin-dependent phosphodiesterase with the incorporation of 1 mol of phosphate/mol of subunit. For example, PDE1A is phosphorylated by cAMP-dependent protein kinase, and thus its affinity for calmodulin is reduced. PDE1A and PDE1C are phosphorylated by cAMP-dependent protein kinase (PKA), whereas PDE1B is phosphorylated by 2+-calmodulin-dependent protein kinase II | info:pmid/8569735#abs:1, info:pmid/7982300#abs:4, info:pmid/1646004#abs:3, info:pmid/10906126#body:369, info:pmid/15763421#body:81, info:pmid/8537356#body:426, info:pmid/15350849#body:10, info:pmid/16102838#body:51, info:pmid/8663227#body:45 | 9 | Unknown | | Phosphorylation |
| PKA → CaM-dependent cyclic nucleotide PDE | ProtModification | Another main difference is that 60 kDa PDE1 isozyme is a substrate of cAMP-dependent protein kinase, whereas, 63 kDa PDE1 isozyme is phosphorylated by calmodulin-dependent protein kinase | info:pmid/22900295#abs:6, info:pmid/12060846#abs:5, info:pmid/9406155#abs:4, info:pmid/9880329#body:255, info:pmid/23459256#cont:72, info:pmid/2986124#title:1, info:pmid/21386978#cont:30 | 7 | Unknown | | Phosphorylation |

(continued)

**Table 13.1** (continued)

| Relation | Type | Description | TextRef | # of references | Effect | Source | Mechanism |
|---|---|---|---|---|---|---|---|
| Calmodulin → PDE1B | DirectRegulation | The activity of one subclass (phosphodiesterase IB) was stimulated severalfold by calmodulin and selectively inhibited by the phosphodiesterase inhibitor TCV-3B, Thus, we may conclude that the basic regulatory properties of the tandem calmodulin-binding domains from Phosphodiesterase1A3 and PDE1B1 were retained in the chimeras, The fully Ca2+−calmodulin-stimulated cGMP hydrolytic activity of PDE1B was comparable with the cGMP hydrolytic activity of PDE5, as measured at 1 μM cGMP, Calcium-bound-calmodulin binds to and activates several molecules in the striatum, including phosphodiesterase 1B, calcineurin, and calcium-calmodulin-dependent protein kinase type II | info:pmid/2158939#abs:2, info:pmid/22484154#body:94, info:pmid/12878685#body:159, info:pmid/22346744#cont:52, info:pmid/10737760#body:119, info:doi:10.1016/j.cellsig.2012.03.019#body:94 | 6 | Positive | | Direct interaction |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CaM kinase II→ CaM-dependent cyclic nucleotide PDE | ProtModification | The sustained calmodulin-dependent protein kinase II activation depended on synergistic actions of two positive-feedback reactions, calmodulin-dependent protein kinase II autophosphorylation and calmodulin-dependent protein kinase II-mediated inhibition of a CaM-dependent phosphodiesterase, PDE1, Model simulation predicted the following cascade as a candidate mechanism for the calmodulin-dependent protein kinase II contribution to Long-term depression: calmodulin-dependent protein kinase II negatively regulates phosphodiesterase 1, subsequently facilitates the cGMP/protein kinase G signalling pathway and down-regulates protein phosphatase 2A (PP-2A), thus supporting the Long-term depression-inducing positive feedback loop consisting of mutual activation of protein kinase C and mitogen-activated protein kinase | info:pmid/19536203#abs:6, info:pmid/23297306#abs:4, info:pmid/2543685#title:1, info:pmid/15944388#body:201 | 4 | Unknown | | Phosphorylation |
| CaM kinase II→PDE1B | ProtModification | Similarly, phosphorylation of PDE1B by calmodulin kinase II reduces the affinity of PDE1B for calmodulin by 6-fold (Hashimoto et al. JBC 264, 10884-10888, 1989), PDE1A and PDE1C are phosphorylated by cAMP-dependent protein kinase (PKA), whereas PDE1B is phosphorylated by 2+−calmodulin-dependent protein kinase II, cyclic-3',5'-phosphodiesterase1A1 from heart and cyclic-3',5'-phosphodiesterase1A2 from brain are phosphorylated by PKA, whereas PDE1B is phosphorylated by calmodulin-dependent protein kinase II, and all of these phosphorylations can be reversed by calcineurin, a Ca/calmodulin-dependent phosphatase | info:pmid/16968949#body:355, info:pmid/15763421#body:81, info:pmid/9893113#body:209 | 3 | Unknown | | Phosphorylation |

(continued)

**Table 13.1** (continued)

| Relation | Type | Description | TextRef | # of references | Effect | Source | Mechanism |
|---|---|---|---|---|---|---|---|
| CAMK → CaM-dependent cyclic nucleotide PDE | ProtModification | Another main difference is that 60 kDa PDE1 isozyme is a substrate of cAMP-dependent protein kinase, whereas, 63 kDa PDE1 isozyme is phosphorylated by calmodulin-dependent protein kinase. Cardiomyocyte contains both a CaMK-regulated isoform of adenylyl cyclase (type III) (23) and a CaM-dependent isoform of cyclic nucleotide phosphodiesterase | info:pmid/22900295#abs:6, info:pmid/10935538#body:113, info:pmid/9138729#body:7 | 3 | Unknown | | Phosphorylation |
| PDE1B → PPP1R1B | ProtModification | Phosphodiesterase 1B and activation of calcineurin that dephosphorylates phosphoThr34 DARPP-32. The activity of DARPP-32, in turn, is regulated by PKA and PKG phosphorylation and, recently, it has been shown that PDE1B is also involved in the regulation of DARPP-32 activity | info:pmid/22346744#cont:805, info:pmid/12967715#body:210, info:pmid/17027094#body:100 | 3 | Unknown | | Dephosphorylation |
| CaM kinase II → PDE1A | ProtModification | The brain 63- and 61-kDa calmodulin-dependent phosphodiesterase isoenzymes (phosphodiesterase1) are phosphorylated in vitro by calmodulin-dependent kinase II and PKA, respectively | info:pmid/8663227#body:45, info:pmid/10799557#body:238 | 2 | Unknown | | Phosphorylation |
| PDE1B → YWHAZ | Binding | | info:pmid/16615898, info:pmid/16959763 | 2 | | PINA | |
| PDE1B → PDE1A | ProtModification | Furthermore, the phosphorylation of PDE1A1 (59 kDa) and PDE1A2 (61 kDa) by PKA and of PDE1B1 (63 kDa) by CaM Kinase II decreases their sensitivity to calmodulin activation | info:pmid/16102838#body:51, info:pmid/9102399#body:68 | 2 | Unknown | | Phosphorylation |
| APOA1 → PDE1A | Binding | | info:pmid/11991719 | 1 | | PINA | |
| APP → PDE1A | Binding | | | 1 | | PINA | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ARRB2 → PDE1C | Binding | This may, however, change as a recent study has demonstrated that ß-arrestin2 can co-localize with PDE1C in the olfactory epithelia of rodents (Menco B. J Neuro cytology, 34: 11–36, 2009) | info:pmid/24292836#cont:322 | 1 | | | |
| Calcineurin → PDE1C | ProtModification | The resulting Ca 2+ influx would activate calcineurin, which would in turn dephosphorylate and reactivate PDE1C, causing a rapid termination of the cAMP signal | info:pmid/15763421#body:89 | 1 | | | Dephosphorylation |
| CALM1 → PDE1A | Binding | | info:pmid/12135876 | 1 | | PINA | |
| CALM2 → PDE1A | Binding | | info:pmid/12135876 | 1 | | PINA | |
| Calponin → PDE1A | Binding | Using primary vascular smooth muscle cells, we show that cytoplasmic and nuclear PDE1A were associated with a contractile marker (SM-calponin) and a growth marker (Ki-67), respectively | info:pmid/16514069#abs:5 | 1 | | | |
| CaM-dependent cyclic nucleotide PDE → ENPP1 | Binding | Similarly, unlike NPP1, cell-associated nucleotide pyrophosphatase/ Phosphodiesterase1-2-2 and nucleotide pyrophosphatase/Phosphodiesterase1-2-1 did not display any nucleotide phosphodiesterase activity | info:pmid/15096095#body:151 | 1 | | | |
| CaM-dependent cyclic nucleotide PDE → GPHA2 | Binding | Moreover, we have provided enticing genetic evidence that Pde1 interacts with Gpa2 in C. albicans but not in S. cerevisiae | info:pmid/20558315#body:182 | 1 | | | |
| CaM-dependent cyclic nucleotide PDE → GPR1 | Binding | Furthermore we have preliminary evidence (D. Wilson, PhD thesis) that PDE1 also interacts genetically with Gpr1, suggesting that inhibitors of Pde1 when combined with those against Gpa2 and/or Gpr1 could prove effective for combinatorial treatment of C. albicans infections | info:pmid/20558315#body:179 | 1 | | | |

(continued)

**Table 13.1** (continued)

| Relation | Type | Description | TextRef | # of references | Effect | Source | Mechanism |
|---|---|---|---|---|---|---|---|
| CaM-dependent cyclic nucleotide PDE→LIG3 | Binding | A recent study showed DNA ligase III directly interacts with tyrosyl phosphodiesterase 1 in the single-stranded break repair complex, which is important for maintaining the genomic integrity of developing neurons | info:pmid/16648486#body:59 | 1 | | | |
| CaM-dependent cyclic nucleotide PDE→Microtubule | Binding | In bovine brain, PDE1 appears to be associated with neurofilaments and microtubules | info:pmid/15763421#body:98 | 1 | | | |
| CaM-dependent cyclic nucleotide PDE→ Neurofilament | Binding | In bovine brain, PDE1 appears to be associated with neurofilaments and microtubules | info:pmid/15763421#body:98 | 1 | | | |
| CaM-dependent cyclic nucleotide PDE→PARP1 | Binding | Once damage has been recognized, PARP binds to the DNA and recruits X-ray repair complementation group 1(XRCC1) and tyrosol DNA phosphodiesterase 1 (TDP1) to remove the damaged region of DNA, enabling repair proteins to fill-in the missing nucleotides | info:pmid/25774912#cont:36 | 1 | | | |
| CAMK→PDE1A | ProtModification | For example, phosphorylation of PDE1A by Ca2+/calmodulin-dependent protein kinase or PDE1B by protein kinase A reduces affinity of both phosphodiesterase1 isoforms for calmodulin | info:pmid/9102399#body:68 | 1 | | | Phosphorylation |
| Deaminase→ PDE1A | Binding | Membrane-bound 3'.5'-cyclic nucleotide phosphodiesterase (EC 3.1.4.17) is closely associated physically with nucleotidase and deaminase, thus forming an enzyme cluster of unique catalytic behaviour | info:pmid/6293512#abs:1 | 1 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| LRP1 → PDE1C | Binding | Mechanistic studies revealed that PDE1C plays a critical role in regulating the stability of growth factor receptors, such as PDGF-receptor-beta (PDGFR? known to be important in pathological vascular remodeling. PDE1C interacts with LDL-receptor-related-protein-1 (LRP1) and PDGFRß, thus regulating PDGFRß endocytosis and lysosome-dependent degradation in an LRP1-dependent manner. A transmembrane-adenylyl-cyclase (tmAC)-cAMP-PKA cascade modulated by PDE1C is critical in regulating PDGFRß degradation. Conclusions: these findings demonstrated that PDE1C is an important regulator of smooth muscle cell proliferation, migration, and neointimal hyperplasia, in part through modulating endosome/lysosome dependent PDGFRß protein degradation via LRP1 | info:pmid/25608528#abs:10 | 1 | | | |
| m_Ubb → PDE1A | Binding | | | 1 | | PINA | |
| Nucleotidase → PDE1A | Binding | Membrane-bound 3'.5'-cyclic nucleotide phosphodiesterase (EC 3.1.4.17) is closely associated physically with nucleotidase and deaminase, thus forming an enzyme cluster of unique catalytic behaviour | info:pmid/6293512#abs:1 | 1 | | | |
| PDE1A → RPS27A | Binding | | | 1 | | PINA | |
| PDE1A → UBA52 | Binding | | | 1 | | PINA | |
| PDE1A → UBC | Binding | | | 1 | | PINA | |
| PDE1A → YWHAZ | Binding | | info:pmid/16959763 | 1 | | PINA | |
| PDE1B → POT1 | Binding | | | 1 | | PINA | |
| PDE1B → UBE3A | Binding | | | 1 | | PINA | |

(continued)

**Table 13.1** (continued)

| Relation | Type | Description | TextRef | # of references | Effect | Source | Mechanism |
|---|---|---|---|---|---|---|---|
| PDE1C → PDGFRB | Binding | Mechanistic studies revealed that PDE1C plays a critical role in regulating the stability of growth factor receptors, such as PDGF-receptor-beta (PDGFR? known to be important in pathological vascular remodeling. PDE1C interacts with LDL-receptor-related-protein-1 (LRP1) and PDGFRß, thus regulating PDGFRß endocytosis and lysosome-dependent degradation in an LRP1-dependent manner. A transmembrane-adenylyl-cyclase (tmAC)-cAMP-PKA cascade modulated by PDE1C is critical in regulating PDGFRß degradation. Conclusions: these findings demonstrated that PDE1C is an important regulator of smooth muscle cell proliferation, migration, and neointimal hyperplasia, in part through modulating endosome/lysosome dependent PDGFRß protein degradation via LRP1 | info:pmid/25608528#abs:10 | 1 | | | |
| PDPK1 → CaM-dependent cyclic nucleotide PDE | ProtModification | The pyruvate dehydrogenase kinase isoenzyme 1 (PDK1) phosphorylates the enzyme of the same name (specifically PDE1), which is the major component of the pyruvate dehydrogenase complex | info:pmid/24086109#cont:155 | 1 | | | Phosphorylation |
| PKG → CaM-dependent cyclic nucleotide PDE | ProtModification | Several PDEs including PDE1, PDE3, PDE4, PDE5 and PDE11 are phosphorylated and regulated by cAMP/cGMP-dependent protein kinase, and at least PDE4 is also regulated by tyrosine phosphorylation | info:pmid/11738832#body:154 | 1 | | | Phosphorylation |

*PINA* Protein Interaction Network Analysis platform (http://omics.bjcancer.org/pina/interactome; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3244997/) Cowley et al. 2012

**Table 13.2** $K_m$ and $V_{max}$ data for PDE1 isoforms (Sharma and Kalra 1994; Yan et al. 1995)

| | $K_m$ ($\mu M$) | | | | $V_{max}$ ratio *cAMP/cGMP* | |
|---|---|---|---|---|---|---|
| | cAMP | | cGMP | | | |
| Isoenzyme | Sharma and Kalra (1994) | Yan et al. (1995) | Sharma and Kalra (1994) | Yan et al. (1995) | Sharma and Kalra (1994) | Yan et al. (1995) |
| Bovine PDE1A2 | 40.0 | 112.7 | 3.2 | 5.1 | 3.0 | 2.9 |
| Bovine PDE1B1 | 12.0 | 24.3 | 1.2 | 2.7 | 0.3 | 0.9 |
| Rat PDE1C2 | | 1.2 ± 0.1 | | 1.1 ± 0.2 | | 1.2 ± 0.1 |

In addition to control via binding of calmodulin, PDE1 activity is inhibited by phosphorylation of the enzyme. Phosphorylation of PDE1A by protein kinase A reduces its affinity for $Ca^{2+}$/CAM; the EC50 for half-maximal stimulation of cAMP hydrolysis goes from 0.51 to 9.1 nM calmodulin (Sharma and Wang 1985). The phosphorylation site for PKA was mapped to Serine 120 of PDE1A1 enzyme, a site between the two calmodulin binding sites (Florio et al. 1994). PDE1C activity is modulated by phosphorylation by PKA (Loughney et al. 1996; Yan et al. 1996).

The N-terminal control elements in PDE1 have an inhibitory effect on enzyme activity. Proteolytic removal of this region with m-calpain made the enzyme calmodulin-independent, with a $K_m$ and $V_{max}$ (as measured with cAMP) very close to the fully stimulated full-length enzyme (Kakkar et al. 1998). Subsequent molecular cloning and expression has confirmed that the PDE1 catalytic core is constitutively active (Zhang et al. 2004). Specific inhibitors of the PDE1 family enzyme, developed in our laboratories, have equivalent potencies against the $Ca^{2+}$/CAM-bound holoenzyme and catalytic core.

Interestingly, control of the PDE1B isoform is modulated by phosphorylation by calmodulin-dependent protein kinase (CamKII) (Kakkar et al. 1996). Given the localization of CamKII in neurons to dendritic spines and post-synaptic densities, this may reflect a calcium-dependent feedback loop that would dampen PDE1B activity in this subcellular region.

## 13.4 Tissue Distribution of PDE1 Enzymes

The distribution of various PDE1 family members has been studied using a variety of qualitative, semi-quantitative and quantitative methods including: isolation and enzymatic characterization, immunohistochemistry, microarray expression profiling and real-time polymerase chain reaction technology (RT-PCR). The literature on PDE1 tissue distribution is rather complicated as the nomenclature have evolved over time. In addition, other factors should be taken into account to judge the importance of the various PDE1 isoforms in different tissues. First, the relation between

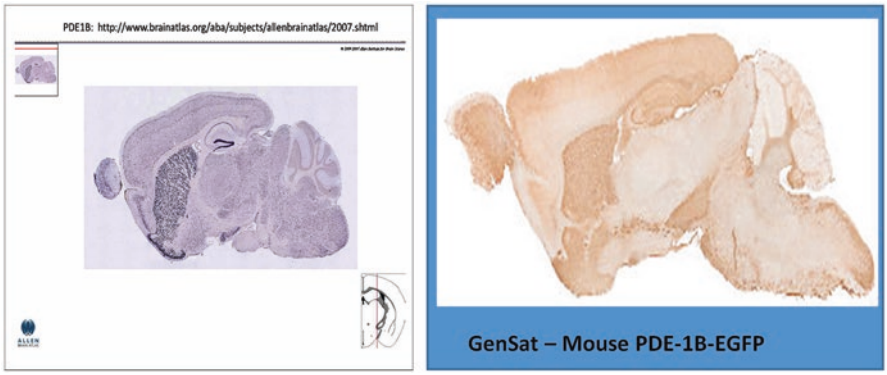



**Fig. 13.7** Distribution of PDE1B in mouse brain as revealed by the Allen brain atlas (*left*) and GenSat (*right*) Technologies

mRNA levels quantitated by RT-PCR may not correlate with enzyme levels. Second, tissues contain various cell types. In the heart, cardiomyoctes, endothelial, smooth muscle and, particularly in disease populations, fibroblasts cell types all present different spectrum of PDE1 and other PDE enzymes. Furthermore, cellular compartments undoubtedly exist, as is the case in cardiomyocytes, which may contain higher levels of certain enzymes. Finally, the kinetics of different enzymes and isoforms influence the importance of the various isoforms. PDE1C, with high mRNA levels in the heart, has significantly higher affinity for cAMP versus PDE1A and PDE1B.

Particularly informative information has been collected in human tissues by RT-PCR (Lakics et al. 2010). PDE1B is highly expressed in the brain in the striatum, hippocampus and pre-frontal cortex, where it is highly-co-localized with the dopamine D1 receptor. PDE1B is richly expressed in dopamine-responsive neurons of the caudate putamen and nucleus accumbens. It is also expressed in macrophage cells (Bender and Beavo 2006b). PDE1C is more ubiquitous in the brain, present in the olfactory tubercle, and found abundantly in the cardiomyocyte, lung and heart tissue. PDE1C is a major PDE in the human and rat heart (Sonnenburg et al. 1998) and the major cyclic nucleotide hydrolyzing activity in cardiomyocytes (Vandeput et al. 2007), where PDE3 is also abundantly expressed (Murata et al. 2009). PDE1A is enriched in the brain and, depending on the species (Miller et al. 2011), in the heart. PDE1A is present in activated cardiac fibroblasts (Miller et al. 2011). An interesting variant of PDE1A was described in sperm and kidney by Vasta et al. (2005). Figure 13.7 depicts the localization of PDE-1B in the mouse brain as defined in the Allen Brain Atlas (http://www.brain-map.org/) using in-situ-hybridization, and in the GenSat Atlas (http://www.gensat.org/index.html) using a reporter gene. Enrichment in the striatum of PDE1B implicates a potential role in Parkinson's disease and for use of PDE1 inhibitors in disorders of motor control. The high enrichment in the cardiomyocyte of PDE1C as well a substantial animal model validation reported in the literature, implicates PDE1 inhibitors for heart failure indications (Miller and Yan 2010). There is an interesting literature describing cognitive dysfunction associated with heart failure (Alosco et al. 2014; Feola et al. 2013;

Garcia et al. 2012; Knecht et al. 2012). Additionally, PDE1 enzymes have been found in vascular endothelial cells, smooth muscle cells, fibroblasts and motor neurons.

As mentioned above, the PDE1 enzyme class is known from biochemical isolation studies to be a mainly cytosolic enzyme (Sonnenburg et al. 1998). According to Pathway Studio knowledgebase from Elsevier, some 40 protein-protein interactions of PDE1 isoforms have been documented in the literature (Table 13.1). PDE1A is found in membrane fractions of bovine tracheal smooth muscle and associated with muscarinic M2 acetylcholine receptors in that tissue (Mastromatteo-Alberga et al. 2015). This set of protein interactions is a rather small set, chiefly comprised of calmodulin and relevant kinases, and indicates more work should be done to identify protein-protein interactions of the PDE1 enzyme family.

The subcellular localization of PDE1 enzymes inside neurons and other cells is poorly researched (Beltman et al. 1993; Sonnenburg et al. 1998). The possibility exists of high enrichment of this enzyme in particular microenvironments (Goraya and Cooper 2005). Calcium calmodulin activated kinase II (CAMKII), as an example, is heavily localized with calmodulin in dendritic spines (Lu et al. 2014), reaching high local concentrations, where it plays an important role in synaptic plasticity and memory formation. Calmodulin concentrations in dendritic spines has been estimated to be very high, at around 100 micromolar (Faas et al. 2011). This compares to a half-maximum activation by calmodulin for PDE1 isoforms of approximately 10 nM (Sharma and Kalra 1994). As shown in Table 13.1, a clear interaction is known to occur between calmodulin and PDE1 enzymes, which could serve to concentrate this enzyme family in this microenvironment, contributing to a critical role for PDE1 enzyme in cognitive function.

The relative importance of various PDEs in the heart has been extensively studied (Lee and Kass 2012). This tissue has significant PDE1, 2, 3, 4 and 5 enzymes present and levels vary upon aging and in disease states. Levels of PDE1A and PDE1C vary across species. As mentioned above, in human heart, PDE1C is the predominant PDE1 isoform as measured by RT-PCR. (Lakics et al. 2010) In isolated human cardiomyocytes (as well as Guinea pig, but not rat) PDE1 is the predominant cAMP and cGMP hydrolyzing activity, as reported by Johnson et al. (2012) using a PDE1-selective inhibitor UK90234. Vandeput et al. characterized PDE1C1 subcellular distribution in human myocardium and concluded it to be the major cAMP and cGMP hydrolyzing activity in soluble compartments. PDE3 was found to be the predominant cAMP hydrolyzing activity in microsomal fractions (Vandeput et al. 2007).

## 13.5 Genomics

As mentioned earlier, catalytic and $Ca^{2+}$/CaM binding domains of the PDE1 genes are highly conserved between species (Zhang et al. 2000) and across the PDE1 subfamily (Zhao et al. 1997). The National Center for Biotechnology Information

(NCBI) has a substantial and well-organized summary of each of the identified human genes to date (http://www.ncbi.nlm.nih.gov/gene): PDE1A gene has 5 mRNA transcript variants as a result of alternative splicing (Michibata et al. 2001), and they differ mostly in the 5′ and 3′ untranslated regions (UTRs). The sequences encoding the catalytic cores and metal binding sites are entirely conserved. Isoform 1 of PDE1A is encoded by transcript variant 1, represents the longest transcript, and is also considered the "canonical" transcript. Of the species with genome sequences available thus far, 148 organisms have an ortholog of the human PDE1A gene, and slightly fewer organisms have an ortholog for human PDE1B or PDE1C. The human PDE1B gene has 2 transcript variants, with transcript variant 1 encoding isoform 1, the canonical transcript. The human PDE1C gene has 5 transcript variants, and the canonical transcript variant 3 encodes isoform 3. Based on the Genome Reference Consortium's current Human Build 28 patch release 2 (GRCh38.p2), the gene locations for each of the human PDE isoforms are as follows:

PDE1A: Chromosome 2; 182,140,035…182,522,845; 382,811 base pair length
PDE1B: Chromosome 12; 54,549,393…54,579,239; 29,847 base pair length
PDE1C: Chromosome 7; 31,616,777…32,299,404; 682,628 base pair length

Splice variants of the human PDE1A transcript were identified using a cDNA cloning and bioinformatics approach (Michibata et al. 2001). These variants differed in their N-terminal and C-terminal regions. Southern blot analysis of different tissues revealed that certain variants were widely expressed throughout most of the body, while others, such as a variant referred to PDE1A10, were confined to one tissue type (Michibata et al. 2001). Variants all share exons 4–12 of the gene's 17 exons, as exons 6–12 encode the catalytic domain.

A separate, 11.5 kb downstream first exon distinguishes PDE1B2 from PDE1B1 (Bender et al. 2004), and PDE1B2 and PDE1B1 have separate promoters. These promoters are differentially regulated in monocytes versus other cell types. Activation of transcription, rather than post-translational modulation, is primarily responsible for PDE1B2 up-regulation in monocytes. Granulocyte-macrophage colony-stimulating factor (GM-CSF) selectively stimulates transcription of PDE1B2 at a transcriptional start site unique to PDE1B2 (Bender et al. 2004).

Common human trans-acting factor AP-1 is reported to be involved in PDE1B transcriptional regulation in monocytes and CHO cells (Spence et al. 1995). Two specific protein kinase C (PKC) isoforms selectively induce production of PDE1B mRNA as an early response to treatment of CHO cells with the tumor-promoting compound phorbol 12-myristate 13-acetate (PMA). This compound is known to signal via activation of PKC and subsequently AP-1 (Spence et al. 1997). Many alterations in PDE1 expression occur at a post-transcriptional stage, for instance, in incidences of traumatic brain injury (TBI) (Oliva et al. 2012).

Transcriptional regulation of each of the PDE1 isoforms has been characterized to some extent. The excitatory effects of cytokine release on PDE1A activity are suppressed in mouse longitudinal smooth muscles. Inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), suggests that this protein complex is involved in upregulation of PDE1A transcription in mouse smooth mus-

cle (Rajagopal et al. 2014). Drug treatment with vasodilators induces PDE1A1 expression, which contributes to nitrate tolerance, as shown in rat aortic VSMSs (Kim et al. 2001). GM-CSF stimulates transcription of PDE1B1 at a start site activated upon monocyte differentiation. Macrophage colony-stimulating factor (M-CSF) stimulation, however, preferentially induces transcriptional upregulation of PDE2 over PDE1B (Bender et al. 2004). Transcription of PDE1C is induced in proliferative human smooth muscle cells (SMCs), and expression is down-regulated upon cellular quiescence (Rybalkin et al. 2003), indicating that PDE1C plays a regulatory role in the cell cycle of SMCs. 7-oxo-prostacyclin treatment was found to increase the transcriptional levels of PDE1C in the rat heart (Kostic et al. 1997).

The roles of PDE1 isoforms in epigenetic mechanisms are unclear. Because PDE1 activity lowers cAMP, it may indirectly inhibit activation of the common transcription factors cAMP/$Ca^{2+}$ response element-binding (CREB) protein and serum response factor (SRF) (Paul et al. 2010). Furthermore, PDE1A is suggested to be an epigenetic regulator of cell cycle growth and proliferation by targeting the epigenetic integrator UHRF1 (Ubiquitin-like, PHD Ring Finger 1). Down-regulation of PDE1A mRNA expression inhibits UHRF-1 and activates the p73 tumor-suppressor protein (Alhosin et al. 2010). RNA-interference knock-down of PDE1A expression in the acute lymphoblastic leukemia Jurkat cell line triggered cell cycle arrest and apoptosis through regulation of these two proteins.

There is little evidence of PDE1 genetic links to CNS disease to date. PDE1 has few SNPs that have been shown conclusively to be linked to disease. However, a recent human genome-wide association study found SNPs in PDE1A that were associated with diastolic blood pressure and carotid intima-media thickness (Nino et al. 2015). Elevated PDE1A and PDE1C mRNA levels were found to be linked with markers of cellular senescence in vascular smooth muscle cells (Nino et al. 2015; Yan 2015). The term senescence refers to a concept of irreparable chromosomal breaks associated with extensive cell passages in culture or age-related vascular disease and mimicked in mouse knockout models lacking nucleotide excision repair genes. A posttranslational regulatory role of PDE1A localization in determining vascular smooth muscle growth has been described (Nagel et al. 2006).

Initially it was proposed that there may be a linkage between PDEs and Major Depressive Disorder. A particular PDE1A variant, rs1549870, was reported to have a significant effect on antidepressant drug response (Wong et al. 2006). Later studies failed to replicate these results (Cabanero et al. 2009; Perlis et al. 2010).

The recent influx of sequencing data regarding the human genome has made it possible to search online databases and compile genetic risk variants within a population. Table 13.3 describes the variants found among 60,706 sequenced human genomes compiled by the Broad Institute's Exome Aggregation Consortium (Lek et al. 2016). The locations of mutations and their consequences are based on the reference genome build GRCh37/hg19. Of note, fewer variations in each of the PDE1 genes have been observed than what would be expected from random mutation rates. PDE1B in particular is predicted to be highly intolerant to loss of function variations (Lek et al. 2016). The generally low frequency of SNPs for this class of enzymes may reflect the vital roles they play. In summary, little information to date

**Table 13.3** Highest-frequency variants resulting in amino acid sequence changes or intronic mutations

| | PDE1A | | | | PDE1B | | | | PDE1C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variant | Consequence | Allele Freq. (%) | POI | Variant | Consequence | Allele Freq. (%) | POI | Variant | Consequence | Allele Freq. (%) | POI |
| 1 | 2:183387027 T/C | p.Lys26Arg | 0.144 | Euro (0.2%) | 12:54963142 C/T | p.Ser155Leu[†] | 0.287 | Euro (0.4%) | 7:32209425 A/G | p.Ser94Pro | 99.98 | |
| 2 | 2:183106619 AGTTT/A | p.Lys7TyrfsTer7[a] | 0.137 | Euro (0.4%) | 12:54969777 G/T | p.Gly423Gly[b] | 0.203 | Euro (0.3%) | 7:32338337 G/A | p.Ala4Val | 80.61 | |
| 3 | 2:183106640 A/C | p.Met1? | 0.100 | E Asian (3.5%) | 12:54967452 G/C | p.Gln341His[b] | 0.177 | Euro (0.3%) | 7:31862756 T/C | p.Ser565Gly | 0.809 | Euro (1.2%), Latino (0.9%) |
| 4 | 2:183387015 C/T | p.Arg30His | 0.086 | Euro (0.1%) | 12:54969851 C/T | p.Ala448Val[b] | 0.173 | Euro (0.3%), S Asian (0.2%) | 7:31855625 C/G | p.Val636Leu | 0.576 | Euro (0.9%) |
| 5 | 2:183106638 C/T | p.Gly2Ser | 0.079 | African (1.3%) | 12:54955390 C/T | p.Gln8Ter[a,†] | 0.098 | S Asian (0.2%) | 7:31793149 C/T | p.Arg720His | 0.573 | Euro (Finnish) (2.6%) |
| 6 | 2:183050553 T/C | p.Ile544Val | 0.074 | Euro (0.1%), Latino (0.2%) | 12:54963045 C/G | p.Ala102Gly | 0.075 | African (0.4%) | 7:31815326 C/T | p.Gly698Ser | 0.525 | E Asian (6.7%) |
| 7 | 2:183095749 C/T | p.Arg192His[b] | 0.034 | Latino (0.3%) | 12:54970470 G/A | p.Glu498Lys | 0.065 | | 7:31855577 A/C | p.Ser652Ala | 0.386 | Euro (0.6%) |
| 8 | 2:183050536 A/C | p.Phe549Leu | 0.017 | | 12:54971056 G/A | p.Glu519Lys | 0.036 | | 7:31918714 G/C | p.Ala167Gly | 0.343 | E Asian (1.1%) |

| | PDE1A | | | | PDE1B | | | | PDE1C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variant | Consequence | Allele Freq. (%) | POI | Variant | Consequence | Allele Freq. (%) | POI | Variant | Consequence | Allele Freq. (%) | POI |
| 9 | 2:183129104 C/T | p.Val47Ile | 0.017 | S Asian (0.1%) | 12:54963333 G/A | p.Met138Ile[c] | 0.033 | S Asian (0.2%) | 7:31793046 G/C | p.Ile754Met | 0.119 | African (1.4%) |
| 10 | 2:183099170 C/T | p.Val152Ile[b] | 0.015 | | 12:54943682 C/T | p.Pro9Leu | 0.026 | African (0.3%) | 7:32209545 A/T | p.Trp54Arg | 0.052 | |
| 11 | 2:183033002 G/A | p.Ser527Leu | 0.014 | | 12:54966485 C/T | p.Thr232Ile[b] | 0.021 | | 7:31815334 C/T | p.Arg695His | 0.044 | |
| 12 | 2:183050795 A/G | p.Ile463Thr | 0.012 | | 12:54962982 C/T | p.Thr81Met | 0.017 | | 7:31862774 T/C | p.Ile559Val | 0.040 | Latino (0.4%) |
| 13 | 2:183051198 C/T | p.Ser458Asn | 0.011 | | 12:54955411 C/A | p.Pro15Thr[†] | 0.011 | | 7:31920469 G/A | p.Arg105Trp | 0.037 | |
| 14 | 2:183011873 G/A | p.Ser524Phe | 0.010 | | 12:54963034 G/A | p.Gly119Glu[c,†] | 0.011 | | 7:32338278 A/G | p.Tyr24His | 0.035 | Latino (0.1%) |
| 15 | 2:183104889 G/T | p.Pro116Thr | 0.010 | | 12:54964027 C/T | p.Thr181Ile[†] | 0.010 | | 7:31867930 T/G | p.Thr481Pro | 0.035 | |
| 1 | 2:183070619 A/T | c.950 + 48 T > A | 31.8 | | 12:54955461 A/G | c.114-5297A > G | 70.0 | | 7:31876766 T/C | | 74.1 | |
| 2 | 2:183053658 C/G | c.1255 + 48G > C | 18.8 | | 12:54955324 T/C | c.-45 T > C | 66.8 | | 7:32209376 T/C | | 15.0 | |
| 3 | 2:183066391 C/A | c.1052 + 24G > T | 11.5 | | 12:54971347 C/G | c.*17 + 218C > G | 50.8 | | 7:32209359 A/G | | 8.1 | |
| 4 | 2:183066394 C/A | c.1052 + 21G > T | 6.8 | | 12:54943775 T/A | c.113 + 6 T > A | 1.7 | | 7:32249155 G/GA | c.86-5dupT | 7.4 | |
| 5 | 2:183066390 ACAAC/A | c.1052 + 21_1052 + 25delGTTGTinsT | 3.2 | | 12:54943620 C/A | c.-13-24C > A | 1.7 | | 7:31848763 T/C | | 5.2 | |

**Table 13.3** (continued)

| | PDE1A | | | | PDE1B | | | | PDE1C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variant | Consequence | Allele Freq. (%) | POI | Variant | Consequence | Allele Freq. (%) | POI | Variant | Consequence | Allele Freq. (%) | POI |
| 6 | 2:183066388 A/C | c.1052 + 24_ 1052 + 27delGTTTinsGTTG | 2.4 | | 12:54969936 GGA/G | c.1376 + 53_1376 + 54delGA | 1.2 | | 7:32091215 A/T | | 4.1 | |
| 7 | 2:183066385 A/AC | c.1052 + 30delTinsGT | 2.4 | | | | | | 7:32249155 GA/G | c.86-5delT | 3.8 | |

*POI* Population of interest

The EXAC database (http://exact.broadinstitute.org) lists 540 PDE1A variants, 521 PDE1B variants, and 807 PDE1C variants with different frequencies. Listed are the top amino acid changes (*p*) and coding (*c*) changes based upon relative frequencies. Of the 121,412 alleles sequences covered in this database, an allele frequency of 1% would indicate that the variant appeared 1214 times. As with other such sequence databases, the frequencies may be influenced by different consortiums contributing to the database. For example, variant 183387027 is listed at a frequency of 0.144, but in European Finnish populations the frequency is 0.42. In the EXAC database, a plurality of the individuals were from either the Myocardial Infarction Genetics Consortium or the Swedish Schizophrenia and Bipolar Studies Consortium. Reference (Wong et al. 2006), describing the EXACT database can be found at: http://biorxiv.org/content/early/2015/10/30/030338

[a] Loss of function mutation

[b] In the catalytic domain

[c] In a $Ca^{2+}$/CaM-Binding domain

[†] Not in canonical transcript (ENST00000243052)

*p.* Indicates change at a protein sequence

*c.* Indicates change in the coding sequence

*p.Ser527Leu* Leucine replaces Serine at residue 527

*2:183051198 C/T* Notation for variant given as chromosome: location/nucleotide variation

*Ter* Translation stop codon

*p.Lys7TyrfsTer7* Tyrosine replaces Lysine, causing a frame shift mutation with stop codon at location 7 in new frame

*c.114-5297A>G* A to G substitution at nucleotide -5297 from the end of an intron (in the coding DNA positioned between nucleotides 113 and 114)

*c.950+48T>A* T to A substitution at nucleotide +48 from the start of an intron (in the coding DNA positioned between nucleotides 950 and 951)

*c.*17+218C>G* Nucleotide change 17 base pairs 3′ of the stop codon

*c.-45T>C* Nucleotide change from T to C at 45 base pairs 5′ of the initiation codon

links PDE1 polymorphism to human disease, based on recent considerable human genome-wide sequencing data. However, this issue is still under-studied. As more information is obtained of this type is developed in disease-specific databases, such as for Parkinson's disease, schizophrenia, and heart failure, it will be interesting to continue to track genetic variations and their potential indications for both disease pathogenesis and drug response.

## 13.6 Inhibitors in the Public Domain

Until recently, research into PDE1 had lagged behind other PDE families (2–11) in part due the lack of potent and specific inhibitors. The literature on PDE1 was confused by the improper identification of compounds, such as vinpocetine, as selective PDE1 inhibitors. In fact, vinpocetine is a non-selective agent that inhibits other targets with higher affinity than PDE1. In spite of the confusion generated by early studies, there is now a substantial and growing literature implicating selective PDE1 inhibitors as agents for the treatment of cognitive dysfunction, as therapies for neurodegenerative diseases including Parkinson's disease, as well as for disorders of the cardiovascular system. In addition, PDE1B is involved in activation of monocytes to macrophages, which is relevant to inflammatory responses associated with degenerative diseases (Bender and Beavo; Bender et al. 2004; Bender et al. 2005). This literature will be reviewed below. PDE1 is present in activated fibroblasts and contributes to fibrotic diseases as documented in heart failure (Miller and Yan 2010). Recently, Intra-Cellular Therapies, Inc. announced completion of a series of Phase 1 human clinical studies with the clinical candidate ITI-214, a potent and selective PDE1 inhibitor. Manuscripts describing this agent's chemistry (Li et al. 2016) recently was published.

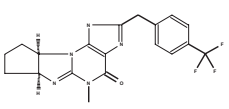
As mentioned, vinpocetine should not be considered a specific PDE1 inhibitor. Curiously, this agent is sold in health food stores as a promoter of memory function. The natural product has been evaluated in six human clinical trials and shown to increase brain vascular blood flow (Patyar et al. 2011). It is not clear which of the target activities of vinpocetine is responsible for this effect (Patyar et al. 2011; Kemeny et al. 2005; Szilagyi et al. 2005; Szatmari and Whitehouse 2003). As shown in Table 13.4 vinpocetine is a very weak PDE1 inhibitor; it interacts with ion channels at nanomolar concentrations. A 2011 review of vinpocetine covers a list of various targets including; voltage-sensitive sodium channels, mitochondrial transition pores, antioxidant properties, and inhibition of the interaction between IκB and IκB kinase (IKK) (Patyar et al. 2011).

Early potent and somewhat selective PDE1 inhibitors were published from efforts at Pfizer and Schering-Plough (Table 13.4). Later efforts at Galapagos and Pfizer demonstrated more potent and more highly selective PDE1 inhibitors. In the 2008 Galapagos patent (WO 2008071650) the authors claimed PDE1C selectivity over PDE1A + B. Our efforts, which started in 2003, led to the discovery of ITI-214 as a potent and highly selective PDE1 enzyme inhibitor with picomolar inhibitory

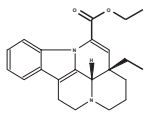
potency and selectivity of 2700-fold versus the first off-target system, PDE4. If one considers a requirement of "greater than 10-fold selectivity" to define a specific inhibitor for one PDE1 isoform (for example PDE1C over PDE1A and PDE1B), we have not seen PDE1 isoform specificity with our proprietary agents, nor when we synthesize and test the literature agents claiming selectivity. As mentioned above, the close PDE1 isoform sequence conservation leads to the prediction that isoform selectivity will be a difficult goal. Importantly, we have seen no downside to hitting all three PDE1 isoforms. Rather, we consider hitting PDE1 A, B and C as beneficial to therapeutic utility with our current PDE1 inhibitors. The rationale for this consideration is that there is no known undesirable effect of this redundancy, and by hitting all three isoforms you can overcome redundancy of isoforms in certain cell types. In human clinical trials, ITI-214 was safe and well tolerated, as described in the clinical trials section below, and reached high plasma drug levels.

**Table 13.4** Structures of PDE1 inhibitors

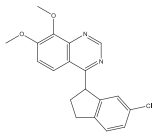
| Compilation of Literature PDE1 Inhibitors IC50 (nM) | | | | | | |
|---|---|---|---|---|---|---|
| | PDE1 | PDE4 | PDE5 | PDE6 | Ratio 5/1 | Reference |
| 8-MeO-IBMX | 1800 | | | | | |
| Vinpocetine | 58900 | | >300,000 | | | Ahn et al. 1997 |
| UK-90234 | 35.4 | >1000 | 1106 | 8650 | 31 | |
| SCH51866 | 70 | | 60 | | 1 | |
| ICOS (IC295?) | 490 | | 390000 | 154000 | 796 | |
| PF04822163 | 2-7 | 7620 | >30,000 | >29,000 | | Humphrey et al. 2114 |
| SP31 | 0.07 | 700 | 305 | | 4357 | Ahn et al. 1997 |
| SP33 | 0.6 | 200 | 200 | | 333 | Ahn et al. 1997 |
| Galapagos 2008 | 16-83 | | | | | Unpublished |
| ITI-214 | 0.058 | 158 | 632 | 324 | 10897 | Li et al. 2016 |



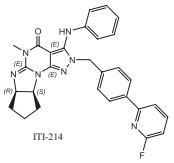
SCH51866



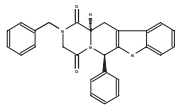
Vinpocetine



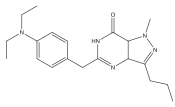
Pfizer PF04822163



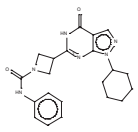
ITI-214



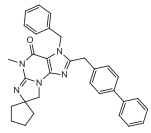
ICOS (IC295)



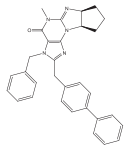
Pfizer UK-90234



Galapagos 2010



Schering Plough 33



Schering Plough 31

The ICOS compound IC295 referred to in the literature was never identified, and so this structure is a representative example from ICOS patent literature

## 13.7 Pharmacology in Animal Models

Cyclic adenosine monophosphate (cAMP) is the primary intracellular signaling system for the D1 dopamine receptor (Mailman et al. 1986) (as well as for a number of other receptor systems including beta-adrenergic receptors, histamine H2, and various peptide hormone receptors (CGRP, CRF, Melanocortin, and VIP)). The close co-localization in the brain of PDE1B with dopamine D1 receptor has driven efforts to exploit PDE1 inhibitors as cognitive enhancing agents. Cyclic GMP and cAMP are intimately involved in Long-Term potentiation (Kleppisch and Feil 2009), involved in memory consolidation. By amplification of cAMP second messengers, PDE1 inhibitors are likely to be neuroprotective, a subject researched extensively by the Filbin Laboratory (Hannila and Filbin 2008). By amplification of the second messenger signaling systems involving cGMP and cAMP, PDE1 inhibitors have positive effects on memory acquisition, consolidation and retrieval. Cyclic GMP is the primary intracellular signaling system of the atrial natriuretic peptide hormone receptors (Duda et al. 2014; Leitman and Murad 1987), and is also produced by soluble guanylate cyclase via activation of nitric oxide synthase in response to elevated intracellular calcium. In contrast, cAMP is made by adenylate cyclases in response to a number of G-protein coupled plasma membrane receptors. Importantly, the temporal and special concentrations of cyclic nucleotides will vary tremendously, depending on the cell type and stimulus.

PDE1A, B and C knockout mice have been produced and are healthy (Siuciak et al. 2007; Cygnar and Zhao 2009; Ye et al. 2016). PDE1B knockout mice display an interesting "on-demand" phenotype when challenged with a sub-optimal dose of dopamine D1 agonist (Reed et al. 2002; Ehrman et al. 2006).

There are rather few reports in the literature of the use of potent and selective PDE1 inhibitors in animal models of CNS diseases. This is in large part due to the lack of potent and selective inhibitors available in the public domain. In addition to the work using novel object recognition (NOR) tests in rats, which we have done, we have investigated reversal of catalepsy induced by haloperidol, a potent dopamine D2 receptor antagonist used as an antipsychotic. Haloperidol induces serious extra-pyramidal side effects (EPS) and leads to tardive dyskinesia, a major downside of the potent D2 receptors used to treat schizophrenics. While we have seen that PDE1 inhibitors reverse catalepsy induced by haloperidol when tested in mouse models, PDE10 inhibitors actually exacerbate catalepsy in this assay. This is a major distinction between PDE1 and PDE10. We have generated data indicating wakefulness-promoting properties of PDE1 inhibitors in mouse models, as measured by EEG. Lastly, PDE1 inhibitors are able to potentiate the beneficial effects of sub-maximal L-DOPA when tested in a unilateral 6-hydroxy dopamine lesion model that scores restoration of use of the affected contralateral limb. This set of data has given us optimism that PDE1 inhibitors will potentially treat CNS disorders involving cognitive function, Parkinson's disease and problems in wakefulness. Neurodegenerative diseases could theoretically be treated with PDE1 inhibitors, but this area is under studied.

In contrast, a substantial validation for the use of PDE1 inhibitors to treat heart failure exists in the literature. This work comes mainly from work done by Chen

Yan at Rochester University. It includes use of ICOS PDE1 inhibitors (no structure revealed), PDE1C and PDE1A knock-out mice studies, cellular models, and RNA interference studies (Miller et al. 2011; Miller and Yan 2010; Miller et al. 2009). In addition to effects on cardiomyocyte hypertrophy, this laboratory has documented reversal of fibrosis in heart failure models. Ahn and colleagues working at Schering Plough, published small decreases of blood pressure after treating spontaneously hypertensive rat models with their PDE1 and dual PDE1-PDE5 inhibitors (1997). While more work needs to be done in the areas of heart failure, this work holds promise that PDE1 inhibitors will be effective in heart failure.

## 13.8 Potential for Cognitive Enhancement in Mental Diseases

Much of the research into cognitive dysfunction in mental disease has focused on the involvement of the pre-frontal cortex (PFC) (Goldman-Rakic 1995; Goldman-Rakic 1994; Goldman-Rakic 1987). In this brain area, well established circuits of pyramidal neurons exist that signal via NMDA glutamate receptors. (Somewhat similar circuits exist in hippocampal regions involved in working memory as described by Tamminga and co-workers (Samudra et al. 2015; Tamminga et al. 2010)). The activity of pre-frontal cortical circuits is dampened by the action of GABA interneurons. The activity of the pyramidal cells is dampened, particularly under stressful conditions, by activation of voltage sensitive potassium channels called hyperpolarization-activated cyclic nucleotide-gated (HCN) and KCNQ channels, both of which are activated by cAMP. These potassium channels, when activated in stressful conditions, can effectively shut down the pyramidal circuits and severely impact memory, particularly in the schizophrenic brain (Arnsten and Jin 2014; Yang et al. 2013).

There is a large literature that indicates hypo-functionality of dopamine D1 receptor in the pre-frontal cortex in patients with schizophrenia (Slifstein et al. 2015; Thompson et al. 2014). As this dopamine receptor is intimately involved in working memory, this hypo-functionality is felt to contribute to the cognitive dysfunction. Importantly, the dopamine D1 receptor plays a pivotal role in many aspects of cognitive function including: speed of processing, attention, vigilance, working memory, reasoning and problem solving (Goldman-Rakic 1996; Goldman-Rakic 1998; Goldman-Rakic 1999). A well supported theory of the etiology of Schizophrenia has proposed cognitive dysfunction as a root cause (Nelson et al. 2009). Moreover, cognitive dysfunction is associated with multiple disorders of the CNS and is well recognized as a component of the cardiovascular disease of heart failure (Moraska et al. 2013). Cognitive dysfunction is compounded by excessive awareness of sensory input, generally suppressed in normal individuals, resulting in overwhelming noise in the PFC.

Over the past decades, substantial efforts have been made to treat schizophrenics with direct-acting dopamine D1 receptors. These efforts have generally failed

(Zhang et al. 2009). These failures are attributed to poor drug bioavailability of first generation agonists such as dihydrexidine (Mottola et al. 1992). In other efforts, the D1 receptor agonists A-86929 (Martin 2011; Giardina and Williams 2001), dinapsoline, dinoxyline, and doxanthrine, were discovered and tested clinically. However, interactions of D1 receptor agonists with D1 receptors in the periphery often lead to side effects resulting in hypotension and tachycardia (Huang et al. 2001). In addition, the failure of these drugs to achieve clinical efficacy may be associated with a diminution of initial positive effects of direct D1 agonists due to receptor desensitization. Dopamine action in this brain area is well known to have an inverted U-shaped activity/[dopamine agonist] relationship. It may be that a rather narrow U-shape response curve contributes to the difficulty of this approach. Apomorphine is a non-selective dopamine agonist with highest potency to D2 receptors, used in the treatment of Parkinson's disease. However, Apomorphine causes emesis, limiting its use.

PDE1 receptor inhibitors act as indirect dopamine D1 receptor agonists and should avoid the problems associated with directly acting D1 receptor agonists. The interest in PDE1 inhibitors revolves around the signal transduction pathway of the D1 receptor (Boyd and Mailman 2012). Dopamine D1 receptors signal via activation of Gs G-proteins to stimulate adenylate cyclase to produce intracellular cAMP (Mailman and Huang 2007). This fact and the co-localization of PDE1B enzyme with the D1 receptor in the pre-frontal cortex indicates PDE1 as a major "turn off" mechanism of the D1 receptor, via hydrolysis of cAMP. Therefore the use of PDE1 inhibitors, by preventing local dampening of cAMP signal transduction, represents an indirectly-acting D1 receptor agonist.

Consistent with these hypotheses, a number of PDE inhibitors have been shown effective in animal models of cognition. These models include the Novel Object Recognition (NOR) model (Reneerkens et al. 2009). The NOR model has a number of advantages as rodents are not perturbed by chemicals or pre-conditioning. This test utilizes the inherent tendency of rodents to explore novel objects. In rats, the recognition of such objects disappears in roughly 4 h. In our standard protocol, a 24 h delay is used and activity at this delay time indicates significant cognitive enhancement. Enhancement of NOR has been demonstrated by a number of PDE inhibitors which include PDE1, PDE2, PDE4, PDE9 and PDE10 (Reneerkens et al. 2009; Bollen and Prickaerts 2012; Reneerkens et al. 2013). Our work has focused on PDE1 inhibitors and we have found a large number of excellent enhancers of NOR, consistent with potency to inhibit PDE1 and to oral bioavailability. PDE inhibitory activity influences multiple aspects of NOR, including cGMP influences on early consolidation and cAMP influences on late consolidation (Bollen et al. 2015).

## 13.9 Diverse Therapeutic Indications

Based on the animal studies and theoretical arguments, there are a number of potential CNS and non-CNS disorders potentially treated by PDE1 inhibitors. Cognitive dysfunction in Schizophrenia, as discussed above, is well supported by animal

studies. Utility of PDE1 inhibitors as dose-sparing adjuncts to L-DOPA treatment in Parkinson's disease is a second very interesting indication, as is use of PDE1 inhibitors in motor disturbances of a variety of etiologies. More work will need to be done to realize the full potential of these possibilities.

Degenerative disorders of the CNS and the periphery remain attractive potential indications for PDE1 inhibitors. The enormous unmet need for treatments of heart failure justifies continued studies. PDE1 acts particularly in excitatory cellular systems such as neurons and cardiomyocytes where repeated cycles of calcium entry occur during the inherent repetitive cellular excitation. Importantly, the role of PDE1 enzyme during cell excitation is one integrated over the course of a lifetime and so it is particularly relevant to degenerative diseases where excessive intracellular calcium is felt to be responsible, in large part, with the progression of these diseases. Mitochondrial ATP generation is known to be influenced by cAMP (Acin-Perez et al. 2009). Indeed, motor neuron survival and regeneration is clearly benefited by cAMP (Qiu et al. 2002). The important motor neuron survival gene (SMN) has a cAMP-response element (CRE-II), giving further credence to the beneficial role of cAMP to skeletal muscle motor neuron function and survival (Hannila and Filbin 2008; Hannila et al. 2007). These properties of PDE1 indicate that PDE1 inhibitors may additionally have use in motor neuronal survival in spinal muscular atrophy (SMA) and more generally in degenerative disease treatments.

## 13.10 Clinical Trial Histories

A series of phase 1 human clinical trials with ITI-214, a potent and selective PDE1 inhibitor, were reported in a press release by Intra-Cellular Therapies, Inc. recently (http://ir.intracellulartherapies.com/releasedetail.cfm?ReleaseID=932472). The clinical candidate, ITI-214, was shown safe and well tolerated in normal healthy volunteers over a wide range of doses and in repeat dose studies. High drug levels were found in plasma after oral administration. This study serves to dispel any notion that PDE1 inhibitors will have any obvious liability. To our knowledge, no other potent and selective PDE1 inhibitor has been tested in humans.

In contrast, there are over 1000 clinical trials found in the "ClinicalTrials.gov" database when searched using the keyword "phosphodiesterase". Seventy-seven percent were for cardiovascular indications and 20% for disorders of the CNS. Ten percent of trials were for PDE3 inhibitors, 14% for PDE4 inhibitors, 46% for PDE5 inhibitors and 1% for PDE10.

Clinical trials covering potential cognitive enhancing drugs is a large area, covering a diverse set of targets. Over 1891 trials are listed in clinicaltrials.gov when searched with the term "cognitive dysfunction". Target mechanisms include cholinergic receptors, glutamatergic receptors, phosphodiesterase inhibitors (PDE9, 10, 5 and 4), serotonergic receptors, histaminergic H3 receptor agents and dopamine D1 agonists. However, the lack of new drug therapies to date for cognitive dysfunction indicates this objective may be a difficult one to demonstrate.

## 13.11 Summary

This review has focused on the potential use of PDE1 inhibitors for various diseases and has attempted to highlight the importance of this target for degenerative disorders and disorders of cognition.

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**Conflict of Interest Statement** All authors are full-time employees of Intra-Cellular Therapies, Inc.

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# Chapter 14
# PDE Inhibitors for the Treatment of Schizophrenia

**Gretchen L. Snyder and Kimberly E. Vanover**

**Abstract** Schizophrenia is a pervasive neuropsychiatric disorder affecting over 1% of the world's population. Dopamine system dysfunction is strongly implicated in the etiology of schizophrenia. Data support the long-standing concept of schizophrenia as a disease characterized by hyperactivity within midbrain (striatal D2) dopamine systems. In addition, there is now considerable evidence that glutamate neurotransmission, mediated through NMDA-type receptors, is deficient in patients with schizophrenia and that hypoactivity in cortical dopamine and glutamate pathways is a key feature of this serious mental disorder. While current antipsychotic medications—with a common mechanism involving dopamine D2 receptor antagonism or pre-synaptic partial agonism—adequately address positive symptoms of the disease, such as the acute hallucinations and delusions, they fail to substantially improve negative features, such as social isolation, and can further compromise poor cognitive function associated with schizophrenia. In fact, cognitive impairment is a core feature of schizophrenia. The treatment of cognitive impairment and other residual symptoms associated with schizophrenia, therefore, remains a significant unmet medical need. With current cell-surface receptor-based pharmacology falling short of addressing these core cognitive symptoms, more recent approaches to treatment development have focused on processes within the cell. In this review, we discuss the importance of cyclic nucleotide (cNT) phosphodiestereases (PDEs)—intracellular enzymes that control the activity of key second messenger signaling pathways in the brain—which have been proposed as targets for new schizophrenia therapies. We also discuss the challenge facing those developing drugs to target specific PDE enzymes involved in psychopathology without involving other systems that produce concomitant side effects.

**Keywords** Antipsychotic • Dopamine • Glutamate • Prefrontal cortex • Cognition

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## 14.1 The Neurochemistry of Schizophrenia

Schizophrenia is a pervasive neuropsychiatric disorder affecting over 1% of the world's population (www.cdc.gov). Individuals with schizophrenia experience hallucinations and delusions, referred to as 'positive' symptoms, and a variety of other debilitating symptoms, including decreased social function and speech, flat affect, disorganized thought, and low motivation, collectively referred to as 'negative' symptoms. Cognitive impairment is also a core feature of schizophrenia (Tandon et al. 2009). Dopamine system dysfunction is strongly implicated in the etiology of schizophrenia based on the observation that medications most effective in combating psychotic hallucinations and delusions are potent antagonists of striatal dopamine D2-type receptor (Creese et al. 1976; see also Meltzer et al. 1989; Meltzer and Fatemi 1996). These data support the long-standing concept of schizophrenia as a disease characterized by hyperactivity within midbrain (striatal D2) dopamine systems (Davis et al. 1991).

Dopamine dysfunction alone, however, fails to encapsulate the complex features of schizophrenia. For instance, antipsychotic medications that adequately address positive symptoms of the disease, such as the acute hallucinations and delusions, fail to substantially improve negative features and can further compromise poor cognitive function in patients with schizophrenia (Meltzer and Fatemi 1996). Negative symptoms and cognitive disability that often persist even as the positive symptoms are attenuated by current antipsychotic therapy contribute substantially to the long term social and work disability of patients with schizophrenia and diminish their quality of life (Green 1996; Tamminga et al. 1998; Kirkpatrick et al. 2006; Tandon et al. 2009). The negative symptoms and cognitive impairment, together with depression and insomnia, make up the core symptoms of what is sometimes referred to as residual phase schizophrenia that goes largely untreated (Laughren and Levin 2011). There is now considerable evidence that glutamate neurotransmission, mediated through NMDA-type receptors, is deficient in schizophrenia (Javitt 2004) and that hypoactivity in cortical dopamine and glutamate pathways is a key feature of the schizophrenic brain (Davis et al. 1991; Laruelle et al. 2003, 2005). In support of this model, NMDA receptor antagonists, (e.g., ketamine) given at subanesthetic doses, induce psychotomimetic symptoms in humans (Krystal et al. 1994). Further, treatments that increase NMDA receptor activity, either directly by increasing glutamate availability, like inhibitors of the glutamate transporter, GlyT-1, indirectly by activating pre-synaptic metabotropic receptors (i.e., mGluR2/3) that promote glutamate release, or indirectly by increasing phosphorylation of GluN2B receptors for enhanced glutamatergic neurotransmission via increased cell surface expression of the receptors, have efficacy in preclinical screens for antipsychotic activity (Schwartz et al. 2012; Snyder et al. 2014). Thus, increasing NMDA receptor activity would be expected to contribute positively to the treatment of psychosis, and perhaps to the treatment of resistant residual phase negative symptoms and cognitive impairments.

Cognitive deficits, among the core features of schizophrenia, are poorly addressed by existing therapies. Patients with first-episode schizophrenia score one to two standard deviations below healthy control subjects on a variety of cognitive tasks

(Hoff et al. 1999). In an effort to raise awareness and facilitate treatment research in the field, cognitive impairment associated with schizophrenia (CIAS) was characterized into seven differentiable domains by the collaborative Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) program (Green et al. 2004). These seven domains include speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition. More recently, various neurocognitive domains have been shown to be highly correlated with each other (August et al. 2012) and it has been proposed that the cognitive impairment associated with schizophrenia might best be described as deficits in three correlated key areas of processing speed, attention and working memory, and learning (Burton et al. 2013). Despite the intentions of MATRICS over a decade ago to facilitate the development of new therapeutics by outlining neurocognitive measures that map onto the seven domains, that could be implemented in clinical trials, and that would be acceptable by regulatory authorities, no pharmaceutical agents have been approved for the treatment CIAS and this remains a major unmet medical need.

Currently, major challenges exist for the future management of schizophrenia. New molecular targets and treatments are actively being sought for management of positive symptoms of the disorder without the persistent side effects of existing drugs, including movement disorders and metabolic syndrome. Further, novel treatments that would effectively address negative symptoms, mood disorders, and cognitive impairment are a major priority for current drug development in schizophrenia.

## 14.2 Phosphodiesterases as Targets for Schizophrenia

*PDE enzyme classification and control*. The cyclic nucleotide second messengers, cyclic AMP (cAMP) and cyclic GMP (cGMP), have been well-characterized as intracellular signaling molecules in the brain and in peripheral organs [for review, see (Beavo and Brunton 2002)]. Cyclic nucleotides (cNTs) are known to be key participants in signaling cascades that integrate the actions of neurochemical pathways in the brain, including dopamine- and glutamate-containing neurons, which underlie neural defects occurring in the schizophrenic brain. Cyclic nucleotide (cNT) levels are, in turn, determined by the balance between the adenylyl and guanylyl cyclases that catalyze their formation under the control of dopamine and glutamate receptors and the phosphodiesterase (PDE) enzymes, which catalyze their hydrolysis and inactivation. PDEs convert active cyclic nucleotide molecules to inactive 5′ nucleotide forms, thereby terminating their ability to recruit their respective target proteins, the cAMP- and cGMP-dependent protein kinases. To date, eleven families of PDE enzymes have been identified, encoded by 21 genes (Beavo 1995; Conti and Beavo 2007; Menniti et al. 2007; Francis et al. 2011). Individual PDE enzyme families have been classified based on substrate specificities (e.g., cAMP only, cGMP only, dual-substrate or cAMP/cGMP, and either cAMP- or cGMP-preferring) and regulatory factors (e.g., activation or inhibition by cAMP or cGMP, activation by

calcium/calmodulin, and regulation by phosphorylation). These enzymes display unique tissue distributions which distinguish individual PDE families and, sometimes, structurally-related isoforms within the same enzyme family.

*Rationale for PDEs as targets for therapy development in schizophrenia*. PDEs have been increasingly recognized as desirable targets for therapy development in psychiatry. There are several reasons why therapeutics, based on PDE enzymes, may be useful for the treatment of schizophrenia. First of all, several PDE enzyme families are expressed in abundance in the brain (Conti and Beavo 2007; Menniti et al. 2007; Francis et al. 2011), and in particular, in brain regions, including the hippocampus, caudate-putamen, and cortex, which are known to be involved in schizophrenia. Further, these PDE enzymes play a key role in controlling the activity of cAMP and cGMP signaling pathways downstream of dopamine and glutamate receptors that are well-known to be aberrantly affected in the brains of schizophrenic patients (see above) [for reviews, see Ramirez and Smith (2014); Duinen et al. 2015); Shim et al. 2016)]. Finally, the diversity of PDE enzyme families and isoforms present in the brain and the complexity of their regulation offers opportunities to design therapeutic molecules to selectively intervene in dopamine- and glutamate-mediated signaling cascades in a regionally-restricted manner. To date, several PDE enzymes have been investigated preclinically as novel targets for therapy development in psychiatric diseases, like schizophrenia. Table 14.1 briefly describes these PDE enzymes according to their specificity for cAMP and/or cGMP substrates, regulatory factors, known small-molecule inhibitors, as well as their distribution within dopamine-rich brain regions involved in the psychiatric diseases, like schizophrenia, and associated features of the disease, including cognitive function.

In this review, we summarize the available preclinical animal data—and, where available, clinical results—implicating PDE enzymes in the symptomatology and potential treatment of schizophrenia. We have focused attention on the PDE isoforms, namely PDE4, 10A, 1, 2A, 9A, and 11A, that have been most extensively studied with regard to psychiatric disease. The data summarized here include studies of isoform-specific PDE inhibitors (or genetic manipulations of PDEs) that impact measures of antipsychotic activity (i.e., related to the positive symptoms of the disease), affective or social behavior (i.e., related to negative symptoms of the disease), and/or on cognition. A brief overview of key studies relating each of these PDE isoforms with salient effects in schizophrenia-relevant models is provided in Table 14.2.

### *14.2.1 PDE4 Isoforms*

The PDE4 family of enzymes is perhaps the best studied of the all PDE superfamilies with regard to anatomical localization and function (Conti and Beavo 2007) and its involvement in various symptoms of schizophrenia. The PDE4 family is composed of four isoforms (A, B, C, and D) and numerous splice variants (Houslay and Adams 2003). PDE4 enzymes selectively inactivate cAMP (cAMP-specific), and are insensitive to regulation by calcium levels. Certain PDE4 enzymes associate with scaffolding proteins, including RACK1 (i.e., receptor for activated C kinase) and the AKAPs

**Table 14.1** Brain distribution of PDE isoforms implicated in certain features of schizophrenia.

| PDE | Substrate | Modulated by | Inhibitors | CNS Distribution |
|---|---|---|---|---|
| PDE1 (A-C) | cGMP > cAMP | $Ca^{2+}$/calmodulin | IC2224, IC86340, ITI-214 | Striatum, hippocampus |
| PDE2A | cAMP and cGMP | cGMP stimulated | BAY 60-7550 | Broadly throughout the brain, highest in hippocampus |
| PDE4A | cAMP | Phosphorylation | Rolipram | Olfactory tubercle, olfactory bulb, layer V cortical cells |
| PDE4B | cAMP | Phosphorylation | Rolipram | Nucleus accumbens, cerebellum, hypothalamus, cortex |
| PDE4D | cAMP | Phosphorylation | Rolipram | Hippocampus cerebellum, thalamus |
| PDE9A | cGMP | – | BAY 73-6991 | Broadly throughout the brain |
| PDE10A | cGMP/cAMP | cAMP phosphorylation | Papaverine, MP-10, TP-10 | Striatum |
| PDE11A | cAMP/cGMP | cGMP | Tadalifil | Hippocampus |

(i.e., A-kinase anchoring proteins), which mediate cell-compartment-specific aspects of intracellular signaling (Colledge and Scott 1999; Houslay et al. 2005), and NUDEL and Lis1, which are involved in neurodevelopment (Brandon et al. 2004).

*Brain distribution of PDE4 enzymes.* PDE4 was first identified in *Drosophila* based on homology to the *dunce* gene (Cherry and Davis 1995), which is involved in learning and memory. In mammalian brain, PDE4 enzymes localize to brain regions subserving memory function, including cortex and hippocampus. PDE4B is expressed at significant levels in the dopamine-rich striatum, substantia nigra, and nucleus accumbens (Cherry and Davis 1999), a constellation of brain regions subserving functions related to affect, emotion, and motivation (Wise 2004). PDE4D is the PDE4 isoform most highly expressed in hippocampus, where it is localized to CA2/3 pyramidal cells, with significant expression in cerebellum, thalamus, and thalamic output pathways. PDE4A displays the most restricted distribution of the three isoforms in mouse brain, with major expression in olfactory tubercle, olfactory bulb, and layer V cells of the cerebral cortex (Cherry and Davis 1999). Little is known regarding the cellular distribution of PDE4C and its relation to other PDE isoforms.

*PDE4—Effects on cAMP and Cognition in Schizophrenia*: Functionally, inhibition of pan-PDE4 activity, using small molecule inhibitors, or isoform-specific inhibition of PDE4 using genetic strategies, has strongly implicated these enzymes in some of the key features of schizophrenia, particularly in cognitive function. Severely impaired working memory function, which is a prominent and enduring feature of schizophrenia, is largely refractive to treatment by current antipsychotic medications with potent D2 receptor antagonist activity. Activation of dopamine D1-type receptors within prefrontal cortical circuits, however, promotes normal

**Table 14.2** Summary of PDE isoform effects relevant to features of schizophrenia

| Isoform | Manipulation | Schizophrenia symptom domains | | | References |
|---|---|---|---|---|---|
| | | Positive symptoms | Negative symptoms | Cognitive performance | |
| 1 (A-C) | ITI-214 | | | Enhance NOR performance | Snyder et al. (2016) |
| 2A | BAY60-7550 | | Anxiolytic/ antidepressant-like activity | | Masood et al. (2009), Xu et al. (2015) |
| | | | | Reverse NOR deficits—Scopolamine/MK-801 models | Rutten et al. (2007), Reneerkens et al. (2013) |
| 4 | Rolipram; PDE4B KO | | | Reverse working memory deficits—Aged animals | Bach et al. (1999), McGirr et al. (2015) |
| | PDE4B KO | Block condition avoidance responding (CAR); block psychostimulant-induced hyperlocomotion | | | Siuciak et al. (2007a) |
| 9A | BAY63-6991 | | | Enhance NOR performance | Van der Staay et al. (2008), Reneerkens et al. (2013) |
| 10A | Papaverine | | | Reverse PCP deficits in working memory/NOR | Rodefer et al. (2005), Grauer et al. (2009), Smith et al. (2013) |
| | MP-10 or PDE10A KO | Block psychostimulant-induced hyperlocomotion | | | Siuciak et al. (2006), Grauer et al. (2009) |
| | LuAF33241 dual 10A/2A) | Block condition avoidance responding (CAR) | | Enhance NOR performance | Redrobe et al. (2015) |
| | THPP-1 | | | Enhance NOR performance | Smith et al. (2013) |
| 11A | PDE11A KO | | Deficits in social odor recognition (SOR) | | Kelly et al. (2010) |

Effect of isoform-selective inhibitors or genetic manipulations are shown. The relevance of the behavioral effects for positive, negative, or cognitive features of schizophrenia is noted, including pertinent references

working memory function (Sawaguchi and Goldman-Rakic 1991; Chudasama and Robbins 2004; Goldman-Rakic et al. 2004; Fletcher et al. 2005). In fact, dopamine D1 receptors have been identified as by the MATRICS initiative as targets of special interest for development of new neurocognitive approaches to treatment of schizophrenia (Harvey 2009). PDE4 isoforms, including PDE4B and PDE4D are expressed in cortical pyramidal cells that receive dopamine inputs, in hippocampal cell populations that are essential for normal spatial memory function (Cherry and Davis 1999). There is evidence that PDE4B, localized to cortical neurons, exerts strong biochemical control over D1 receptor/cAMP activity in cortex based on increases in D1 receptor signaling and phosphorylation of the dopamine and cAMP-regulated phosphoprotein (Mr = 32 kDa) (DARPP-32) in response to the pan-PDE4 inhibitor, rolipram (Kuroiwa et al. 2012).

Though these observations suggest a role for PDE4 in supporting D1 signaling, and thereby memory function, ascertaining the optimal level of activity within D1 receptor/PKA-dependent pathways for normal working memory performance is complex. Current data support an "inverted-U" response where prefrontal memory function is impaired by abnormal increases or decreases in cAMP activity (Bourtchouladze et al. 1998; Taylor et al. 1999; Williams and Castner 2006). Nonetheless, impaired working memory can be improved by activation of the D1 receptor/cAMP pathway within prefrontal cortex (Barad et al. 1998). Age-dependent deficits in spatial memory function in rats are also attenuated by increases in dopamine D1 activity in the hippocampus (Bach et al. 1999). Inhibition of all PDE4 isoforms with rolipram mimics the effect of a D1 agonist, repairing hippocampal long-term potentiation (LTP) deficits and spatial memory loss in aged animals (Bach et al. 1999; Barad et al. 1998). Recently, selective inhibition of the PDE4B isoform activity, achieved in mice bearing mutant PDE4B enzyme with low cAMP catalytic activity, enhanced memory performance across a range of learning tasks, including working memory and spatial memory paradigms (McGirr et al. 2015). These data support the concept that PDE4 enzyme families modulate D1 receptor-dependent memory circuits in cortex and hippocampus that are impaired by aging and disease. Further, the data suggest that the activity of the PDE4B isoform may play a prominent role in these cognition effects. This quality distinguishes the approach of using PDE4 inhibitors from that of using traditional antipsychotic drugs that further impair cognition (Nagai et al. 2009).

*PDE4 and Antipsychotic Activity*. Pharmacological and genetic inhibition of PDE4 isoforms also results in behavioral effects in animals that are predictive of antipsychotic benefit against positive symptoms of the disease. For example, rolipram mimics some of the biochemical effects of dopamine D2 receptor antagonists used as antipsychotic agents. Inhibition of PDE4 activity with rolipram increases dopamine synthesis, release and turnover in presynaptic dopamine-containing terminals in the striatum in a manner similar to dopamine D2 antagonists, like the antipsychotic medication, haloperidol (West and Galloway 1996; Yamashita et al. 1997; Nishi et al. 2008). Interestingly, rolipram treatment preferentially regulates dopamine signaling in the sub-set of striatal medium spiny-type neurons (MSNs) that express dopamine D2 receptors and project to globus pallidus (i.e., striatopallidal)

rather than the striatal MSNs that enrich dopamine D1 receptors and project to substantial nigra (i.e., striatonigral) (Nishi et al. 2008). The overall effect of rolipram is to mimic the behavioral profile of an antipsychotic drug (Siuciak et al. 2007a). For instance, rolipram blocks the hyperlocomotion caused either by dopamine overactivity (i.e., amphetamine) or by NMDA receptor blockade (i.e., PCP) and reverses deficits in sensorimotor processing produced by amphetamine treatment, as measured in the prepulse inhibition paradigm (Kanes et al. 2006). It also suppresses responding in the conditioned avoidance response (CAR) paradigm (Siuciak et al. 2007a), a model that is particularly sensitive to pharmacological effects of D2 antagonists (Wadenberg and Hicks 1999). The suppression of hyperlocomotion and avoidance responding in the various antipsychotic behavioral screens can result from drug effects on motor performance. This does not appear to account for the behavioral effects of rolipram, as the drug produces only modest reductions in spontaneous motor activity at dose levels far exceeding those used in CAR and hyperlocomotion assays (Siuciak et al. 2007a). The data support the hypothesis that PDE4 inhibition normalizes aberrant dopamine and glutamate neurotransmission to address symptoms of psychosis with minimal effect on movement. The relative lack of motor interference found with PDE inhibition compared with dopamine receptor blockade, despite profound changes in dopamine signaling pathways, is an attractive, yet poorly understood, effect of PDE inhibitors.

*PDE4B Interactions with DICS1:* A link between PDE4 enzymes and schizophrenia is further supported by the association of the PDE4B isoform with DISC1, a 100 kDa adapter protein encoded by a major schizophrenia susceptibility gene, called *Disrupted in schizophrenia-1* (*DISC1*). The biology of DISC1 and its relation to PDE4 and psychiatric illness is reviewed elsewhere (Brandon 2015 and chapters in this volume), and only briefly reviewed here. The *DISC1* gene was first identified as a factor co-segregating with psychiatric illnesses in the pedigree of a large Scottish family (Millar et al. 2000). The DISC1 protein was subsequently shown to directly bind PDE4B to regulate cAMP signaling (Millar et al. 2005). Available data support the idea that DISC1 functions as a targeting protein for PDEs analogous to the well-known targeting proteins for PKA, called AKAPs to sequester PDE4 enzymes, and thus, control their access to cyclic nucleotides within specific cellular compartments to effect the regulation of sub-cellular actions of cAMP in neurons (Houslay and Adams 2003; Conti et al. 2003; Houslay et al. 2005; Brandon 2015). DISC1 activity has also recently been implicated as a mediator of stress-induced cognitive dysfunction, as viral knockdown of the *DISC1* gene transcription specifically in PFC increased the susceptibility of rats to cognitive deficits after stress (Gamo et al. 2013). This observation may be particularly important considering the role of stress in precipitating and exacerbating the symptoms of schizophrenic episodes (Arnsten 2009; Breier et al. 1991).

*Functional Implications of PDE4 Gene Knockout for Schizophrenia:* Since other chapters in this volume provide a detailed review of PDE4, garnered in part from knockout mouse studies, this chapter will focus briefly on data from PDE4 gene knockouts relevant to specific models of antipsychotic activity. As noted above,

non-selective PDE4 inhibitors, (rolipram) mimic certain biochemical and behavioral effects of dopamine D2 antagonists and D1 agonists relevant to schizophrenia. Gene knockout mice have been used in an effort to attribute specific behavioral roles relevant to schizophrenia to individual PDE4 enzymes. Knockout mouse lines have been evaluated for behavioral effects of the two prominent striatal PDE4 isoforms, PDE4B and PDE4D (Siuciak et al. 2008; Zhang et al. 2008; Rutten et al. 2008). Surprisingly, PDE4B knockout mouse and rolipram elicit opposing phenotypes in a number of behavioral models. For example, knockout mice display impaired PPI responses, and enhanced locomotor responses to amphetamine that are accompanied by reduced tissue levels and turnover of DA and 5-HT (Siuciak et al. 2008) suggesting that a down-regulation of monoamines occurs in PDE4B-deficient mice, compared with increased dopamine turnover after PDE4 inhibition (Yamashita et al. 1997). The surprising "pro-psychotic" phenotypes seen in PDE4B KO mice are similar to the reported effect of certain DISC1 missense mutations. For example, the L100P mutation, induced in mice using the technique of *N*-nitroso-*N*-ethylurea (ENU) mutagenesis (Coghill et al. 2002), results in the loss of DISC-1 binding to PDE4B (Clapcote et al. 2007). Mice expressing DISC-1 bearing the L100P mutation display impaired PPI responses indicative of a "pro-psychotic" phenotype, which was partially reversed by either atypical (clozapine) or typical (haloperidol) antipsychotic drugs. Impaired PPI in DISC-1 mutant mice is fully restored to normal levels by treatment with rolipram (Clapcote et al. 2007). In a separate study, PDE4B knockout displayed anxiogenic behaviors (Zhang et al. 2008) not seen in DISC-1 mutant mice (Clapcote et al. 2007). PDE4D knockout mice exhibit deficits in LTP rather than the enhanced LTP seen after rolipram treatment in wild type mice (Rutten et al. 2008). Thus, to date, pharmacological tools and genetic approaches used to discern the impact of individual PDE family enzymes on schizophrenia-like behaviors have revealed complex effects of these enzymes on behavior. However, together with data from DISC-1 mutant mice, results suggest that the selective loss of normal PDE4B activity, mediated either by PDE4B gene knockout or loss of its normal PDE4B sequestration by adaptor proteins such as DISC-1, reveals psychosis-like behaviors.

Drug development efforts to discover PDE4 inhibitors for human use have been extremely challenging. Decades of work on these enzymes has yet to yield small-molecule inhibitors for human clinical evaluation, due in large part to the difficulties in designing agents with minimal interaction with the PDE4D subunit, which mediates (through poorly defined mechanisms) severe side effects, including nausea and vomiting, in animals and humans (Robichaud et al. 2001). Recent work, guided by structural data for the PDE4D isoform (Burgin et al. 2010), has enabled several groups to discover and publish on small molecules with minimal side effects. These efforts involve molecules with selective binding for PDE4B (Hagen et al. 2014) over PDE4D, or molecules capable of exerting negative allosteric modulation (NAM) of PDE4D (Fox et al. 2014). Some of these inhibitors appear poised for preclinical development and human clinical evaluation for cognition enhancement, providing the possibility that the role of PDE4 in cognition impairment in diseases like schizophrenia may eventually be tested.

### *14.2.2 PDE10A Isoform*

*Brain Distribution of PDE10A:* PDE10A is dual-substrate PDE (Fujishige et al. 1999; Loughney et al. 1999). PDE10A brain expression differs significantly from that of the PDE4 enzymes, with expression mostly restricted to the caudate-putamen (in humans), or striatum (in lower animals). PDE10A mRNA and protein are abundant within MSNs of striatum and nucleus accumbens in addition to other structures with connections to the basal ganglia, including the olfactory tubercle (Seeger et al. 2003). It is notable that the highly restricted brain distribution of PDE10A (and PDE1B, as well, see below) is strikingly similar to the brain abundance of dopamine innervation (e.g., high in striatum, lower in cortex) and the composite expression pattern for D1-type and D2-type dopamine receptors. PDE10A immunoreactivity is undetectable in striatal interneurons, suggesting a selective expression of this PDE in dopaminoceptive neurons, specifically GABAergic MSNs expressing dopamine receptors (Coskran et al. 2006). Subcellular fractionation studies reveal that PDE10A (specifically the PDE10A2 splice variant) is associated with synaptosomal fractions and bound to membrane surfaces of striatal dendrites and dendritic spines (Xie et al. 2006) as a result of post-translational modification by palmitoylation and phosphorylation, which controls the enzyme's transit between plasma membrane and cytosol (Kotera et al. 2004; Charych et al. 2010).

*Inhibitors of PDE10A Mimic Antipsychotic Effects of D2 Receptor Antagonists*: PDE10A was first investigated as a possible target for psychiatric diseases, like schizophrenia, based on its highly discrete brain distribution to dopamine and dopamine receptor-enriched target regions. It soon became clear that tool inhibitors of PDE10A activity, like papaverine, mimic the ability of antipsychotic medications to block dopamine D2 receptor intracellular signaling cascades in striatal neurons (Schmidt et al. 2008; Nishi et al. 2008; Grauer et al. 2009) and elicit certain behavioral responses predictive of antipsychotic agent activity. For example, selective PD10A inhibitors like MP-10 and genetic knockout models for PDE10A reverse sensorimotor gating deficits caused by the NMDA receptor antagonist, MK-801 (Siuciak et al. 2006; Grauer et al. 2009; Rodefer et al. 2005). PDE10A inhibitors block hyperlocomotion induced by psychostimulant drugs, such as amphetamine. Importantly, both PDE10A inhibitors and PDE10A gene knockout reduce spontaneous locomotor activity in mice and can result in catalepsy reminiscent of the motor side effects that are a common feature of typical antipsychotic drugs like haloperidol (Smith et al. 2013). PDE10A inhibitors potentiate the cataleptic effects of low doses of haloperidol and PDE10A knockout mice display an exaggerated sensitivity to the cataleptic actions of this drug (Siuciak et al. 2006). Thus, interference with PDE10A activity mimics many of the biochemical and behavioral actions of antipsychotic agents, including motor side effects (Siuciak et al. 2006; Schmidt et al. 2008).

*PDE10A Inhibitors Preferentially Target D2 receptor-enriched Striatopallidal Neurons*: The efficacy of PDE10A inhibitors in behavioral assays for antipsychotic activity is likely explained by the ability of these molecules to biochemically mimic the (cAMP) signaling effects of dopamine D2 antagonists (for review, see Schmidt

et al. 2008). PDE10A inhibitors, including papaverine and MP-10, induce increased phosphorylation of cAMP-dependent targets like DARPP-32, CREB and the GluR1-type glutamate receptor in striatum (Nishi et al. 2008; Grauer et al. 2009; Rodefer et al. 2005). Importantly, data from biochemical, gene expression, and electrophysiological studies conclude that these D2 antagonist-like actions arise predominantly from D2 receptor-enriched striatopallidal neurons. Biochemical studies using BAC transgenic mice engineered to express tagged-DARPP-32 under the control of D1-receptor promoters in striatonigral and striatopallidal neurons, respectively (i.e., D1-flag DARPP-32/D2-myc-DARPP-32 mice), robust (several-fold) increases to PDE10A inhibition in cAMP-dependent DARPP-32 phosphorylation in D2R-expressing (myc-tagged) neurons, but only modest (<twofold) increases in neurons D1R-expressing (flag-tagged) neurons (Nishi et al. 2008). Interestingly, this pathway-specific effect of a PDE10A inhibitor is seen with antipsychotic drugs; typical and atypical medications selectively increase DARPP-32 phosphorylation in D2 receptor-expressing striatal neurons in mice (Bateup et al. 2008). Gene expression, in response to PDE10A inhibition, are similarly more robustly regulated in D2R-expressing neurons of the striatopallidal pathway than in D1R-expressing striatonigral neurons (Wilson et al. 2015; Polito et al. 2015). As shown by Wilson and colleagues, c-fos induction after MP-10 administration to rodents is more prominent in striatopallidal neurons. PDE10A inhibition also preferentially enhances the responsiveness of striatopallidal neurons to activation of cortical inputs. Threlfell and colleagues demonstrate that intra-striatal infusion of the PDE10A inhibitor, TP-10, increased activity of striatal neurons in response to stimulation of cortical afferents, specifically increasing the probability of firing after activation of corticostriatal inputs that were identified as striatopallidal neurons (Threlfell et al. 2009). These data support the idea that functional activation of striatopallidal neurons may predominate in the presence of PDE10A inhibition, owing to the special electrophysiological properties of D2 receptor-containing neurons, and suggesting that a selective targeting of this PDE within D2 receptor-containing neurons may be responsible.

*PDE10A Inhibitors as Therapies for Cognitive and Negative Symptoms*: Traditional D2 receptor antagonists, including many current antipsychotic medications, do not adequately address the negative symptoms and cognitive dysfunction in schizophrenia (Tsapakis et al. 2015). Certain of the cognitive deficits, typical in schizophrenia patients, can be modeled in animals using a sub-chronic regimen of treatment with the NMDA-receptor antagonist, phencyclidine (PCP) (Jentsch et al. 1997, 1999), a compound that also induces a psychosis-like state (i.e., hallucinations) in humans (Krystal et al. 1994). Some evidence supports the contention that inhibition of PDE10A can reverse both the cognitive deficits induced by phencyclidine (PCP), in addition to providing activity against positive symptoms. Rodefer et al. (2005) have reported that deficits in executive function (i.e., attentional set shifting) induced by sub-chronic PCP treatment in rats, are attenuated by treatment with papaverine. Papaverine treatment also enhanced memory performance in rats, as measured in the novel object recognition (NOR) task (Grauer et al. 2009). Memory was enhanced by papaverine across a broad range of doses and at dose

levels effective in antipsychotic screens. Papaverine also improved memory performance in mice, as assayed in the social odor recognition (SOR) paradigm (Grauer et al. 2009). Recently, a novel and more potent PDE10A inhibitor, THPP-1, was shown to enhance performance of rats in the novel object recognition (NOR) paradigm (Smith et al. 2013). THPP-1 also proved effective in reversing ketamine-induced memory deficits in an object retrieval detour task in non-human primates (NHPs). Interestingly, in contrast to the effects of papaverine and THPP-1, the PDE10A inhibitor, MP-10, tested at a range of doses, did not significantly alter memory performance in NOR and had only modest positive effects in SOR (Grauer et al. 2009). The basis for the observed differences in effects of PDE10A inhibitors of varying potency and selectivity will require further investigation.

The development of small-molecule inhibitors of PDE10A has been an area of intense pharmaceutical industry effort over the past decade. During this time, several pharmaceutical companies have developed potent PD10A inhibitors (Kehler and Kilburn 2009; Kehler 2013). The first, and best characterized of these efforts found in the clinical literature is that of Pfizer's efforts with their PDE10A inhibitor, MP-10. Advanced into Phase II human clinical evaluation for treatment of schizophrenia, MP-10 reportedly failed to demonstrate efficacy against positive symptoms of schizophrenia and was associated with motor side effects (Wilson and Brandon 2015). Efforts to advance other candidate compounds into clinic continue, including programs by Roche, Merck, FORUM (formerly EnVivo), and Takeda. A recent review by Wilson and Brandon (2015) provides a succinct summary of the current status of PDE10A as a therapeutic target for schizophrenia and other psychiatric and movement disorders.

In summary, several dual-specificity PDE isoforms, exemplified by PDE4B/D and PDE10A, have been intensively studied as potential targets for schizophrenia based in part on their enrichment in dopamine-receptive neurons and their linkage to disease-related genes. Their respective preclinical effects on cognitive function (Barad et al. 1998; Simpson et al. 2010; Rodefer et al. 2005; Smith et al. 2013) and on dopamine and glutamate signaling pathways involved in antipsychotic drug action (Schmidt et al. 2008; Nishi et al. 2008; Grauer et al. 2009; Kuroiwa et al. 2012) suggest their inhibitors may have efficacy for treating the positive symptoms of hallucinations and delusions as well as cognitive impairment in the disease, provided that side effects related to motor and nausea can be overcome.

### *14.2.3 Other PDE Enzyme Families with Relevance for Schizophrenia*

While PDE4 and PDE10A enzymes have been the first and most aggressively pursued for therapeutic development related to schizophrenia, other PDE family enzymes have been reported to show preclinical efficacy for significant—and currently poorly treated—symptoms of schizophrenia. Here, we will explore the evidence that PDE2A and PD11A families of enzymes may target mood and social

interaction deficits, common in schizophrenics. Other families, including PDE1, display preclinical efficacy for enhancement of memory performance, and are candidates for treatment of cognition impairment associated with schizophrenia (CIAS).

### *14.2.4 PDE2A and PDE11A Isoforms: Effects on Social Interaction/Negative Symptoms*

Some of the most debilitating features of schizophrenia include what are referred to as the 'negative symptoms', including decreased social function and speech, flat affect, disorganized thought, and low motivation (Tamminga et al. 1998; Laughren and Levin 2011). Despite the fact that 'positive symptoms' of the disease usually improve with antipsychotic mediation, negative features are typically resistant to improvement with medication and contribute to an enduring social and work disability of patients with schizophrenia (Meltzer and Fatemi 1996; Tamminga et al. 1998; Tsapakis et al. 2015). Both PDE2A and PDE11A have been investigated as possible targets for the mood disturbances and social isolation behaviors such as those which contribute to long-term disability in patients with schizophrenia.

PDE2A is a cGMP-stimulated, dual-substrate PDE that is broadly expressed in the brain. The enzyme is most abundant in brain regions known to be involved in learning and memory and the control of motivated behaviors, including hippocampus, cortex, striatum, substantia nigra, olfactory bulb, and amygdala (Stephenson et al. 2009; Lakics et al. 2010; Stephenson et al. 2012. In contrast with other PDE isoforms, (e.g., some PDE4 isoforms) hippocampal PDE2A is absent in dentate granule cells and CA2/3 pyramidal cells, but enriched in the hilus and molecular layer of the dentate gyrus, and in CA3 mossy fibers and the subiculum. Thus, PDE2A appears to display a cellular distribution that complements the expression pattern for other PDEs in hippocampus (Stephenson et al. 2009; Fernández-Fernández et al. 2015). High levels are also present in the substantia nigra. In contrast to PDE10A which is expressed relatively equally within striatonigral and striatopallidal MSNs and their fiber projections, PDE2A immunoreactivity appears strongest in nigrostriatal fibers and dopamine cell body regions in the pars compacta regions of the nigra. The available data suggest that PDE2A is present in dopamine-containing pathways innervating striatum, placing this PDE in a position to modulate pre-synaptic dopamine activity in a manner which is similar to PDE4 (Yamashita et al. 1997; West and Galloway 1996; Stephenson et al. 2012). PDE2A is also expressed in a subset of neurons in the ventral tegmental area (VTA), suggesting an additional role in the regulation of mesocortical and mesolimbic regions innervated by VTA. Cortical immunoreactivity for PDE2A appears in several layers with prominent staining in bipolar neurons of layer V. Significantly, strong expression of PDE2A is detected in human prefrontal cortex, a key region involved in the control of working memory in primates and rodents, as determined by western blotting of postmortem tissue samples (Stephenson et al. 2009). PDE2A2, a specific isoform

that co-localizes with proteins in the mitochondrial matrix, may also be an interesting therapeutic target for modulating mitochondrial function as it relates to cognition and age-related disease processes (Acin-Perez et al. 2011).

PDE2A has been investigated as a target for treatment of mood disorders, including anxiety and depression, and for cognitive enhancement. Biochemically, inhibition of PDE2A activity with the potent tool inhibitors, like BAY60-7550, preferentially controls neuronal cGMP levels (Boess et al. 2004). For example, PDE2 inhibitors, such as ND-7001, EHNA and 4N-716, increase basal and NMDA-stimulated cGMP in primary hippocampal neurons. Further, the cGMP changes produced by PDE2A inhibitors are required for anxiolytic effects of in rodent models of anxiety (Masood et al. 2009). Studies in rodents also implicate PDE2A activity in stress-induced depression-like behaviors. Chronic stress results in a significant increase in immobility time of mice in the tail suspension test which is blocked by pretreatment of mice with the PDE2A inhibitors, BAY 60-7550 or ND-7001, to an extent comparable to the classical antidepressant desipramine. These PDE2A inhibitors also produced antidepressant- and anxiolytic-like effects on chronic stress-induced depression- and anxiety-like behaviors in the novelty suppressed feeding test as both drugs reversed the stress-induced increase in the latency to feed. In both behavioral paradigms, effects of PDE2A inhibition were dependent upon intact cGMP signaling, supporting the idea that cGMP availability and resulting signaling pathways are essential for these effects on depression- and anxiety-like behaviors (Masood et al. 2009; Xu et al. 2015).

Several companies have reported preclinical efforts to discover selective inhibitors against PDE2A and/or advance PDE2A inhibitors through preclinical development. Bayer has published on early-stage, potent and selective, small-molecule inhibitors toward the enzyme (Boess et al. 2004), which, to date, have not advanced to clinic. More recently, Pfizer has also reported the discovery and optimization of a portfolio of novel PDE2A inhibitors with efficacy in a non-CNS indication, using an osteoarthritis model (Plummer et al. 2013a, b). The only human clinical evaluation reported, to date, with PDE2A inhibitors is one involving Pfizer compounds for treatment of migraine (www.clinical trials.gov).

*PDE11A:* The dual-specificity PDE, PDE11A, is found as three major variants with expression in several peripheral tissues, including skeletal muscle, prostate, kidney, and testes (Fawcett et al. 2000). At least one splice variant (i.e., PDE11A2) has been localized to rat brain (Yuasa et al. 2001). PDE11A message and protein is present at low level in the brain, but with an interesting restricted enrichment in rodents in the ventral hippocampus (Kelly et al. 2010). Localization of the enzyme to ventral hippocampus is of particular interest for schizophrenia, as this brain region has developmentally-important influences on the appearance of schizophrenia-like phenotypes in rodent models (Lipska and Weinberger 2002). Further, PDE11A knockout mice display subtle deficits in certain social behaviors, including social odor recognition memory that could signal a role for the enzyme in governance of social interaction behaviors which are fundamentally disrupted in schizophrenia (Kelly et al. 2010). The mice also demonstrate a pharmacological supersensitivity to blockade of NMDA-type glutamate receptors with agents that induce psychosis-like

behaviors in humans (Krystal et al. 1994). For example, mice deficient in PDE11A, untreated with drugs, display an unexpected locomotor hyperactivity in open field activity tests and an exaggerated hyperactivity response to the NMDA receptor antagonist, MK-801, compared with wild-type mice. The available data indicate a role for PDE11A in maintaining normal glutamatergic neurotransmission in regions of the hippocampus and extended amygdala that may contribute to social interactions disrupted in schizophrenia. While studies in the PDE11A knockout mouse implicate PDE11A in social behaviors that are fundamentally disrupted in schizophrenia it remains to be seen whether pharmacological manipulation of the enzyme will be a useful approach to addressing the development or symptomatic treatment of schizophrenia.

### *14.2.5 Cognition Impairment in Schizophrenia: PDE1, PDE9, PDE2A Isoforms*

Cognitive impairment is a major predictor of long-term disability in individuals suffering from schizophrenia (Tamminga et al. 1998). Several PDE isoforms have been implicated in the control of cognitive performance. For example, as described above, PDE10A inhibitors have been shown to address psychostimulant-induced deficits in memory function (Rodefer et al. 2005) which are believed to mirror certain cognitive impairments seen in individual with schizophrenia (Jentsch et al. 1997, 1999). Other PDE isoform families, as described below, have also been implicated in the enhancement of memory performance in normal or cognitively-impaired animals using tests such as novel object recognition (NOR), a paradigm measuring short-term memory, which is relevant to memory acquisition processes (Akkerman et al. 2016) and is in the domain of cognitive functions known to be impaired in schizophrenia (Targum and Keefe 2008). It should be noted that while a number of preclinical animal models have been used to evaluate therapeutics for cognition impairment, none of these models have translated into cognitive benefit for schizophrenia, in clinic. Further, no small molecule therapies have been convincingly shown enhance cognitive performance in normal individual or patients, in clinic, to date. Ultimately, the utility of PDE inhibitors for improving cognitive performance in normal individuals and in patients suffering from cognitive impairments associated with distinct CNS disorders, including schizophrenia, Alzheimer's disease and Parkinson's disease, will need to be established through well-planned clinical trials. That being said, several families of PDE enzymes, described below, have been investigated preclinically for specific pro-cognitive effects in animals.

*PDE1*: The PDE1 family of enzymes includes three structurally-homologous isoforms, PDE1A, 1B, and 1C, which are regulated by the presence of calcium and the calcium-binding protein, calmodulin (Bentley et al. 1992; Sonnenburg et al. 1993; reviewed by Wennogle et al., this volume). In fact, the dependence of PDE1 activity on cellular calcium/calmodulin availability is unique among the PDE families (Francis et al. 2011). The calcium-dependence of PDE1, in concert with the

biological data from PDE1-deficient gene models (reviewed below), further indicates that the enzyme is selectively recruited under conditions of neuronal activation in which intracellular calcium levels rise. This implies that PDE1 modulates phasic-endogenous activity rather than producing tonic effects in overall cellular activity (see Reed et al. 2002; Siuciak et al. 2007b). Therefore, development of inhibitors to PDE1 as a drug development strategy may be a way to subtly amplify endogenous signaling pathways without over-driving the system in an unbalanced way. Though all PDE1 isoforms are dual-specificity, hydrolyzing both cAMP and cGMP, they are cGMP-preferring enzymes. Messenger RNAs (mRNAs) for distinct PDE1 isoforms is abundant in several regions of the brain involved in learning and memory and volitional behavior. The PDE1B isoform, in particular, is highly abundant in basal ganglia structures, including striatum, substantia nigra and olfactory tubercle (Yan et al. 1995; Lakics et al. 2010). The highest levels of the PDE1B isoform in the brain are found in MSNs of the striatum (Polli and Kincaid 1992). PDE1B immunoreactivity is also prominent in the hippocampus, with high levels expressed in dentate granule cells, and in pyramidal cells within layers V and VI of the cerebral cortex (Polli and Kincaid 1992; Lakics et al. 2010). PDE1A mRNA is prominent in deep layers of the cerebral cortex and dentate gyrus of the hippocampus, with low levels apparent throughout the mouse brain. In contrast, PDE1C mRNA is expressed at highest levels in mouse cerebellum, with low diffuse staining present in cortex and hippocampus (Lakics et al. 2010).

In the absence of potent and specific inhibitors of PDE1 enzymes, studies utilizing a PDE1B knockout have provided some data to indicate a role for PDE1 in the regulation of dopamine D1 receptor signaling and related behaviors. PDE1B has been localized to DARPP-32-positive MSNs (Polli and Kincaid 1992). Striatal tissue from mice deficient in PDE1B shows an increased protein phosphorylation response at PKA-dependent sites on DARPP-32 and the GluR1 AMPA-type glutamate receptor upon addition of a dopamine D1 receptor agonist (i.e., SKF81297) *in vitro*, compared with tissue from wild-type mice (Reed et al. 2002). The basal level of phosphorylation at these sites, however, was unaffected by the loss of PDE1B expression. Behaviorally, PDE1B knockout mice also demonstrate an accentuated locomotor response to dopamine agonists, in the absence of effects on spontaneous locomotor activity, which has been replicated in several studies with this knockout model (Reed et al. 2002; Siuciak et al. 2007b; Ehrman et al. 2006). Taken together the accentuated biochemical and locomotor response of PDE1B mice to dopamine agonists is consistent with the activity-dependent nature of PDE1B enzyme and the molecular enhancement of dopamine activity through D1 receptor pathways.

*PDE1B Knockout and Memory Performance:* The role played by PDE1 enzymes in memory and learning has been far more complicated to study, compared with locomotor activity responses. A genetic model lacking all three PDE1 isoforms—in which pan-PDE1 function could be studied—is not currently available. To date, only PDE1B knockout mice have been studied with respect to central nervous system function and behavior. Based on the prominent expression of PDE1B in hippocampal regions that mediate dopamine effects on spatial memory (Bach et al. 1999), PDE1B mutant and wild type mice were tested for spatial performance in the

Morris water maze paradigm (Reed et al. 2002). Though PDE1B null mice show no difference from wild type mice in time spent to locate the submerged platform or in total distance travelled during the test, they did demonstrate a small, but significant increase in latency to learn the maze position under both the task acquisition phase and in a reversal paradigm. Factors that can influence memory performance, including anxiety, are not affected by gene mutation (Siuciak et al. 2007b; Ehrman et al. 2006). PDE1B null mice perform normally in elevated plus maze and other anxiety screens, including conditioned avoidance responding (CAR) (Siuciak et al. 2007b).

One factor to consider in evaluating the biochemistry and behavior of the PDE1B knockout mouse is the potential for redundant function of the other PDE1 family members. As reviewed above, PDE1A and PDE1C isoforms are expressed within the same brain regions, and quite possibly the same cells, as PDE1B (Lakics et al. 2010). For example, mRNA for PDE1A, PDE1B, and PDE1C isoforms is present within hippocampal dentate gyrus and in deep layers of the cortex—areas that subsume prominent roles in the memory tasks in which PDE1B null mice have been tested (Devan et al. 1996; Devan and White 1999). Thus, gene knockout of PDE1B alone may be insufficient to reveal the biological role of PDE1 enzymes in memory performance due to residual effects of PDE1A and PDE1C isoforms in hippocampus and striatum. Recently, the development of pan-PDE1 inhibitors with high potency and isoform selectivity has revealed a role for PDE1 in memory performance (Snyder et al. 2016). ITI-214, an PDE1 inhibitor with >1000-fold selectivity for PDE1 versus other PDE enzymes families, was found to enhance memory performance in rats, as measured by NOR. ITI-214 has effects across a broad range of doses on memory acquisition, consolidation, and retrieval processes (Snyder et al. 2016) supporting the role of PDE1 in cognitive performance.

The development of high-potency inhibitors of PDE1 enzymes with selectivity over other PDE enzyme families, like ITI-214, will likely offer opportunities to study PDE1 enzyme family effects on other domains of cognitive function, like working memory, which are implicated in the cognitive impairments seen in schizophrenia. Further, Intra-Cellular Therapies (ITI) has advanced ITI-214 into clinical development—the first example of a PDE1 inhibitor under human clinical evaluation as a potential treatment for CIAS. To date, ITI-214 has successfully completed four Phase I safety studies in healthy volunteers and patients with schizophrenia and is reportedly progressing in clinical development (http://www.intracellulartherapies.com/).

*PDE9A:* PDE9A is a cGMP-specific PDE with the highest affinity for cGMP reported for any PDE enzyme (Km = 170 nM) (Fischer et al. 1998; Van Staveren et al. 2002). Like PDE1B, PDE9A is prominently expressed in cerebellar granule cells, dentate granule cells of hippocampus, olfactory tubercle, and layer V pyramidal cells of cerebral cortex (Van Staveren et al. 2002) and shares a brain expression pattern similar to that of the soluble form of guanylyl cyclase (Polli and Kincaid 1992; Walaas et al. 1989; Van Staveren et al. 2002). Small-molecule inhibitors of PDE9A, available as tool compounds, like BAY63-6991, are effective in enhancing the performance of rats in NOR (Van der Staay et al. 2008; Reneerkens et al. 2009).

*PDE2A*: As noted above, inhibitors of PDE2A activity, such as BAY60-7550, preferentially control neuronal cGMP levels (Boess et al. 2004; Masood et al. 2009; Xu et al. 2013) to result in antidepressant-like and antianxiety-like effects in rodent models. These inhibitors also have potent effects on memory performance in paradigms such as NOR (Rutten et al. 2007; Reneerkens et al. 2013; Xu et al. 2015). For example, Reneerkens et al. (2013) investigated the memory sparing effects of BAY60-7550 in a model of memory impairment, using pharmacological treatment with either the muscarinic cholinergic antagonist, scopolamine, or the NMDA receptor antagonist, MK-801. BAY60-7550 pretreatment reversed memory deficits seen in rats after either scopolamine or MK-801, supporting the idea that PDE2A inhibition may be a suitable approach for addressing memory deficits in various disease states. Recently, Sierksma and colleagues report improvement in memory performance in the APPswe/PS1dE9 mouse model of Alzheimer's disease using PDE2A inhibitors (Sierksma et al. 2013). Of particular relevance to the treatment of cognition in schizophrenia, Lundbeck has reported preclinical data on effects of a combined PDE2A/PDE10A inhibitor, Lu AF33241 in tests of antipsychotic activity (conditioned avoidance response, CAR) and cognition (NOR) (Redrobe et al. 2015). Janssen has also reported novel, dual PDE2A/PDE10A inhibitors (Andres et al. 2013), though no preclinical data are available for those compounds.

## 14.3 Summary

Schizophrenia is a pervasive neuropsychiatric disorder affecting over 1% of the world's population. Though some disease features can be controlled by available medications for some patients, major challenges still exist in addressing resistant (negative) features, including social withdrawal/isolation, flat affect, depression, lack of motivation, etc., which hinder the social integration of patients into families and into the community. Several families of key intracellular enzymes, known as phosphodiesterases (PDEs), control the second messenger (cAMP and/or cGMP)-dependent intracellular signaling pathways within brain glutamatergic and dopaminergic system central to the underlying neurochemistry of schizophrenia. Evidence from pharmacological and genetic studies implicate activities of distinct PDE enzymes in the positive features of schizophrenia (e.g., hallucinations and delusions) as well as the negative features of the disease, including mood disorders and cognition impairment. Research is continuing in the search for small molecule inhibitors capable of translating exciting preclinical observations on PDE enzyme biology into therapeutic proof of concept studies in humans.

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

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# Part V
# PDEs and Others

# Chapter 15
# Targeting Phosphodiesterases in Pharmacotherapy for Substance Dependence

**Rui-Ting Wen, Jian-Hui Liang, and Han-Ting Zhang**

**Abstract** Substance dependence is a chronic relapsing brain disorder associated with adaptational changes in synaptic plasticity and neuronal functions. The high levels of substance consumption and relapse rate suggest more reliable medications are in need to better address the underlying causes of this disease. It has been well established that the intracellular second messengers cyclic AMP (cAMP) and cyclic GMP (cGMP) and their signaling systems play an important role in the molecular mechanisms of substance taking behaviors. On this basis, the phosphodiesterase (PDE) superfamily, which crucially controls cyclic nucleotide levels by catalyzing their hydrolysis, has been proposed as a novel class of therapeutic targets for substance use disorders. This chapter reviews the expression patterns of PDEs in the brain with regard to neural structures underlying the dependent process and highlights available evidence for a modulatory role of PDEs in substance dependence.

**Keywords** Substance dependence • Central nervous system • cAMP • cGMP • Signal transduction • Phosphodiesterase

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## 15.1 Introduction

Substance dependence, also known as drug addiction, is a chronic relapsing brain disorder. It can be characterized by compulsive and repetitive use of alcohol, nicotine or other drugs of abuse despite negative consequences, as well as multiple physical and psychological signs indicating tolerance and withdrawal (American Psychiatric Association 2000; World Health Organization 2004). Misuse of substances constitutes one of the most serious public health issues worldwide, which requires more reliable medical approaches to better control substance consumption and the high relapse rates. Exploration into specific mechanisms of the dependent process may contribute to uncover novel therapeutic targets for medication development.

From a neurobiological perspective, dependence can be defined as an adaptive state of the central nervous system (CNS) (Koob and Le Moal 1997; Koob 2003a). This process is associated with abnormal synaptic plasticity and a series of neuronal dysfunctions that possibly develop as early as the first exposure to addictive substances (Wang et al. 2014; Pandey et al. 2005a; Jing et al. 2011; Qin et al. 2013; Liu et al. 2012; Luo et al. 2011). The best established molecular mechanism of the adaptational changes in individual neuron involves dysregulated intracellular signal transduction, especially in the second messengers cyclic AMP (cAMP) and cyclic GMP (cGMP) as well as their signaling systems (Peregud et al. 2013; Pandey et al. 2005a; Nestler 2004; Javadi et al. 2013). Accumulating evidence indicates that altered activity of cAMP and/or cGMP signaling plays an important role in the motivational aspects, rewarding properties, and relapsing features of substance taking behaviors (Pandey et al. 2001, b; Wen et al. 2015; Kleppisch and Feil 2009).

Intracellular cAMP and cGMP signal transduction is triggered by elevated levels of cAMP and cGMP, which are generated by adenylyl cyclase (AC) and guanylyl cyclase (GC), respectively. However, the concentrations of these cyclic nucleotides are crucially determined by the activity of phosphodiesterases (PDEs), which represent the only known enzyme superfamily that catalyze the hydrolysis of cAMP and cGMP. PDEs have been studied for about six decades and consist of more than 100 different protein products transcribed from at least 21 genes in human genome (Lugnier 2006). All PDE isoforms can be identified in 11 families (PDE 1–11) according to their structural and functional characteristics (Lugnier 2006; Bender and Beavo 2006), and are classified into three groups based on their substrates: cAMP-specific PDEs (PDE4, PDE7, PDE8), cGMP-specific PDEs (PDE5, PDE6, PDE9), or dual substrate PDEs (PDE1, PDE2, PDE3, PDE10, PDE11). Most mammalian cell types express PDEs. In the CNS, which contains all the PDE isoforms (Menniti et al. 2006), the activity of PDEs have been reported as essential regulators for multiple CNS functions, including synaptic plasticity (Sanderson and Sher 2013), learning and memory (Blokland et al. 2006; Rose et al. 2005). Based on the significant involvement of cAMP and cGMP signaling in the key features of substance taking behaviors, PDEs have been proposed as potential therapeutic targets for substance use and abuse (Logrip 2015; Mu et al. 2014; Lai et al. 2014; Thompson

et al. 2004; Iyo et al. 1995; Wen et al. 2015). This chapter provides an overview of PDE expression profiles in the brain with regard to neural structures underlying the dependent process and highlights recent evidence that implicates a regulatory role of PDEs in substance dependence.

## 15.2 Neural Structures Underlying Substance Dependence

Neurobiological responses to addictive substances involve complex interactions between different parts of the CNS (Daglish et al. 2005). Although differing in primary actions on the brain, addictive substances are likely to share similar neural mechanisms underlying their rewarding properties and dependent process (Li et al. 2008; Nestler 2005). The limbic corticostriatal circuitry, consisting of neural circuit across multiple limbic cortical brain regions, has been considered as the key neural system mediating the motivation, rewarding, and behavior response to substances of abuse (Lingford-Hughes et al. 2010). The development of dependence can be seen as a dysfunction of these processes. Among the key brain structures in this circuitry, the nucleus accumbens (NAc), ventral tegmental area (VTA), amygdala, and hippocampus are of most importance to the dependent process. Their general function and neurochemical modulation will be briefly discussed here.

In the initial steps of dependent behaviors, psychoactive substances exert their euphoric and rewarding effects mainly through activation of the mesolimbic dopaminergic system, which begins in the VTA of the midbrain and projects to the NAc and prefrontal cortex (Moore and Bloom 1978). Preclinical studies have shown that increased dopamine (DA) release in the NAc represents the primary regulator of rewarding properties for nearly all substances of misuse (Hyman and Malenka 2001; Chao and Nestler 2004; Imperato and Di Chiara 1986). This up-regulation of DA levels can be attributed to stimulation of dopaminergic neurons in the VTA (e.g. opiates, alcohol), DA reuptake blockade in the NAc (e.g. cocaine), or blockade combined with DA release from neuronal terminals (e.g. amphetamine) (Lingford-Hughes et al. 2010). The NAc can be divided into core (NAcC) and shell (NAcSh) subregions based on different morphology and functions (Heimer et al. 1991). The NAcC, which is linked to the caudate putamen and substantia nigra, is involved in motor function that facilitates reward acquisition (Heimer et al. 1997). However, the NAcSh shares similar afferent projections and neurochemical modulation with the central nucleus of the amygdala (CeA), leading to its inclusion in the "extended amygdala" (EA) structure (Koob 2003a). Addictive substances usually cause higher DA release in the NAcSh than in the NAcC (Zocchi et al. 2003). Therefore, the NAc, especially the NAcSh, takes part in the initial positive reinforcement of addictive substance exposure, contributing to habit-forming patterns of substance use.

Studies to date indicate that, despite positive reinforcing properties of addictive substances, the anhedonic or dysphoric states (e.g. anxiety, depression) derived from pre-existing conditions or substance withdrawal are also implicated as a negative reinforcer in substance use and abuse (Koob 2003b; Thompson et al. 2012;

Koob and Kreek 2007). Continuous substance consumption or relapse is considered as a way to alleviate these aversive conditions (Koob et al. 1993; Pandey 2003). The amygdala appears to be closely involved in modulating the negative emotional states (Koob et al. 1998; Pandey 2004). The interconnected nuclei of the amygdala can be grouped into the central, medial, and basolateral divisions, each with different afferent and efferent projections (Pitkanen et al. 1997). The central and medial amygdala (CeA and MeA, respectively), which represent major components of the EA structure, are shown to be critical for the innate and withdrawal-induced dysphoric reactions, especially anxiety-like behaviors (Koob et al. 1998; Moonat et al. 2010). Dysregulated neural functions in the CeA and MeA can offer genetic predisposition to excessive substance use in animal models (Moonat et al. 2013; Pandey et al. 2005a, b; Prakash et al. 2008). On the other hand, the basolateral nuclei of amygdala (BLA) is necessary for motivational value or sensory specific properties of substance reinforcers (Fuchs and See 2002; Fuchs et al. 2006). Lesions of the BLA disrupt drug-seeking behavior in rodent models (Fuchs and See 2002; Meil and See 1997). Moreover, the nonadrenergic nucleus of locus coeruleus (LC) is specifically involved in the development of opiate dependence and withdrawal. Biochemical changes in LC regulate chronic actions of opiates and attenuate somatic signs of opiate withdrawal symptoms (Han et al. 2006; Lane-Ladd et al. 1997; Punch et al. 1997; Guitart et al. 1992).

The hippocampus is an important brain structure for declarative memory, i.e. memory of events and facts. To some extent, physical and psychological dependence to addictive substances can be conceptualized as maladaptive memories obtained from repeated substance exposure (Milton and Everitt 2012). It has been well-established that exposure to contexts previously associated with drug use can promote relapse in both human and animal models (O'Brien et al. 1992; Kearns and Weiss 2007). Inactivation of the dorsal hippocampus leads to reduction in the context-induced reinstatement of drug seeking in rodents (Fuchs et al. 2007; Fuchs et al. 2005). Meanwhile, neurogenesis in the hippocampus has been shown to alleviate anxiety and depression, two important factors that can promote substance taking behaviors (Li et al. 2009; Li et al. 2011).

## 15.3 Biodistribution of PDEs in the Brain

In situ hybridization is widely used in examining mRNA expression of a single PDE subtype in different brain regions, and shows more consistent results than immunohistochemistry detecting PDE protein expression (Bender and Beavo 2006). The later application of quantitative real-time polymerase chain reaction (RT-PCR) has enabled quantitative comparison of the expression levels for all PDEs in different tissues (Lakics et al. 2010). Available data shows that PDEs are widely distributed in the brain in a tissue- and cell-specific manner, and their expression patterns are distinct among different subtypes.

### 15.3.1 cAMP-Specific PDEs

Among the 11 PDE families, PDE4 is most important in the control of intracellular cAMP (Zhang 2009). There are four subtypes (PDE4A, 4B, 4C, and 4D) and at least 25 different splice variants of PDE4, among which PDE4B is most highly expressed across the human brain (Lakics et al. 2010). PDE4B is abundantly expressed in the cortex, amygdala, striatum, hippocampus, hypothalamus, and cerebellum, suggesting its possible role in DA-associated and emotion-related processes (Zhang 2009). PDE4A and PDE4D share a similar distribution pattern with PDE4B but at lower expression levels in DA-enriched brain regions. Relative abundant expression of PDE4D in area postrema and nucleus of solitary tract may account for the side effects of nausea and emesis associated with PDE4 inhibitor treatment (Perez-Torres et al. 2000). In contrast to these PDE4 subtypes, PDE4C is predominantly located in peripheral tissues, with little CNS functions.

The PDE7 family is encoded by two genes, PDE7A and 7B. Relatively low levels of PDE7 mRNA are detected in the hippocampus, cortex, striatum, and hypothalamus, with PDE7B as the major isoform (Reyes-Irisarri et al. 2005; Lakics et al. 2010). PDE7B has a very selective and high expression in the Purkinje cells.

PDE8 expression is detected throughout the human brain, albeit at relative low levels. PDE8 has two subtypes, PDE8A and 8B. PDE8A mRNA levels are similar to PDE8B in the cerebellum, thalamus, and substantia nigra, but lower in all other CNS tissues. To date, no data regarding the effects of PDE8 inhibitors have been reported.

### 15.3.2 cGMP-Specific PDEs

PDE5A, the only isoform of PDE5, is expressed in cerebellar Purkinje neurons. Its expression level appears to be very low in the human brain compared with other PDEs.

PDE6 expression is restricted to the retina and pineal gland and appears to play no direct role in neural functions (Bender and Beavo 2006).

Moderate levels of PDE9A mRNA are present in cerebellar Purkinje cells, hippocampus, hypothalamus, and substantia nigra. A selective PDE9 inhibitor has been shown to improve learning and memory in rodents (van der Staay et al. 2008).

### 15.3.3 PDEs with Dual Enzyme Specificity

Three subtypes of PDE1 (PDE1A, 1B, and 1C) have been shown to exhibit comparable distribution patterns in the human brain, with PDE1A expressed at the lowest levels. PDE1B and PDE1C are highly distributed in the cortex, hippocampus, striatum, substantia nigra, and cerebellum. PDE1B is the most prevalent PDE isoform in

the NAc, while PDE1B together with PDE10A is the most abundant in the caudate nucleus. The high levels of PDE1B in the caudate putamen and NAc indicate its possible enrollment in rewarding and motivational behaviors. Compared to PDE1B, PDE1C has much higher expression levels in the substantia nigra and hypothalamus and similar expression levels in the cortex, hippocampus, and cerebellum.

PDE2A is found highly expressed across the human brain. It represents the most prevalent PDE in hippocampal and cortical regions and second highest in the NAc, supporting its role in learning, memory, and rewarding properties (Boess et al. 2004).

PDE3 is comprised of PDE3A and PDE3B. It shows relatively high levels only in the cerebellum, with negligible expression in other parts of the brain.

PDE10A shows high levels of expression in the striatum, substantia nigra, cerebellum, and hypothalamus. In the caudate nucleus, it is one of the two most prevalent PDE, the other being PDE1B. Selective inhibition of PDE10 has been shown to exhibit antipsychotic activity in rodent models, indicating therapeutic potential of PDE10 inhibitors in schizophrenia (Siuciak et al. 2006; Schmidt et al. 2008).

PDE11 is the most recently described PDE family, with PED11A being the only isoform. PDE11A is present at particularly low levels in most brain regions in human except the dorsal root ganglia.

The distinct distribution patterns and substrate-specific modulation of PDE isoenzymes indicate their potential regulation of different neural functions. Therapeutic effects may be achieved via chemical or biological manipulation of specific PDE isoforms, although studies regarding their CNS functions are needed for further demonstrations.

## 15.4 Cyclic Nucleotide Signaling in Substance Dependence

The main cellular signal pathways sensitive to PDE regulation are the second messenger cAMP and cGMP signal transduction together with the cyclic nucleotide-gated ion channels (Podda and Grassi 2014). In the cAMP signal system, AC catalyzed cAMP generation is functionally coupled to multiple neuronal receptors via guanine nucleotide binding proteins (G proteins). Elevated cAMP levels ultimately lead to phosphorylation of the gene transcription factor cAMP response element-binding protein (CREB) via cAMP-dependent protein kinase (PKA). CREB occupies a central position for the interaction of multiple intracellular signal cascades, including the signal pathways from Ras to extracellular regulated kinases (ERK1/2) and p38 mitogen activated protein kinase (MAPK) (Impey et al. 1999; Mayr and Montminy 2001; Lonze and Ginty 2002). Phosphorylated CREB (pCREB) regulates gene expression by binding to the cAMP response element (CRE) region in the promoter regions of their target genes (Shaywitz and Greenberg 1999). The above impacts of the cAMP signaling render it a critical modulator in experience-based neuroadaptations. On the other hand, intracellular cGMP is synthesized by cytosolic soluble guanylyl cyclases (sGCs) and membrane-bound particulate guanylyl cyclases (pGCs) in response to nitric oxide (NO) and natriuretic peptides

(NP), respectively. Elevated cGMP levels activate cGMP-dependent protein kinase (PKG) and alter cellular functions via phosphorylation of substrate proteins. In comparison with PKA, PKG plays only a minor role in the regulation of CRE-dependent gene transcription (Collins and Uhler 1999). However, both cAMP- and cGMP-mediated signals have been shown to play an integral role in patterning behavior responses to substance exposure.

### *15.4.1 cAMP Signal Transduction*

Signal transduction triggered by cAMP, $Ca^{2+}$, neurotrophic factors, or other cellular stimuli ultimately culminates in specific gene expression patterns via CREB phosphorylation or dephosphorylation (Xing et al. 1998; Impey et al. 1999; Lonze and Ginty 2002). For addictive substances with primary actions on G protein coupled receptors (GPCRs) (e.g. opiates and cannabis), stimulatory or inhibitory G-proteins (Gs or Gi, respectively) mediates their post-receptor actions via CREB phosphorylation or dephosphorylation. Likewise, substances with primary actions on other types of targets (e.g. alcohol and cocaine) also induce functional changes in cAMP signal system. Since CREB has been implicated in the expression of many immediate early genes (e.g. c-fos) and neuropeptide genes [e.g. neuropeptide Y (NPY)], CREB phosphorylation may be an important early nuclear event mediating long-term consequences of substance use and abuse. Moreover, DA represent the common modulator for euphoric effects of most addictive substances, with its $D_1$ (D1, D5)- and $D_2$ (D2, D3 and D4)-like receptors all coupled to G proteins (Hopf et al. 2003; Kebabian and Greengard 1971). On these grounds, it is hypothesized that the cAMP signaling represents a common route for intracellular actions induced by addictive substances in the CNS (Table 15.1).

The three types of opioid receptors (μ, δ, and κ) all belong to the GPCR superfamily. Opiates mainly act on the μ-opioid receptors (MORs) which are negatively coupled to AC-activated cAMP generation via inhibitory Gi proteins (Childers 1991). Acute morphine exposure *in vitro* down-regulates cAMP signal transduction in neurons and brain tissues of the striatum, frontal cortex, LC, and dorsal raphe (Kaplan et al. 1998; Duman et al. 1988). Compounds that increase intracellular cAMP levels attenuate morphine-induced discriminative-stimulus effects in the rat models, indicating they decrease the reinforcing properties of morphine (Yan et al. 2006). On the contrary, chronic opiate exposure leads to a compensatory up-regulation of the cAMP signaling in a brain region-specific manner (Nestler and Aghajanian 1997; Kaplan et al. 1998; Duman et al. 1988). The LC and dorsal root ganglion/spinal cord exhibit enhanced levels of AC and PKA after chronic morphine exposure, while the NAc and amygdala show increased AC and PKA activity, and the thalamus shows increased PKA activity only (Nestler 2015; Kaplan et al. 1998; Duman et al. 1988). Activated cAMP transduction is also detected following opiate removal or naloxone administration, which may contribute to features of withdrawal (Kaplan et al. 1998; Guitart et al. 1992). Decreased CREB levels in the

**Table 15.1** Main types of addictive substances and their impact on cAMP signal transduction

| Category | Substances | Primary action target | cAMP signal transduction | | |
|---|---|---|---|---|---|
| | | | Acute exposure | Chronic exposure | Withdrawal |
| Opiates | Morphine | MOR (GPCR) | ↓ | ↑ | ↑ |
| | Heroin | MOR (GPCR) | | ↑ | ↑ |
| Cannabis | | CB1 receptor (GPCR) | ↓ | ↑/↓ | |
| Sedatives | Alcohol | GABA/glutamate receptor | ↑ | ↓/– | ↓/↑ |
| Stimulants | Amphetamine | | ↑ | ↓ | ↓ |
| | Methamphetamine | | ↑ | ↑ | ↑/↓ |
| | Cocaine | DA transporter | ↑ | ↑/↓ | ↑/↓ |
| | Nicotine | N-AChR | – | ↓ | ↑/↓ |

↑ increase in levels or function,↓ decrease in levels or function, – remain unchanged, *MOR* μ-opioid receptor, *GPCR* G-protein coupled receptor, *GABA* γ-aminobutyric acid, *N-AChR* nicotinic acetylcholine receptor

LC via genetic CREB knockout or knockdown attenuate the severity of opiate withdrawal symptoms (Lane-Ladd et al. 1997; Punch et al. 1997; Maldonado et al. 1996; Han et al. 2006). Thus, alterations in the cAMP pathway are involved in both the acute reinforcing effects of and long-term adaptive responses to opiate exposure (Lane-Ladd et al. 1997; Punch et al. 1997), supporting a crucial role of the cAMP signals in opiate dependent process. Similar cAMP modulation is observed with heroin. In rodents exhibiting heroin-seeking behaviors, activated cAMP signaling can reduce the rewarding properties of heroin (Sun et al. 2015). Chronic heroin treatment increases AC activity, cAMP generation and CREB phosphorylation in the rat NAc, while spontaneous withdrawal increases pCREB in the caudate putamen (Jiang et al. 2012; Edwards et al. 2009). Agents that inhibit cAMP signal transduction alleviate heroin withdrawal symptoms (Jiang et al. 2012). However, in humans, chronic heroin consumption leads to decreased amount and activity of AC in the temporal cortex (TC), but not the NAc, of heroin addicted brains, indicating a down-regulating mechanism of cAMP signaling (Shichinohe et al. 2001; Shichinohe et al. 1998).

The endogenous cannabinoid (CB) receptors, including CB1, CB2 receptors, and GPR55, are all functionally coupled to G proteins (Baker et al. 2006; Munro et al. 1993). Upon cannabis derivative binding, CB receptors negatively regulate AC activity and inhibit cAMP signal transduction via Gi proteins. CB1 (the brain type) receptors, with confined expression on presynaptic terminals, represent the predominant type of CB receptors expressed in the CNS, while CB2 (the peripheral type) receptors are mainly located in leukocytes of peripheral tissues (Pertwee et al. 2010). Activation of CB1 receptors usually causes inhibition of neurotransmitter release. The reduction of inhibitory neurotransmitters, such as γ-aminobutyric acid (GABA), is critically responsible for stress and reward mechanisms related to cannabis dependence.

Alcohol has many different effects on the CNS. The pleasurable effects are thought to be mediated via MORs in the VTA, while other effects, such as ataxia, sedation and anxiolysis, are mediated through the GABA-benzodiazepine receptor complex (Lingford-Hughes et al. 2010). Acute ethanol exposure causes activation in cAMP signal transduction both *in vitro* (Asher et al. 2002; Constantinescu et al. 2002; Gordon et al. 1986) and *in vivo* (Asyyed et al. 2006; Yang et al. 1996), which might be attributed to stimulation in AC activity (Nelson et al. 2003; Yoshimura and Tabakoff 1995). Among the nine membrane-bound AC isoforms (AC1–9), the activity of AC7 is most sensitive to ethanol exposure with 2–3 fold greater cAMP generation than other isoforms (Yoshimura and Tabakoff 1995; Yoshimura and Tabakoff 1999). On the other hand, chronic ethanol treatment attenuates acute ethanol induced rapid increase in cAMP signal transduction. Decreased cAMP signaling is detected in the mouse cortex (Saito et al. 1987) and hippocampus (Valverius et al. 1989) as well as in the rat cerebellum (Yang et al. 1996, Yang et al. 1998a, b) and striatum (Yang et al. 1998a, b) after long-term alcohol exposure. It should be noted that chronic voluntary ethanol intake decreases CREB phosphorylation in the NAcSh, but not the NAcC, in rats (Li et al. 2003; Misra et al. 2001). Down-regulated cAMP signaling is also found in the rat cortex and CeA in response to ethanol withdrawal, while CREB phosphorylation and CRE-DNA binding ability in the cortical structure remain unaffected during long-term ethanol exposure (Pandey et al. 2001a, b; Pandey et al. 2003; Pandey et al. 1999a, b). In contrast, hippocampal pCREB levels in the rat brain are increased during ethanol withdrawal after being decreased by chronic ethanol treatment (Bison and Crews 2003). Thus, different neurons or brain regions may differ in intracellular cAMP signal transduction in response to ethanol exposure, which ultimately results in distinct alcohol-induced effects.

In addition to mediating the intracellular actions of alcohol, key elements of the cAMP signaling have been proposed to act as genetic factors for the predisposition and modulation of alcohol tolerance and dependence. AC1 and AC8 are the only AC isoforms primarily activated by calcium through activation of calmodulin. Decreased AC1 levels have been found in cortical structures of postmortem brains from clinical alcoholic patients who have been abstinent from alcohol for at least 6 months (Sohma et al. 1999; Hashimoto et al. 1998). The subsequent RT-PCR analysis has shown that mRNA levels of AC1 and AC8 are lower in blood cells of alcoholics with a positive family history compared to non-drinker controls (Sohma et al. 1999). In preclinical studies, Muglia and colleagues have demonstrated that AC1 knockout mice display enhanced sensitivity to ethanol-induced sedative effect, while AC8 knockout lead to decreased voluntary ethanol intake (Maas et al. 2005). In contrast, mice lacking AC5 display increased ethanol intake and preference as well as reduced sensitivity to ethanol sedation (Kim et al. 2011). The opposite modulation pattern of AC5 compared with AC1 and AC8 may be attributed to its different brain distribution feature. AC5 shows a preferential concentration in the dorsal striatum and NAc (Kim et al. 2008), while calmodulin-sensitive AC1 and AC8 are predominantly expressed in olfactory system and neocortex (Muglia et al. 1999; Xia et al. 1991). On the other hand, Pandey and colleagues have found that CREB, pCREB, and downstream NPY expression are innately lower in the NAcSh, but not in the NAcC,

of alcohol-preferring C57BL/6 (C57) mice compared to non-preferring DBA/2 (DBA) mice (Misra and Pandey 2003; Belknap et al. 1993). Similarly, levels of CREB and pCREB have been shown to be lower in the CeA and MeA, but not in the BLA, in alcohol-preferring (P) rats compared with non-preferring (NP) rats.

A major negative reinforcer of alcohol use and abuse is anxiety. Correspondingly, brain-region specific deficits of CREB function mentioned in the preceding paragraph correlated with anxiety-like behavior and higher alcohol preference in P rats (Pandey et al. 2005a, b; Pandey et al. 1999a, b). Pandey and co-workers have shown increased anxiety-like behavior and higher ethanol preference in CREB-haplodeficient mice compared to wild-type littermates (Pandey et al. 2004); they also demonstrated that infusions of the PKA inhibitor Rp-cAMP into the CeA inhibited CREB phosphorylation provoked anxiety-like behavior and increased ethanol consumption in NP rats (Pandey et al. 2005a, b). In contrast, acute ethanol treatment via voluntary intake or systemic injections increases pCREB levels and produce anxiolytic-like effects in P rats, but not in NP rats. Pharmacological activation of CREB signaling in the CeA by the PKA activator Sp-cAMP or NPY decreases both anxiety levels and ethanol consumption in P rats. These results suggest an important role of CREB function in anxiety-like and alcohol drinking behaviors. In addition to being associated with an innately higher alcohol preference, anxiety is involved in withdrawal symptoms. Sprague-Dawley (SD) rats withdrawn from ethanol after chronic exposure display anxiety-like behavior, which is correlated with decreased levels of pCREB and NPY in the CeA or MeA, while expression of total CREB remains unchanged (Pandey et al. 2003). Infusions of Sp-cAMP directly into the CeA increase pCREB and NPY expression to normal levels, and prevent the development of anxiety-like behavior in response to abstinence in SD rats. In contrast, Rp-cAMP infusions into the CeA decrease CREB phosphorylation and provoke anxiety and increase alcohol preference in normal SD rats (Pandey et al. 2003). Taken together, these results suggest the activity of cAMP signaling is negatively related to alcohol drinking behavior. Deficits of CREB activation in the NAc and/or CeA, either innately or due to alcohol withdrawal, may promote alcohol intake; blocking these deficits may decrease alcohol consumption and prevent alcohol addiction.

Although psychostimulants, such as amphetamine, cocaine, and nicotine, don't directly act on GPCRs, altered cAMP signal transduction is also found as neuronal response to these drug exposure. Acute and chronic amphetamine treatment causes increased CREB phosphorylation and CRE-mediated transcription in rodent striatum and primary striatal cultures (Konradi et al. 1994; Turgeon et al. 1997; Shaw-Lutchman et al. 2003; Cole et al. 1995). CREB has also been shown to be necessary for amphetamine induced *c-fos* gene expression and possibly the long-term adaptive responses of amphetamine administration (Konradi et al. 1994). Similar modulatory patterns have also observed following methamphetamine exposure. Extended access to methamphetamine self-administration causes increased stiatal and hippocampal CREB phosphorylation and downstream gene expression in rat models (Krasnova et al. 2016; Liu et al. 2014). Likewise, both acute and chronic cocaine treatment results in increased cAMP generation and signal transduction in the NAc neurons of rats (Terwilliger et al. 1991; Zhdanova and Giorgetti 2002). However, continuous intracerebroventricular

(ICV) infusions of cocaine decreases CREB phosphorylation in the rat caudate putamen (Di Benedetto et al. 2007), indicating a brain region-specific pattern of cocaine action on cAMP signaling. Chronic intermittent administration of psychostimulants, such as cocaine and amphetamine, can produce a sensitized behavioral response characterized by locomotor hyperactivity and stereotyped behavior in rodent models (Post and Rose 1976). This behavioral sensitization involves the changes in mesolimbic DA systems (Heidbreder et al. 1996; Parsons and Justice 1993; Post and Rose 1976), and is thought to underlie drug craving and relapse (Steketee 2005). Pretreatment with selective AC activator in the VTA induces sensitization to the locomotor stimulant effects of amphetamine and cocaine, while intra-VTA microinjection of PKA inhibitor blocks amphetamine-induced behavioral sensitization (Tolliver et al. 1996). Similarly, concurrent intra-NAc injection of 8-bromo-cAMP, an analogue of cAMP which activates PKA, increases locomotor activity in responses to acute cocaine exposure and the subsequent challenge (Miserendino and Nestler 1995). Moreover, repeated ICV injection of forskolin, a direct AC activator, enhances behavioral sensitization to systemic cocaine administration in rats (Schroeder et al. 2004). These findings suggest that enhanced cAMP signal transduction can potentiate the sensitizing effects of psychostimulants and may underlie a molecular mechanism for the development of behavioral sensitization. Similar with the case in alcohol dependence, down-regulation of CREB-mediated signal transduction via overexpression of a dominant-negative mutant CREB in the NAcSh decrease the threshold of cocaine to induce conditioned place preference (CPP) in rat models; conversely, up-regulated CREB signaling by CREB overexpression in the NAcSh increases cocaine doses to induce CCP and makes low doses of this drug aversive (Carlezon et al. 1998). These results indicate that innate levels of CREB signal transduction in the NAcSh play a critical role in regulating rewarding properties of cocaine. Finally, acute nicotine administration activates neuronal nicotinic acetylcholine receptors (nAChRs), which belong to the ligand-gated ion channel receptor family, but exhibits no impact on PKA activity; whereas chronic nicotine exposure results in nAChR desensitization and decreased PKA activity in the rat brain, suggesting inhibited PKA signaling may be responsible for nicotine tolerance and dependence (Sun et al. 2004).

In summary, the cAMP/PKA/CREB signal pathway is prominently involved in rewarding properties and neuroadaptational responses to addictive substances. Activation of this signaling is considered as an important compensatory mechanism to decrease the motivational properties of drugs of abuse.

### *15.4.2 cGMP Signal Transduction*

Although relatively fewer studies have investigated the involvement of cGMP-mediated signaling in substance use and abuse, components of this signal system may also play a role in the neuronal adaptations and behavioral responses to multiple substances. Most of the cGMP effects are mediated via activation of PKG and its effects on subsequent targets. In the CNS, the canonical NO/sGC/cGMP/PKG pathway modulates

long-term changes in synaptic activity and contributes to many forms of learning and memory processes (Kleppisch and Feil 2009). Studies have shown that cGMP reduces DA release in cells and in brain regions related to addictive behaviors in animal models (Samson et al. 1988; Guevara-Guzman et al. 1994; Thiriet et al. 2001).

Activated cGMP signal transduction via *in situ* injection of cGMP-elevating agents in the median prefrontal cortex, but not the NAc, reduces intravenous cocaine self-administration (Deschatrettes et al. 2013). Besides, stimulation of cGMP signaling in the VTA decreases cocaine-induced locomotor hyperactivity and relative gene expression in dopaminergic brain regions. This effect is reversed by pretreatment with a selective PKG inhibitor (Jouvert et al. 2004). However, systemic increases in NO or cGMP availability promote cocaine-induced behavior sensitization and hippocampal long-term potentiation (LTP) (Gabach et al. 2013). These results indicate that cGMP signaling pathway may have brain region- or system-specific effects on neuroadaptation and behavioral response to cocaine.

Chronic ethanol exposure increases cGMP levels in the rat cortex, striatum, and hippocampus, while cessation of this treatment decreases cortical and striatal cGMP to normal levels (Uzbay et al. 2004). Consistent with previous studies implicating cGMP signaling in the modulation of anxiety (Li et al. 2005; Volke et al. 2003), PKG type II knockout mice show increased anxiety-like behaviors, reduced ethanol's sedative effects, and potentiated voluntary alcohol consumption (Werner et al. 2004). Conversely, pharmacological activation of cGMP signaling in either the VTA or the prefrontal cortex reduces alcohol deprivation and causes higher ethanol intake, an effect that can be reversed by PKG inhibition (Romieu et al. 2008). Thus, the activity of cGMP signaling is negatively correlated to alcohol-drinking behaviors in a way similar to cAMP signaling.

The cGMP signaling pathway has also been implicated in morphine and nicotine use. In an electrophysiological study, GC activation has been shown to initiate a novel form of LTP in GABA-mediated synaptic transmission. Morphine exposure *in vitro* and *in vivo* prevented this type of LTP by inhibiting presynaptic glutamate release or interrupting the signaling from NO to GC, respectively, indicating the involvement of cGMP signaling in neuroadaptations to opioid drugs (Nugent et al. 2007). In addition, opiate withdrawal studies have shown selective sGC inhibition suppressed the behavioral signs of morphine withdrawal precipitated by naloxone (Sullivan et al. 2000). With regards to nicotine, a genome wide study has identified an association of the human PKG type I gene with nicotine dependence (Uhl et al. 2007).

## 15.5 Role of PDEs in the Process of Substance Dependence

The crucial involvement of cAMP and cGMP-mediated signals in substance dependence raises the possibility of their essential modulators, PDEs, as potential therapeutic targets for the treatment of this disease. As the brain expression profile and substrate specificity are distinct among PDE isoforms, it is hypothesized that more than one PDE may be involved in the dependent process. Available evidence has

revealed the enrollment of several subtypes of PDEs in substance dependent animal models (Table 15.2). However, further studies are still needed to verify their modulatory mechanism and the functional roles of other PDEs in substance related disorders.

### *15.5.1 PDE1*

The PDE1 family consists of three subtypes of dual-substrate PDEs, which are activated by calcium and calmodulin (Bender and Beavo 2006). With high expression level in the striatum as well as the regulation of both cAMP and cGMP signaling, PDE1 has been proposed to play a role in substance dependent behaviors. However, the selective PDE1 inhibitor vinpocetine showed no significant effect on alcohol intake in the two-bottle choice test via systemic administration (Blednov et al. 2014). Microinjections of the PDE1 inhibitor into drug dependence-related brain nuclei may aid to better determine whether PDE1 modulates substance actions in the CNS. Nevertheless, it is noteworthy that vinpocetine shows cognitive-enhancing effects by facilitating LTP (Molnar and Gaal 1992) and improving memory consolidation (Deshmukh et al. 2009) in rodent models. Furthermore, it ameliorates learning and memory impairment in rodents exposed to alcohol during fetal development (Filgueiras et al. 2010). It remains to be studied whether this protective effect on cognition is present against other substances or in adulthood as well.

### *15.5.2 PDE3*

PDE3 is expressed at relatively low levels throughout the brain, and mainly known for its cardiovascular modulations. Correspondingly, the PDE3 inhibitors milrinone and olprinone show negative effects on alcohol intake when tested in the two-bottle choice test (Blednov et al. 2014). Further studies are needed to detect the CNS actions of PDE3.

### *15.5.3 PDE4*

Widely distributed in the brain, the cAMP-specific PDE4 exhibits a multitude of effects on the CNS. Pharmacological blockade or genetic knockout of PDE4 produces anti-depressive (Li et al. 2009; Zhang 2009; Zhang et al. 2002), anxiolytic (Siuciak et al. 2007; Rutter et al. 2014; Ankur et al. 2013) and antipsychotic (Kelly et al. 2007) effects; enhances LTP (Chen et al. 2010; Navakkode et al. 2004); and improves performance in learning and memory (Barad et al. 1998; Rutten et al. 2008; Li et al. 2011; Zhang et al. 2000; Zhang et al. 2004). The ability of PDE4

**Table 15.2** Overview of PDEs role in substance dependence

| PDE isoform | Substance | Inhibitor | Animal | Behavioral paradigm | Results | Reference |
|---|---|---|---|---|---|---|
| PDE1 | Alcohol | Vinpocetine | C57BL/6J mice | Two-bottle choice drinking | Vinpocetine did not affect alcohol intake | Blednov et al. (2014) |
| PDE3 | Alcohol | Milrinone | C57BL/6J mice | Two-bottle choice drinking | Milrinone or olprinone showed no significant effecteffect on alcohol intake or preference | Blednov et al. (2014) |
| | | Olprinone | | | | |
| PDE4 | Methamphetamine | Rolipram | Wistar rats | Behavioral sensitization | Rolipram inhibited methamphetamine-induced behavioral sensitization | Iyo et al. (1995, 1996) |
| | Cocaine | Rolipram | Wistar rats | Operant self-administration | Rolipram and Ro-20 1724 suppressed the initiation of cocaine self-administration | Knapp et al. (1999) |
| | | Ro-20 1724 | | | | |
| | | Rolipram | Wistar rats | Discrete trial | Rolipram augmented cocaine's reinforcing effect | Knapp et al. (2001) |
| | | Rolipram | Swiss Webster mice | Behavioral sensitization | Rolipram prevented cocaine-induced locomotor sensitization | Janes et al. (2009) |
| | | Rolipram | Sprague-Dawley rats | CPP[a] | Rolipram impaired the acquisition, but not the expression or extinction, of cocaine-induced CPP | Zhong et al. (2012), Liddie et al. (2012) |
| | | | B6129S mice | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Morphine | Rolipram | C57BL/6J mice | Naloxone precipitated withdrawal | Rolipram blocked withdrawal behavioral manifestations in morphine dependent mice | Hamdy et al. (2001) |
| | | Rolipram Diazepam | Sprague-Dawley rats | Naloxone precipitated withdrawal | Either rolipram or diazepam co-administration attenuated morphine withdrawal symptoms | Gonzalez-Cuello et al. (2007), Nunez et al. (2009) |
| | | Rolipram | Swiss Webster mice | CPP | Rolipram inhibited the acquisition but not expression of morphine-induced CPP | Thompson et al. (2004) |
| | Heroin | Rolipram | Sprague-Dawley rats | Operant self-administration | Rolipram decreased heroin-seeking behaviors | Lai et al. (2014) |
| | Alcohol | Rolipram | C57BL/6J mice | Two-bottle choice drinking | Rolipram, Ro-20 1724, selectively decreased alcohol intake | Hu et al. (2011) |
| | | Ro-20 1724 | | | | |
| | | Rolipram | C57BL/6J mice | Two-bottle choice drinking | Rolipram, piclamilast, CDP840 and mesopram all decreased alcohol intake in both continuous and limited access two bottle choice drinking | Blednov et al. (2014) |
| | | Piclamilast | | | | |
| | | Mesopram | | | | |
| | | CDP840 | | | | |
| | | Rolipram | FH/Wjd rats | Operant self-administration | Rolipram selectively inhibited alcohol seeking and drinking behaviors | Wen et al. (2012) |
| | | | | Two-bottle choice drinking | | |

(continued)

**Table 15.2** (continued)

| PDE isoform | Substance | Inhibitor | Animal | Behavioral paradigm | Results | Reference |
|---|---|---|---|---|---|---|
| PDE5A | Cocaine | Sildenafil | Wistar rats | Behavioral sensitization | Sildenafil potentiated behavioral sensitization to cocaine | Gabach et al. (2013) |
| | | Zaprinast[b] | Wistar rats | Operant self-administration | Zaprinast reduced cocaine self-administration | Deschatrettes et al. (2013) |
| | Alcohol | Zaprinast | C57BL/6J mice | Two-bottle choice drinking | Zaprinast had no effect on alcohol consumption | (Blednov et al. (2014) |
| PDE 9A | Cocaine | BAY-73-6691 | B6129S mice | CPP | BAY-73-6691 facilitated extinction and diminished the reinstatement of cocaine CPP | Liddie et al. 2012) |
| PDE10A | Cocaine | Papaverine | B6129S mice | CPP | Papaverine had no effect on the extinction of cocaine CPP | Liddie et al. (2012) |
| | Alcohol | TP-10 | Scr:sP[c] | Operant self-administration | TP-10 decreases alcohol self-administration in both alcohol-preferring Scf:sP rats and alcohol-dependent or nondependent Wistar rats | Logrip et al. (2014) |
| | | | Wistar rats | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Nonspecific | Cocaine | IBMX[d] | Sprague-Dawley rats | Behavioral sensitization | IBMX attenuated development of cocaine-induced behavioral sensitization | Schroeder et al. (2012) |
| | Alcohol | Propentofylline | C57BL/6J mice | Two-bottle choice drinking | Propentofylline has no effect on alcohol intake | Blednov et al. (2014) |
| | | Ibudilast[e] | P rats | Two-bottle choice drinking | Ibudilast decreased alcohol intake in P rats, HAD1 rats, and C57BL/6J mice | Bell et al. (2015) |
| | | | HAD1 rats | | | |
| | | | C57BL/6J mice | | | |

[a]*CPP* conditioned place preference
[b]Highest selectivity for PDE5, less potent at PDE1, PDE10 and PDE11
[c]Scr:sP: sardinian alcohol-preferring rats of The Scripps Research Institute subline
[d]IBMX, isobutylmethylxanthine, both nonspecific PDE inhibitor and A1 receptor antagonist
[e]Nonspecific but with preference to PDE3, PDE4, PDE10 and PDE11

inhibition to ameliorate negative emotional state and improve memory performance suggests that PDE4 may play a role in substance use disorders.

The functional role of PDE4 has first proved in animal models of methamphetamine-induced behavioral sensitization (Nishikawa et al. 1983). This sensitization appears to be mediated by enhanced DA efflux in mesolimbic and/or nigrostriatal DA systems (Kalivas and Stewart 1991; Akimoto et al. 1990). Co-administration of the selective PDE4 inhibitor rolipram inhibits methamphetamine-induced locomotor sensitization by increasing cAMP levels while not affecting DA release (Iyo et al. 1996). However, stereotyped behavior is not altered by rolipram, indicating PDE4 partly regulates behaviors related to hyperdopaminergic activity.

Consistently, rolipram co-administration prevents the development of locomotor sensitization to cocaine, but has no effect on cocaine-induced activation of the ERK transcriptional pathway in the NAc (Janes et al. 2009), which represents critical neuroadaptation for cocaine-related behavioral plasticity (Girault et al. 2007; Valjent et al. 2006). However, these results conflicts with the above mentioned findings revealing that enhanced cAMP levels increase behavioral sensitization to cocaine and amphetamine (Miserendino and Nestler 1995; Tolliver et al. 1996). These discrepancies may be attributable to different experimental procedures of drug treatment and behavior measurement procedures. In studies involving cocaine self-administration, both rolipram and Ro 20-1724 prolong the latency for cocaine self-administration and reduce the number of cocaine infusions (Knapp et al. 1999). This suppression of cocaine-seeking behavior is consistent with the results obtained from D1-like receptor agonists, indicating a negative modulatory influence of PDE4 inhibitors on motivational systems and mesolimbic dopaminergic neurotransmission. Moreover, intra-VTA or systemic injections of rolipram can attenuate the acquisition, but not the expression, of cocaine-induced CPP (Thompson et al. 2004; Zhong et al. 2012). Systemic rolipram administration shows no effect on the extinction of cocaine CPP (Liddie et al. 2012), but can increase *c-fos* expression in the NAcSh, but not NAcC or caudate putamen (Thompson et al. 2004). Finally, intra-NAc infusions of rolipram produce enhancement of the sensitivity of brain stimulation reward (BSR) pathways, an effect that is potentiated when combined with systemic cocaine administration (Knapp et al. 2001). On the contrary, systemic administration of rolipram blocks the effects of BSR or raises BSR thresholds, indicating a NAc-specific role of rolipram. The above results suggest that PDE4 inhibition can produce suppression in multiple behaviors related to cocaine exposure possibly through activation of cAMP signaling in mesolimbic DA systems, including the VTA and NAc.

Research regarding PDE4 functions in the development of morphine dependence has been mainly focused on morphine withdrawal. Naloxone-participated withdrawal symptoms following cessation of chronic morphine exposure are usually associated with up-regulation of the cAMP and cGMP signal pathways as well as immediate early gene expression in rodent models. Studies from Abe and coworkers demonstrated that the elevated cAMP signaling following morphine withdrawal may be attributed to lack of PDE4 activation (Kimura et al. 2006). A combination of rolipram and morphine chronic treatment significantly reduces

naloxone-precipitated withdrawal manifestations and prevents the increase in brain *c-fos* gene expression in morphine dependent mice (Hamdy et al. 2001). Similar results have also been observed in rat models. Co-administration of the PDE4 inhibitor rolipram or diazepam with morphine during the pre-treatment period significantly reduces withdrawal symptoms as well as the enhanced noradrenaline turnover and cAMP levels in the heart and hypothalamic paraventricular nucleus (PVN) of rats (Gonzalez-Cuello et al. 2007; Nunez et al. 2009). However, cGMP levels are not affected by these inhibitors, and *c-fos* expression is not modified in PVN either. Besides its involvement in the long-term dependent process, PDE4-mediated cAMP signaling has also been implicated in the establishment of reward valence to opiates. Rolipram by systemic administration reduces the acquisition, but not the expression, of morphine-induced CPP by increasing *c-fos* expression in the NAcSh but not NAcC (Thompson et al. 2004). Rolipram also decreases heroin-seeking behaviors, which is correlated with the increases in CREB phosphorylation in the NAc of rats (Lai et al. 2014).

Finally, PDE4 inhibitors have been shown to decrease alcohol seeking and consumption behaviors. Our studies showed that, in C57BL/6J mice and FH/Wjd rats, systemic administration of PDE4 inhibitors rolipram or Ro-20 1724 selectively reduced ethanol intake without altering total fluid or water intake in the two-bottle free-choice drinking paradigm (Hu et al. 2011; Wen et al. 2012). This inhibitory effect on ethanol drinking seemed to mainly result from PDE4 regulation and less likely to be related to taste preference, rolipram-induced sedation or nausea, or interference in alcohol metabolism. These findings were later confirmed by a similar study examining the effects of several selective PDE4 inhibitors, including rolipram, CDP840, piclamilast, and mesopram, in the two-bottle choice test (Blednov et al. 2014). These agents all produce a suppression in ethanol intake of C57BL/6J mice in both long-term and limited-access two-bottle choice drinking. Similar with our results, the effect of single-dose rolipram, CDP840, or piclamilast only lasts for the first 6 h, while mesopram exhibits a long-lasting reduction of ethanol intake. Moreover, rolipram also selectively reduced operant ethanol self-administration without altering sucrose or water seeking in FH/Wjd rats (Wen et al. 2012). All these data strongly support a positive correlation of PDE4 activity with alcohol dependent behaviors.

### *15.5.4 PDE5*

The cGMP-specific PDE5 is a single gene PDE family. Studies of PDE5A have mainly focused on its regulation of smooth muscle vasodilation by using selective PDE5A inhibitors, such as sildenafil, vardenafil, and tadalafil. With the significant expression in cerebellar Purkinje cells, PDE5A has been shown to modulate memory performance (Xu et al. 2011) and produce antidepressant-like effects (Liebenberg et al. 2010).

As cGMP signaling may underlie many substance dependent processes, studies have begun to test the efficacy of PDE5A inhibitors in regulating addictive behaviors. PDE5A blockade by sildenafil increases cGMP availability, potentiates behavioral cocaine sensitization, and reduces threshold to generate hippocampal LTP (Gabach et al. 2013), a regulation pattern opposite to PDE4. Zaprinast, another PDE5A inhibitor, reduces intravenous cocaine self-administration by rats when injected into the prefrontal cortex, but not the NAc (Deschatrettes et al. 2013), which might involve epigenetic modulation in dopaminergic brain regions. Noteworthy, however, though zaprinast has the highest selectivity for PDE5A, it may also elicit effects through inhibition of PDE1, 9, 10 and 11 (Bender and Beavo 2006). Thus, while mainly targeting cGMP hydrolysis, it may also increase cAMP levels to some extent.

### *15.5.5 PDE9A*

Along with high expression in the hippocampus, the expression of cGMP-specific PDE9A is also strong in cerebellar Purkinje cells. The selective PDE9 inhibitor BAY-73-6691 has been shown to enhance long-term memory and attenuate memory deficits associated with aging in rodent models (Domek-Lopacinska and Strosznajder 2010; van der Staay et al. 2008). Thus, PDE9A inhibition is considered as a potential therapeutic manipulation for memory deficits related to neurodegenerative disorders including Alzheimer's disease (Wunder et al. 2005). Likewise, in a mouse CPP model, acute administration of BAY-73-6691 facilitates extinction and diminishes the reinstatement of cocaine-induced place preference (Liddie et al. 2012), possibly by elevating cGMP levels in the amygdala and hippocampus, two areas involved in regulating emotional and spatial learning.

### *15.5.6 PDE10A*

PDE10A is a dual-specificity PDE that regulates both cAMP and cGMP activation. Its relatively high expression levels in striatal brain regions render it as a possible modulator of dopamine-associated and stress-related processes. Both of the PDE10A inhibitors, TP-10 and papaverine, exhibits antipsychotic properties by decreasing psychotic-like behaviors, including phencyclidine- or amphetamine-induced behavioral abnormalities (Grauer et al. 2009; Schmidt et al. 2008; Siuciak et al. 2006). These effects are likely attributed to suppression in mesolimbic dopaminergic neurotransmission (Sotty et al. 2009).

A positive relationship between PDE10A expression levels and stress or alcohol drinking patterns has been well established by Logrip and colleagues. In an operant alcohol self-administration model, the baseline of alcohol lever preference in high and low alcohol-drinking rats is positively correlated with BLA and CeA *Pde10a*

mRNA levels, respectively. Rats with a stress history of repeated footshock during alcohol self-administration training show increased *Pde10a* mRNA expression in the BLA. This stress history ultimately increases 'relapse' of alcohol self-administration in low alcohol-drinking rats following an extinction period; it does not alter alcohol seeking or intake in high alcohol-drinking rats or change sucrose self-administration in either group rats. This protracted effect of stress history shows a positive relationship to *Pde10a* mRNA levels in the prelimbic subdivision of the prefrontal cortex (Logrip and Zorrilla 2012). In addition to further supporting the negative reinforcing effects of aversive affective states on substance use and relapse these results demonstrate the involvement of stress-induced *Pde10a* expression in the motivational aspect of substance taking behaviors. Measurements of *Pde10a* mRNA expression have also been taken in alcohol withdrawal period (Logrip and Zorrilla 2014). In response to acute (8–10 h) alcohol withdrawal, *Pde10a* mRNA levels were increased in the MeA, BLA, as well as the infralimbic and anterior cingulate subdivisions of the prefrontal cortex. Following protracted (6w) withdrawal, *Pde10a* expression was increased only in the BLA, but down-regulated in the MeA, prelimbic prefrontal cortex, and dorsal striatum. These suggest that consistent up regulation of *Pde10a* mRNA expression in the BLA is a lasting neuroadaptation associated with alcohol dependence.

To confirm the above findings, the efficacy of the selective PDE10A inhibitor TP-10 was tested in alcohol self-administration models (Logrip et al. 2014). Relapse-like alcohol seeking and intake were decreased by systemic pretreated TP-10 in rats with or without a stress experience. TP-10 also reduced alcohol self-administration in genetically alcohol-preferring rats (Scr:sP) as well as in alcohol-dependent and non-dependent Wistar rats. Region-specific microinjections of TP-10 implicated the dorsolateral striatum as an additional structure to the NAc involved in modulating the inhibitory effects on alcohol seeking and drinking behavior. However, saccharin self-administration was also inhibited by TP-10, suggesting a nonspecific modulating pattern of PDE10A on reinforcing properties of rewards. On the other hand, the PDE10A inhibitor papaverine, despite increasing both cAMP and cGMP levels in the hippocampus and amygdala, didn't show any significant effect as the PDE9A inhibitor on cocaine-induced place preference after extinction training in a mice CPP model (Liddie et al. 2012). This may be attributable to lower density of PDE10A in the hippocampus, which play a key role in extinction learning, and less efficiency in hydrolyzing cGMP compared to PDE9A.

### *15.5.7 Nonspecific*

Nonspecific PDE inhibitors have also been tested for efficacy in substance related behaviors. For instance, isobutylmethylxanthine (IBMX), a nonspecific PDE inhibitor and an adenosine receptor antagonist, attenuates the development of cocaine-induced behavior sensitization following ICV administration. However, whether this effect is resulted from direct enhancement of cAMP signal transduction or

inhibition of adenosine production along with A1 receptor-mediated noradrenergic activation needs to be further elucidated (Schroeder et al. 2012). In the two-bottle free choice drinking test, propentofylline does not alter alcohol consumption (Blednov et al. 2014), while ibudilast reduces alcohol intake in three different rodent models of alcohol dependence, i.e. alcohol-preferring P rats, high-alcohol drinking HAD1 rats, and alcohol dependent C57BL/6J mice (Bell et al. 2015). The effects observed with ibudilast may derive from its preferential inhibition of PDE3A, PDE4, PDE 10, and PDE11 (Gibson et al. 2006), demonstrating the down-regulating properties of PDE4 and PDE10A in alcohol drinking behavior.

## 15.6 Conclusions and Future Perspectives

As described above, PDEs represent promising therapeutic targets for treatment of substance dependence through regulation of cAMP and cGMP signal transduction. Based on the studies to date, PDE4, PDE5A, PDE9A, and PDE10A appear to play a functional role in modulating behavioral responses and neural adaptation to multiple substances. For the most part, inhibition of these PDEs produces suppression in substance-related behaviors. Additional studies are still needed to get more in-depth knowledge in the mechanisms of PDE mediations. For instance, more sophisticated technology including genetic manipulations should be employed to characterize the involvement and modulation patterns of individual PDE subtypes or isoforms in the dependent behavior. This may aid in the development of novel and specific PDE inhibitors that may reduce the off-target side effects of current PDE inhibitors (e.g. the emetic properties of PDE4 inhibitors), which represent a major hurdle for their clinical use (Rutter et al. 2014). On the other hand, the discovery of broader acting PDE inhibitors with fewer side effects may also be beneficial because substance dependence involves both cAMP and cGMP signaling as well as the interaction among multiple brain regions with different PDE distribution. Together, the available studies raise the possibility of PDEs as potential therapeutic targets for substance-related disorders. Further research into the CNS function of PDEs and the discovery and development of novel PDE inhibitors will provide a better basis for developing therapeutic manipulations with higher translational potential.

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

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# Chapter 16
# Genetic Understanding of Stroke Treatment: Potential Role for Phosphodiesterase Inhibitors

**Anjana Munshi and Satrupa Das**

**Abstract** Phosphodiesterase (PDE) gene family is a large family having at least 21 genes and multiple versions (isoforms) of the phosphodiesterase enzymes. These enzymes catalyze the inactivation of intracellular mediators of signal transduction such as cAMP and cGMP and therefore, play a pivotal role in various cellular functions. PDE inhibitors (PDEI) are drugs that block one or more of the five subtypes of the PDE family and thereby prevent inactivation of the intracellular cAMP and cGMP by the respective PDE-subtypes. The first clinical use of PDEI was reported almost three decades ago. Studies later found the ability of these compounds to increase the levels of ubiquitous secondary messenger molecules that can cause changes in vascular tone, cardiac function and other cellular events and thus these findings paved the way for their use in various medical emergencies. PDEs are found to be distributed in many tissues including brain. Therefore, new therapeutic agents in the form of PDEI are being explored in neurodegenerative diseases including stroke. Although studies have revealed their use in cerebral infarction prevention, their full-fledged application in times of neurological emergency or stroke in specific has been very limited so far. Nevertheless, recent investigations suggest PDE4 and PDE5 inhibitors to play a vital role in mitigating stroke symptoms by modulating signaling mechanisms in PDE pathway. Further, extensive research in terms of their pharmacological properties like dosing, drug specific activities, use of simultaneous medications, ancillary properties of these compounds and studies on adverse drug reactions needs to be carried out to set them as standard drugs of use in stroke.

**Keywords** Phosphodiesterases • Phosphodiesterase inhibitors • Stroke • Rolipram • Therapeutic potential

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## 16.1 Introduction

Stroke is the most common cause of neurological disability and a leading cause of death worldwide. Attack of stroke can result in a variety of symptoms and signs but the most widely seen effect is that of motor impairment that controls the movement of face, arm and leg of one side of body (Lawrence et al. 2001). This clinical syndrome results from a number of different disease processes and is categorized into two types. Approximately 80% of patients have been recorded to suffer from ischemic stroke while the other 20% suffer from hemorrhagic stroke. An ischemic stroke happens when a blood vessel (artery) supplying blood to an area of the brain becomes blocked by a blood clot. A hemorrhagic stroke happens when an artery in the brain leaks or bursts (ruptures). These two basic categories of stroke are further divided into other subtypes depending on several other delineating clinical features. Its prognosis too varies greatly depending on a number of factors like pre-morbid condition, stroke severity, age, and post-stroke complications. Although stroke affects both young and adult individuals, population demographics study has shown that stroke among older people to result in more severe functional loss (Baztán et al. 2007). Stroke rehabilitation has thus focussed on recovery of impaired movement/function to reduce disability resulting from both motor and non-motor impairments post stroke.

Recent reports have focussed on acute management of stroke and a significant amount of progress has been made in this direction. A multitude of studies have assessed novel therapeutic interventions in patients and emerging evidence has revealed the concept of brain repair (neuroplasticity) or endogenous neurorestorative processes that have helped in development of pharmacological and cell-based therapies capable of stimulating neurological recovery after stroke (Hermann and Chopp 2012). Despite novel interventions, drugs have been the main management agents till date that are capable of altering numerous biological processes leading to a stroke attack or worsen the condition of a patient post stroke. Agents such as lipid lowering drugs (statins and recombinant tissue plasminogen activator) and antiplatelet agents (aspirin or ecosprin and clopidogrel) have now long been in use along with management of conventional risk factors like hypertension, diabetes and hyperlipidemia that are the main contributing factors that predisposes one to stroke (Meschia 2007). However, apart from clinical characteristics and environmental factors, genetic factors have been suggested to contribute in the development and worsening of condition. A number of genomic studies in the area of stroke have suggested different candidate genes responsible for stroke. One such widely studied plausible gene is phosphodiesterase 4D (PDE4D) identified by deCODE group in Icelanders. Further, replicative studies among different populations (Gretarsdottir et al. 2003; Munshi et al. 2009; Saleheen et al. 2005; Liu et al. 2013; Staton et al. 2006; Matsushita et al. 2009) and studies on different variants of this gene including various aspects of PDE4 pathway provided a strong evidence for its role in the development of stroke (Das et al. 2016). Interestingly, the Icelandic study by

Gretarsdottir et al. (2003) reported decrease in stroke risk in individuals with PDE4D polymorphisms by PDE4D inhibition using small molecule inhibitors (Gretarsdottir et al. 2003).

With advancement in research, PDE and its inhibitors have been reported to be involved in functional behavior of humans and animals and therefore, PDEI are emerging as therapeutic agents and physiological modifiers (Kuhlenbaumer et al. 2006). Therefore, exploring the use of PDEI in molecular therapeutics for possible diseases would prove to be very helpful.

## 16.2 General Features of PDE and PDEI

PDE or diester-orthophosphoric-phosphohydrolases are basically a group of enzymes capable of hydrolyzing cyclic nucleotides adenosine 3' and guanosine 3' in their inactive form 5'-cyclic monophosphates (cAMP and cGMP). These molecules are essentially secondary messenger molecules and PDEs essentially degrade the phosphodiester bond in these molecules. In mammals PDEs are classified into 11 families (PDE1-PDE11) depending on amino acid sequences, substrate specificities, regulatory properties, pharmacological properties and tissue distribution. Regulation of localization, duration and amplitude of cyclic nucleotide signaling within subcellular domain is their main function (Beavo 1990). Similarly, there are a number of PDEI having various therapeutic implications that have altered response to specific tissues depending on cyclic nucleotides. Initially it was caffeine that was found to act as an inhibitor of PDE following which a lot of nonselective PDE inhibitors such as theophylline (caffeine analogue) entered into clinical use. Later, several isoenzyme-selective PDEI were developed as therapeutics. As therapeutic agents they have been used in control of pathophysiological changes caused due to cyclic nucleotides in central nervous system, lungs, digestive tract and inflammatory processes (Clarke et al. 1994; Cristina and Nagy 2003; Kanes et al. 2007; Li et al. 1994; Lipworth 2005; Wright 2006). Since PDE have unique tissue distribution, structural and functional properties they have been used as successful targets for pharmacological inhibition during times of cardiac failure, pulmonary hypertension, erectile dysfunction etc. (Barnes et al. 1988; Torphy and Undem 1991; Boolell et al. 1996).

Although the use of PDEI in a number of clinical conditions has been well studied, its use in stroke has only recently been explored. PDE1, PDE4, PDE8, PDE9 and PDE10 have been reported to be distributed in brain tissues and therefore, various PDEI have the potential to be used in the treatment of neurodegenerative diseases (Fig. 16.1) (Boswell-Smith et al. 2006). Apart from their distribution in brain, PDE isoforms found on blood platelets (PDE2, PDE3 and PDE5) also offer a novel strategy to deal with stroke (Fig. 16.2). Further, it is to be noted that PDEI generally has multiplicity of effects due to action of drugs on more than one isoform and many tissues harboring more than one isoform.

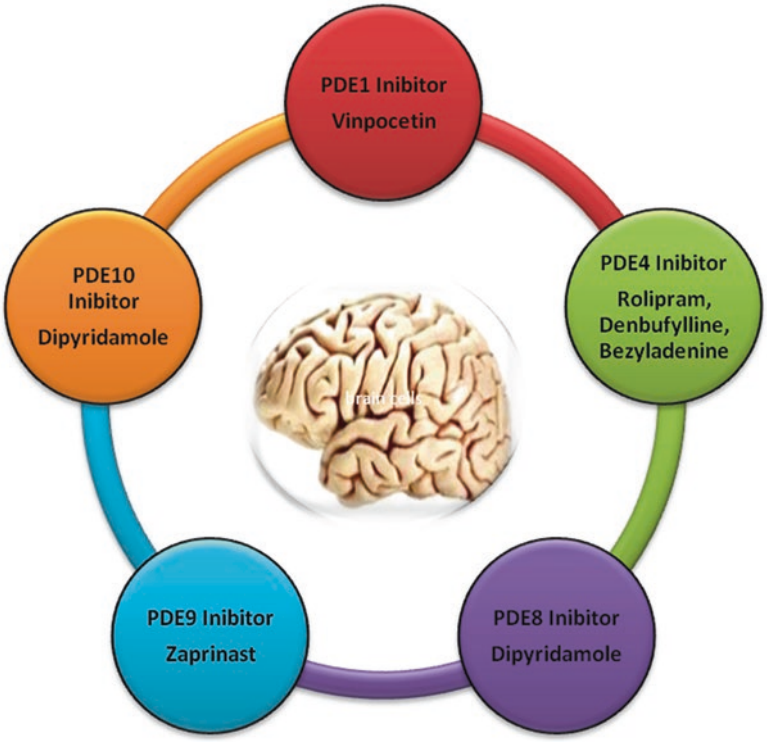


**Fig. 16.1** PDE1, 4, 8, 9, 10 inhibitors affect neuronal tissues, brain cortex cells and cerebral blood flow thus finding its use in neurodegenerative diseases and stroke

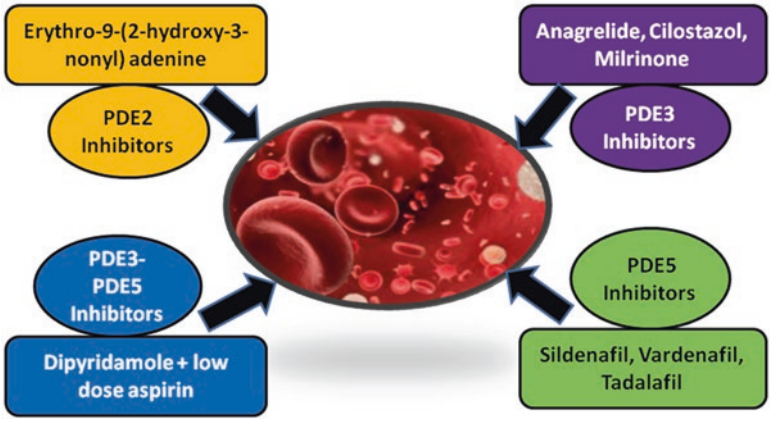


**Fig. 16.2** Inhibition of three PDE isoforms (PDE2, PDE3 and PDE5) found on platelets exerts a strong platelet inhibitory effect. These PDEIs have shown great benefit for the treatment and prevention of stroke

## 16.3 PDEs Localized in Brain Tissues, Their Pharmacology and Inhibitors

PDE1 family is mostly found in cytosolic region and is specifically activated by calcium calmodulin (Ca+2/CaM) and thus named as CaM-PDE (Wells et al. 1975). It hydrolyzes both cAMP and cGMP. PDE1A, PDE1B and PDE1C are the three genes having various splice variants that constitute this family. In brain PDE1A is highly expressed while PDE1B1 mRNA is predominantly found in neuronal cells of the cerebellum, hippocampus, caudate and purkinje cells. Its expression mainly correlates to brain regions having extensive dopaminergic innervations and D1 dopamine receptor mRNA. PDE1C also mainly expresses in brain and heart and is highly expressed in the mouse cerebellar granular cells (Polli and Kincaid 1992; Yu et al. 1997; Loughney et al. 1996; Yan et al. 1996). This family has been implicated in a number of pathological and physiological processes and most likely regulates vascular smooth muscle contraction and induction of apoptosis in human leukemic cells (Jiang et al. 1996). However, the most important feature of PDE1 is the regulation of smooth muscle cells and neuronal signaling. It has been suggested that inhibition of PDE1C can cause beneficial effects by inhibiting proliferation of smooth muscle cell an event that contributes to atherosclerosis one of the main contributing factors for stroke (Sonnenburg et al. 1995). Inhibitor vinpocetin (chemically called as ethyl apovincaminate) is a semisynthetic derivative of vincamine extracted from periwinkle plant that inhibits PDE1 with an $IC_{50}$ of approximately $10^{-5}$ M and is known to increase cerebral blood flow and improves memory (Sonnenburg et al. 1995).

Similarly, PDE4 family represents the largest of all PDE families and constitutes four genes PDE4A, PDE4B, PDE4C and PDE4D with a number of alternative mRNA splice variants of long and short isoenzymes of PDE4 and with 35 different PDE4 proteins (Swinnen et al. 1989; Livi et al. 1990; Bolger et al. 1993; McLaughlin et al. 1993). Its localization is complex and is found in cytosol or associated with cellular membranes and mainly found in brain, smooth muscle, inflammatory cells and cardiovascular tissues. Among the PDE subfamily, PDE4 alone represents 70–80% of PDE activity in neuronal tissue and specifically hydrolyzes cAMP (Beglopoulos and Shen 2006). Studies mostly have focused on PDE4D and PDE4D deficient mice have been known to display delayed growth, reduced viability and an antidepressant profile which revealed the PDE4D-regulated cAMP signaling to play a role in pharmacotherapy of depression (Jin et al. 1999; Zhang et al. 2002a; Zhang et al. 2002b). Studies on stroke pathogenesis had long suggested the vital role of PDE4 pathway in influencing it via an uncertain mechanism. Thus, Yang et al. (2012) investigated specifically the role of tissue plasminogen activator (tPA) on inhibition of PDE4 with the drug rolipram and reported that inhibition of PDE4 and PDE4D to reduce expression of tPA by Epac pathway (Yang et al. 2012). Rolipram the first generation potent inhibitor of PDE4 was the archetype to synthesize new potent and selective PDE4 inhibitors. Although other PDE4 inhibitors like Denbufylline (xanthine derivative) and Benzyladenine derivatives were synthesized as potent inhibitors they were not found to be that effective due to their adverse

emetic effect. These latter drugs also have broad anti-inflammatory and immunomodulatory actions which makes them applicable in other diseases too. However, in neuronal cultures it has been seen that PDE4 tightly regulates cAMP formed by stimulation of N-methyl-D-aspartate receptors and that rolipram due to its ability to cross blood brain barrier decreases ischemic neuronal damage and administration of it in 1 mg/kg significantly enhances hippocampal neurogenesis (Kato et al. 1995; Suvarna and O'Donnell 2002; Nikulina et al. 2004).

Further, recent reports also showed rolipram to promote axonal regeneration, attenuate glial scar formation, enhancement of functional recovery after spinal cord injury and to improve synaptic and cognitive functions (Nikulina et al. 2004; Gong et al. 2004). Additionally the experiment results by Sasaki et al. (2007) revealed rolipram to enhance survival of newborn neurons due to pharmacological activation of cAMP-CREB signaling that may also provide to be an effective therapy for stroke and post stroke complications (Sasaki et al. 2007). Adding to these findings the study carried out by Kraft et al. (2013) found rolipram to improve stroke outcome by modulation of important mechanism of ischemic neurodegeneration such as blood brain barrier disruption, inflammation and thrombosis. Their study revealed rolipram to show multifaceted mode of action and be an important and effective lead compound in stroke therapies when applied 2 h after stroke (Kraft et al. 2013). However, to comment on the safety and efficacy of the drug is too early and intense efforts are required in this direction to determine the same not only in animal experiments but its applicability in human system too. Despite its experimental effectiveness its limitation lies in specific side effects such as gastrointestinal problems, hypotension, fear or flushing which has forced a number of patients to withdraw from clinical trials. To overcome this problem next generation PDE4 inhibitors with improved side effect profiles have been suggested (Dal Piaz and Giovannoni 2000; Pagès et al. 2009). Apart from this, unresolved critical pharmacological issues related to optimum dosage, time point, delivery of the inhibitor (single vs. continuous application), its long term effect in acute ischemic stroke and additional mechanisms such as modulation of endogenous tPA release from cerebral endothelial cells needs to be successfully addressed (Yang et al. 2012).

The other subfamily found in brain is PDE8 that specifically hydrolyzes cAMP and is encoded by two genes PDE8A and PDE8B and has its least amount of mRNA expression in the brain with PDE8B3 being the most abundant form in the brain (Hayashi et al. 2002). Similarly PDE9 subfamily is encoded by a single gene PDE9A with several variants and specifically hydrolyses cGMP. PDE9A gene is known to have complex regulation of expression and more than 20 variants have been observed to exist but its specific function has still not been elucidated. However, its pattern of mRNA expression in brain closely resembles that of soluble form of guanylyl cyclase that suggests a possible functional association in regulation of cGMP levels that play a vital role in behavioral state regulation and learning. On the other hand PDE10 has been reported to be cGMP-sensitive and cAMP selective and is encoded by PDE10A gene with abundant transcripts found in brain. Huntington's chorea a progressive neurodegenerative disease is reported to be associated with PDE10

family (Hebb et al. 2004). For these above three families only the differential sensitivity to inhibitors has been reported. PDE8A was found to be inhibited by dipyridamole; PDE9A was reported to be sensitive to zaprinast and PDE10A is known to be inhibited by dipyridamole.

## 16.4 PDEs Localized in Platelets, Their Pharmacology and Inhibitors

Inhibition of platelet aggregation has been shown to be a great benefit for the treatment and prevention of stroke. This can be achieved either by the blockade of the membrane receptors or by interaction with intracellular signaling pathways. Two critical intracellular secondary messengers' cAMP and cGMP are provided with strong inhibitory activity of fundamental platelet functions. The intracellular levels of cyclic nucleotides are limited by PDEs by catalyzing the hydrolysis of cAMP and cGMP that leads to the regulation of platelet functions. Platelets possess three PDE isoforms i.e. PDE2, PDE3 and PDE 5 and inhibition of these PDEs may therefore, exert a strong platelet inhibitory effect. Non-selective or isozyme-selective PDE inhibitors have been developed and some of them are being used as antiplatelet agents in clinical use.

### *16.4.1 PDE2 Inhibitors*

Inhibitors of PDE2 have been investigated for their effectiveness in memory impairment and prevention of endothelial permeability in inflammation (Bender and Beavo 2006). One of the selective inhibitors called as Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) that inhibits adenosine deaminase (ADA) has reported no direct effect on platelet aggregation but potentiates the inhibition of thrombin-induced platelet aggregation by nitroprusside-a guanylyl cyclase stimulator (Dickinson et al. 1997). A natural product from *Ocotea pretiosa* has also been explored for its antiplatelet activity (Lima et al. 1999). Further, a novel selective compound 9-(6-phenyl-2-oxohex-3-yl)-2-(3, 4- dimethoxybenzyl)-purin-6one (PDP) was recently developed but has not been tested on platelets as of now (Diebold et al. 2009).

### *16.4.2 PDE3 Inhibitors*

PDE3 is known to have two isoforms i.e. PDE3A and PDE3B, of which PDE3B subtype is mainly expressed in platelets (Sun et al. 2007). Anagrelide is a potent and broad-spectrum inhibitor of platelet aggregation but studies involving humans have

shown the drug resulting in thrombocytopenia (Seiler et al. 1987; Thiele et al. 2006) and therefore, it has mainly found its clinical use among patients with essential thrombocythemia (Silverstein et al. 1988). The other well-known specific and strong inhibitor of PDE3 in platelets and smooth muscle cells is drug Cilostazol that causes smooth muscle cell relaxation and inhibition of platelet activation (Shrör 2002). It inhibits primary and secondary platelet aggregation and its use has been suggested over conventional antiplatelet therapy due to its short recovery time of platelet function (Iwamoto et al. 2003). This drug has also been studied for secondary prevention of stroke and studies have shown that use of this drug significantly reduces the recurrence of ischemic stroke, myocardial infarction, transient ischemic attack and intracranial hemorrhage. Data from studies have confirmed a low bleeding risk, fewer hemorrhagic events, significant reduction in risk of cerebrovascular events and prevention of post-stent restenosis (Gotoh et al. 2000; Weintraub et al. 2004; Uchiyama et al. 2009; Shinohara et al. 2010) with adverse effects like headache, tachycardia, palpitations, soft stools and diarrhoea (Sorkin and Markham 1999). Milrinone is another specific PDE3A inhibitor that induces an elevation of intraplatelet cAMP in a dose dependent manner, resulting in inhibition of platelet aggregation but its clinical use has so far been restricted to congestive heart failure (Manns et al. 2002; Colucci 1991).

### *16.4.3 PDE3-PDE5 Inhibitors*

Interestingly enough there are drugs that simultaneously inhibit PDE3 and PDE5. Drug dipyridamole although used initially as a coronary vasodilator, later showed its property of inhibiting platelet aggregation (Born and Cross 1963; Elkeles et al. 1968) and this paved way for its use as antithrombotic agent (Schwartz et al. 1988). It inhibits both PDE3 and PDE5 thus increasing the intraplatelet cAMP and/or cGMP, and it also acts as an antioxidant by scavenging free radicals that inactivate cyclo-oxygenase. Dipyridamole however, inhibits platelet aggregation in whole blood but not in platelet-rich plasma by blocking the reuptake of adenosine (Gresele et al. 1983; Gresele et al. 1986) and the antioxidant property of this drug is known to be better than ascorbic acid, α-tocopherol and probucol (Iuliano et al. 1996; Pascual and Romay 1992). Other pharmacological effects like inhibition of vascular smooth muscle cell proliferation, prevention of endothelium-leukocyte interactions and inhibition of inflammatory gene expression in platelet–monocyte aggregates also help in prevention of atherothrombosis (Kim and Liao 2008; Iimura et al. 1996; Weyrich et al. 2005). However, the clinical evidence of it alone exerting antithrombotic effect is very little and two large studies have shown dipyridamole in combination with low-dose aspirin leads to greater stroke risk reduction in ischemic cerebrovascular disease (Diener et al. 1996; Halkes et al. 2006).

### *16.4.4 PDE5 Inhibitors*

PDE-5 family was originally identified and purified from rat platelets (Coquil et al. 1980) but subsequent studies have showed its distribution in vascular and bronchial smooth muscles, platelets and lungs (coquil et al. 1980; Francis et al. 1980). Its inhibitors result in increased tissue level of cGMP that cause smooth muscle cell relaxation. Three known PDE5 inhibitors sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis) are currently in clinical use. However, the first developed inhibitor of this group was zaprinast that was originally meant for treatment of allergic diseases (Murray 1993). The observation that zaprinast induced elevation of cGMP and caused smooth cell relaxation led to its application in cardiovascular diseases (Rudd et al. 1983) and is known to inhibit human platelet PDE5 with an $IC_{50}$ of 0.3 μM and PDE2A with an $IC_{50}$ of 42 μM. However, this compound was unsuccessful and was modified leading to the identification of sildenafil a 100 times more potent and highly specific drug. Studies found it to have rapid absorption after oral administration with ~40% bioavailability and were shown to significantly increase bleeding time in healthy men 1 h after 100 mg of its intake with a recovery time of 4 h (Berkels et al. 2001). Nevertheless, this drug has relatively low selectivity for PDE5 and thus more potent selective PDE5 inhibitors like vardenafil and tadalafil were later developed (Young 2002; Corbin and Francis 2002).

PDE-5 inhibitors have been suggested to protect the brain against stroke and other neurodegenerative diseases but the mechanisms by which they exert cytoprotective effects are not understood completely. However, it has been hypothesized that the vasodilatory action of PDE-5 inhibitors *in vivo* could release endogenous mediators of pre-conditioning. For example, adenosine and bradykinin (endogenous mediators) from endothelial cells may trigger a signaling cascade activating kinases resulting in phosphorylation of endothelial nitric oxide synthase (eNos), synthesis of eNos and inducible nitric oxide synthase (Rosanio et al. 2006; Das et al. 2005 and Salloum et al. 2003). Further, animal studies on sildenafil report its oral administration for seven consecutive days starting 2–24 h after embolic middle cerebral artery occlusion to enhance neurological recovery without any effect on volume of the infarct (Zhang et al. 2002b). Many other cerebral vascular-protective effects of the drug have been demonstrated in patients suffering from pulmonary hypertension and this drug is also known to affect the cerebral hemodynamics during acute exposure to high altitudes (Rosengarten et al. 2006; Chan et al. 2005).

The first study showing a pre-conditioning like effect of sildenafil against myocardial ischemia/reperfusion therapy was reported by Ockaili et al. (2002). Subsequent studies showed the infract-limiting effect of sildenafil in several models including mouse hearts, infant rabbit hearts and rat hearts (Salloum et al. 2003; Wang et al. 2008; Das et al. 2009; Bremer et al. 2005; Das et al. 2002; du Toit et al. 2005 and Rosanio et al. 2006). The anti-ischemic effects of PDE-5 inhibitors have also been observed against ischemia/reperfusion-triggered ventricular arrhythmias and also the improvement of post ischemic ventricular contractile function

(Das et al. 2002; Nagy et al. 2004; Bremer et al. 2005; Das et al. 2002). Studies have also showed the infarct-limiting effect of sildenafil and vardenafil when these inhibitors were administered just before reperfusion (Elrod et al. 2007; Salloum et al. 2007).

## 16.5 Discussion and Conclusion

Stroke is a leading cause of serious and long term disability and an estimated 5.7 million people die from stroke worldwide (Lopez et al. 2006; Feigin et al. 2003). With the attack of stroke the problem of recurrence in stroke survivors has been estimated to be around 7.7% at 1 year, increasing to 18.3% in 5 years (Feigin et al. 2003). Effective strategies that can prevent stroke recurrence; stroke related morbidity and mortality are a major issue for the healthcare organizations worldwide. Mostly prevention of stroke is done by the use of antiplatelet drugs and anticoagulants useful in primary or secondary prevention of ischemic stroke (Apostolakis et al. 2013). Administration of drug aspirin within 48 h after stroke has been the recommended treatment of action and the other drug clopidogrel mostly used in secondary prevention has complex pharmacokinetics and consequently its early use after stroke attack has not been recommended (Floyd et al. 2012; NICE Guidelines 2008). Nevertheless, despite their use the pharmacological understanding of these drugs has been poor and sometimes their use has to be withdrawn in patients undergoing surgery to prevent bleeding complications.

Platelets although lifesaving during bleeding but over active platelets pose life threatening situation due to severe ischaemic tissue and other devastating complications. Thus, understanding the pharmacokinetics of antiplatelet agents is important since haemostatic properties of platelets are mediated by different receptors and downstream intracellular mechanisms. It has been found that antiplatelet agents act principally on three target molecules i.e. cyclooxygenase-1 (e.g., aspirin), adenosine 5-diphosphate receptor (e.g., clopidogrel, prasugrel, ticagrelor) and glycoprotein IIb/IIIa antagonists (e.g., abciximab, eptifibatide, tirofiban). Even if a particular receptor engaged in platelet aggregation is blocked, residual platelet activity may take place through an alternative pathway and thus more than one antiplatelet agent may be needed to be used simultaneously to achieve platelet inhibition. Therefore, strategies in such treatments should be a tailor made approach depending on individual patient circumstances (Apostolakis et al. 2013). Apart from this the use of intravenous administration of anticoagulants had been a common practice of treatment in acute phase of ischemic stroke but data on use of anticoagulant heparin has mostly been inconclusive (Jauch et al. 2013). Experimental results by International Stroke Trial (IST) on the use of subcutaneous unfractionated heparin reports, reduction of acute recurrent cardioembolic stroke but increase of intracerebral haemorrhage rate to a similar degree (International Stroke Trial Collaborative Group 1997). Similarly, the secondary analysis by trial of ORG 10172 in Acute Stroke Treatment (TOAST) on the beneficial effect of heparinoid danaparoid in

cardioembolic stroke group also reports negative results (The Publications Committee for the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) Investigators 1998). Another study by Heparin in Acute Embolic Stroke Trial (HEAST) also reports aspirin to be a better compound than low molecular weight heparin in acute phase of cardioembolic stroke due to atrial fibrillation (Berge et al. 2000). Thus, the results of these findings do not recommend the use of anticoagulants in clinical management of ischemic stroke. Nevertheless, studies now have suggested other important pathways that if blocked can mitigate the occurrence of stroke symptoms. Thus, new options of management of stroke risk in patients are an urgent need of the hour and therefore, agents like PDEI that interfere with intracellular signaling pathways have theoretically great potential for platelet inhibition.

In view of this, recent studies have thus explored PDEI after the successful use of drugs like theophylline and papaverine (non-selective inhibitors) in a range of diseases. Their real impact on treatment of various diseases has gained importance in last 10 years and its use in stroke is a relatively new concept and yet to be established. A series of PDEI namely milrinone, enoximone, vesnarinone, pentoxifylline, and cilostazol are in use, each having unique pharmacologic properties but so far the application has mostly been restricted to wide use in cardiovascular failure and asthma. These inhibitors mostly promote reduction in cAMP breakdown with variety of tissue specific effects and vasodilation resulting in hypotension particularly in vasoconstricted and hypovolemic patient. Apart from this, these drugs tend to show inotropic effect that improves functional status, reduces inflammation and oxidative stress. Among the various classes of inhibitors PDE4 inhibitors have been reported to have greater effect on inflammation when compared with PDE3 inhibitors. Drugs like roflumilast, cilomilast, and rolipram have been associated with significant anti-inflammatory effects and have received considerable amount of attention but side-effects such as nausea, vomiting and headache have also limited their use (Feneck 2007).

Most of the studies in stroke have focussed on PDE4 pathway and PDE4 inhibitor (rolipram). Subfamilies like PDE2 although reported to be present in brain cortex but their possible functional role has not been studied. PDE3 family has been reported to be found in platelets, heart and liver and thus its inhibitors find application during heart failure. Similarly PDE5 inhibitors mainly have helped in treatment of pulmonary hypertension and respiratory distress (Hansen et al. 2000). Apart from this PDE5 inhibition has shown to improve early memory consolidation of object information and to reduce neurological deficits and evoke neurogenesis (Prickaerts et al. 2004; Zhang et al. 2002b).

In conclusion, devastating neurological emergency like stroke needs to be measurably improved and treatment through PDEI seems to offer quiet a novel approach. Both non-selective and selective inhibitors have been used in a number of medical conditions. However, with respect to stroke, studies so far suggest inhibitors of PDE4 and PDE5 to be most relevant in post stroke management. With this possible strategy the complications also arise due to widespread distribution of PDE in the body that renders it difficult for an effective antiplatelet action without any significant unwanted effects (Gresele et al. 2008). Further, reversibility of effect of most

clinically used PDEI on their target is a serious limitation on their antithrombotic effectiveness for long term secondary prophylaxis. Therefore, a deeper understanding of physiology of PDEs in platelets and other tissues, targeting of PDE inhibition to platelets and development of long term acting selective PDEI are required for an effective antiplatelet therapy. Their full fledged use in stroke, thus, requires a lot of clinical study not only with respect to their pharmacological properties but also in regards to the adverse side reactions that may result from its administration.

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

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# Chapter 17
# A Unique Sub-Pocket for Improvement of Selectivity of Phosphodiesterase Inhibitors in CNS

**Yousheng Wang and Hengming Ke**

**Abstract** This chapter describes crystal structures of phosphodiesterases (PDEs) that are involved in CNS diseases and their interactions with family selective inhibitors. The structural comparison identifies a small hydrophobic pocket next to the active site, which may be valuable for improvement of selectivity of PDE inhibitors.

**Keywords** Phosphodiesterases in CNS • crystal structures • subpocket for inhibitor improvement.

## Abbreviations

| | |
|---|---|
| cAMP | cyclic adenosine monophosphate |
| cGMP | cyclic guanosine monophosphate |
| IBMX | 3-isobutyl-1-methylxanthine |
| PDE | phosphodiesterase |

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## 17.1 Introduction

Phosphodiesterase (PDE) is a superfamily of enzymes hydrolyzing the second messengers cGMP and cAMP. Human genome encodes 21 genes that are categorized into 11 PDE families and express >100 isoforms of proteins (Conti and Beavo 2007; Maurice et al. 2014). PDE molecules contain a conserved catalytic domain at the C-terminus and a variable regulatory domain at the N-terminus. The catalytic domains of PDEs are well conserved but selectively hydrolyze their preferable substrates: PDE5, PDE6, and PDE9 specifically recognize cGMP as their substrate, while PDE4, PDE7, and PDE8 are cAMP-specific. The remaining PDE families are capable of degrading both substrates. For critical roles of the second messengers cAMP and cGMP in physiological processes, PDE inhibitors have been widely studied as therapeutics for treatment of human diseases (Conti and Beavo 2007; Maurice et al. 2014). A well-known example is the PDE5 inhibitor sildenafil that has been approved for the treatment of erectile dysfunction and pulmonary hypertension (Rotella 2002; Galie et al. 2005). This chapter will describe interactions between the inhibitors and PDEs in CNS to provide structural insight into design of PDE inhibitors.

## 17.2 Conservation of the PDE Active Sites for Binding of Substrates and Inhibitors

After the first crystal structure of the PDE4B catalytic domain (Xu et al. 2000), structures of catalytic domains of nine out of eleven PDE families (PDE1–5 and PDE7–10) are available and have greatly guided design of PDE family selective inhibitors (Ke and Wang 2007). The catalytic domains of PDEs contain 300–350 amino acids and their active site pockets are well conserved (Table 17.1).

The active site can be divided into two major sub-pockets respectively for metal and inhibitor binding (Fig. 17.1). Two metal ions occupy the metal binding sub-pocket. A zinc ion was identified by the anomalous scattering experiment and coordinates with four invariant residues in PDE families: His529, His563, Asp 564, and Asp 674 in PDE10A2 (Table 17.1). Another metal ion has been assigned but not confirmed as magnesium or manganese, dependent on PDE family, and forms only one coordination with Asp564. A hydroxide ion bridges the two metal ions and presumably serves as nucleophile to initiate the hydrolysis of the cyclic nucleotide (Xu et al. 2000; Zhan and Zheng 2001; Huai et al. 2003). The two ions chelate with several water molecules and form an octahedral configuration. The sub-pocket for inhibitor binding shows two characteristic features: (1) an invariant glutamine (Gln726 in PDE10A2) forms a hydrogen bond with substrate or inhibitors and (2) a conserved phenylalanine (Phe729 in PDE10A2) stacks against a hydrophobic group of substrates/inhibitors such as adenosine of cAMP (Fig. 17.1b). In addition, two hydrophobic residues (Ile693 and Phe696 in PDE10A2) together with the conserved phenylalanine (Phe729) sandwich the adenosine of cAMP to form a hydrophobic slot.

**Table 17.1** Alignment of residues at the active site of PDE families

| | 524 | 525 | 529 | 563 | 564 | 635 | 674 | 678 | 685 | 689 | 693 | 694 | 696 | 713 | 725 | 726 | 729 | 730 | 762 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PDE10 | Y | H | H[a] | H[a] | D[a] | L | D[a] | V | T | A | I | Y | F | M | G | Q | F | Y | W |
| PDE1b[b] | Y | H | H | H | D | L | D | P | H | T | L | M | F | L | S | Q | F | I | N (SG) |
| PDE2 | Y | H | H | H | D | L | D | Q | T | A | I | Y | F | M | L | Q | F | M | W |
| PDE3 | Y | H | H | H | D | L | D | P | H | T | I | V | F | F | L | Q | F | I | W |
| PDE4 | Y | H | H | H | D | L | D | P | Y | T | I | M | F | M | S | Q | F | I | Y |
| PDE7 | Y | H | H | H | D | L | D | P | S | S | V | T | F | L | I | Q | F | M | W |
| PDE8 | F | H | H | H | D | L | D | P | C | A | I | S | Y | V | S | Q | F | I | W |
| PDE5 | Y | H | H | H | D | L | D | I | Q | A | V | A | F | M | M | Q | F | I | W |
| PDE6 | Y | H | H | H | D | I | D | I | Q | A | V | A | F | M | L | Q | F | I | W |
| PDE9 | F | H | H | H | D | L | D | E | A | V | L | L | Y | F | A | Q | F | I | Y |
| PDE11 | Y | H | H | H | D | L | D | V | S | A | V | T | F | F | L | Q | W | I | W |

[a]Metal chelating residues

[b]SG in parenthesis are corresponding residues of PDE1A and PDE1C

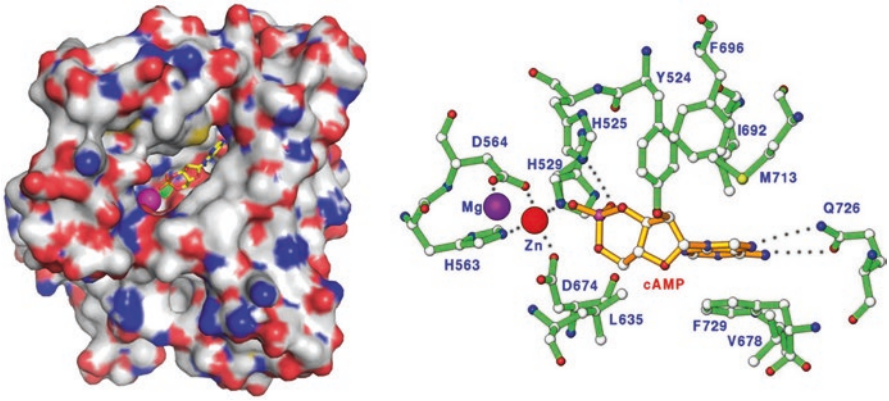


**Fig. 17.1** The active site of PDE10A2 catalytic domain. (**a**) Surface presentation of the active site of PDE10A2. Substrate cAMP is shown as *yellow sticks* and zinc and magnesium are presented with *green* and *pink balls*. (**b**) Ribbon diagram for cAMP binding to PDE10A2. *Dotted lines* represent hydrogen bonds or charge-charge interactions with zinc and magnesium

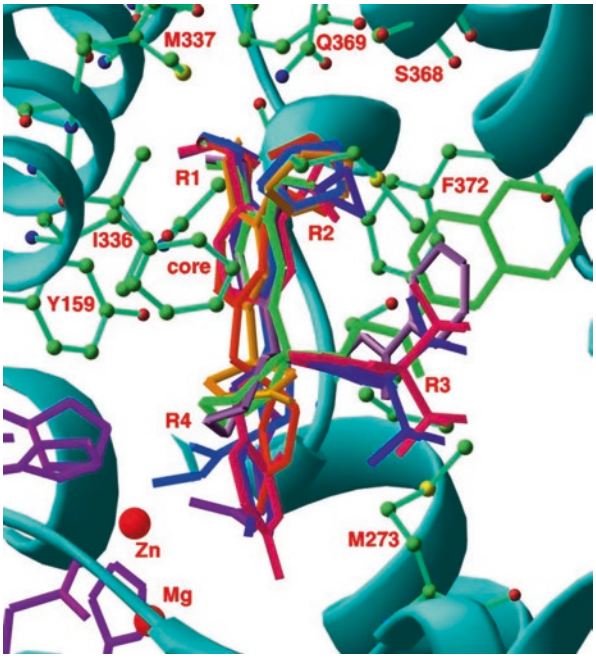


**Fig. 17.2** Overlay of PDE4 inhibitors with catechin scaffold suggests five pharmacophores. The CORE group is well superimposed and stacks against a conserved phenylalanine (Phe372 in PDE4D2), in addition to hydrophobic interactions with conserved Phe341 and Ile336, thus contributing basic affinity for non-selective binding of the inhibitors. Other pharmacophores R1-R4 appear to contribute to the selective binding of the PDE4 inhibitors

The above features are well conserved for binding of most PDE inhibitors. For example, the classic PDE4 inhibitor rolipram uses its catechin group to form two hydrogen bonds with the invariant glutamine (Gln369 in PDE4D2) and stacks against the conserved phenylalanine (Phe372 in PDE4D2, Huai et al. 2003). When the rolipram analogs such as roflumilast, a drug for the treatment of severe chronic obstructive pulmonary disease (Field 2008), are superimposed one another, they can be divided into five pharmacophores of CORE, R1-R4 (Fig. 17.2). The CORE pharmacophore or catechin forms two hydrogen bonds with Gln369 and stacks against Phe372 of PDE4D2 (Fig. 17.2), and may account for basic affinity of most inhibitors. The remaining pharmacophores are located in slightly different environments of PDE families and may thus contribute to selective binding of the inhibitors.

## 17.3 A Unique Subpocket for Improvement of Selective Binding of PDE Inhibitors

An early study on the crystal structure of the *Leishmania major* phosphodiesterase B1 (LmjPDEB1) revealed a small hydrophobic pocket neighboring the active site (Wang et al. 2007). Approach to this subpocket is gated by two residues: Met874 and Gly886 of LmjPDE1 (Fig. 17.3). Since the size of these two residues is apparently large in human PDE families, the subpocket was thought to specially belong to *Leishmania major* phosphodiesterases and was named as the L-pocket (Wang et al. 2007). Later, a careful examination on the structure of *Trypanosoma brucei* phosphodiesterase B1 and the sequences of other parasite PDEs show possible accession to the pocket in the parasite PDEs and thus the pocket was renamed as the P-pocket (Jansen et al. 2013). However, this claim was not completely justified by the crystal structure of human PDE10 catalytic domain in complex with inhibitor


**Fig. 17.3** Sequence alignment for the subpocket neighboring the active sites PDEs. The *green color* highlights helices in the crystal structures. Two residues in *red* gate the pocket

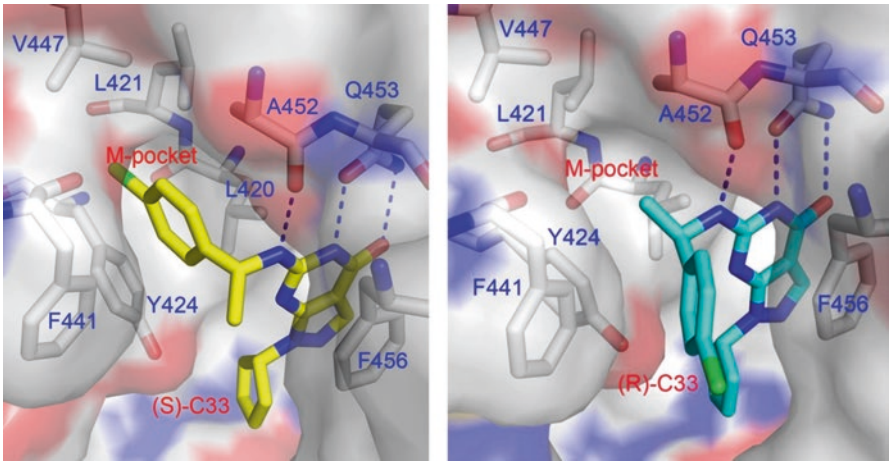


**Fig. 17.4** The inhibitor binding to the M-pocket of PDE9. (**a**) Surface model for binding (S)-C33 (*yellow sticks*) to the M-pocket of PDE9. (**b**) Binding of (R)-C33 (*cyan sticks*) to PDE9A. *Dotted lines* represent hydrogen bonds

PF-2545902, in which a fragment of the PDE10 inhibitors interacts with the P-pocket and thus the pocket was named as the PDE10 selective pocket (Verhoest et al. 2009).

Recently, the crystal structure of PDE9 in complex with inhibitor C33 revealed that the tyrosyl group of (S)-enantiomer of C33 oriented to and interact with a small subpocket that is composed of a portion of helices H14 and H15 and the M-loop (Fig. 17.4), while its (R)-enantiomer adopted different conformation. Since the M-loop is a major contributor, the pocket was named as the M-pocket. Helices H14 (Leu420, Leu421 and Phe425) and H15 contribute two walls of the pocket, while Val447 and backbone of the M-loop form the bottom of the pocket. In fact, the M-pocket is identical to the P-pocket or PDE10-selective pocket, and may thus serve as a general pocket for improvement of selectivity of PDE inhibitors. Sequence alignment reveals that the gating residues vary significantly across PDE families (Fig. 17.3) so as to make the pocket inaccessible for many human PDEs (Wang et al. 2007; Wang et al. 2012).

In addition to the accessibility gated by two residues, the M-pocket shows significant different size, conformation, and sequence components (Fig. 17.3), and thus may serve as an important element for improvement of selective binding of PDE inhibitors. For example, the M-pocket in PDE5, which is composed of Val782, Ala783, Phe786, Phe787 and Ile813 (Fig. 17.5a), is small and slightly deeper than that of PDE9. Two large gating residues of Leu804 and Met816 would allow small group to penetrate into the pocket, such as an ethoxyl fragment in the PDE5-sildenafil structure (Wang et al. 2008). The affinity of the PDE5 inhibitors was significantly improved by the replacement of ethoxyl with propoxy group (Salem et al. 2006). The M-pocket in the PDE8A1 structure, which is made up of residues Ser745, Tyr748, Phe749, Phe767, and Cys772, is much shallower and smaller than that of PDE9 and might not well accommodate their inhibitors (Fig. 17.5b). In the structure of PDE1, the M-loop has a good conservation of amino acids to those of

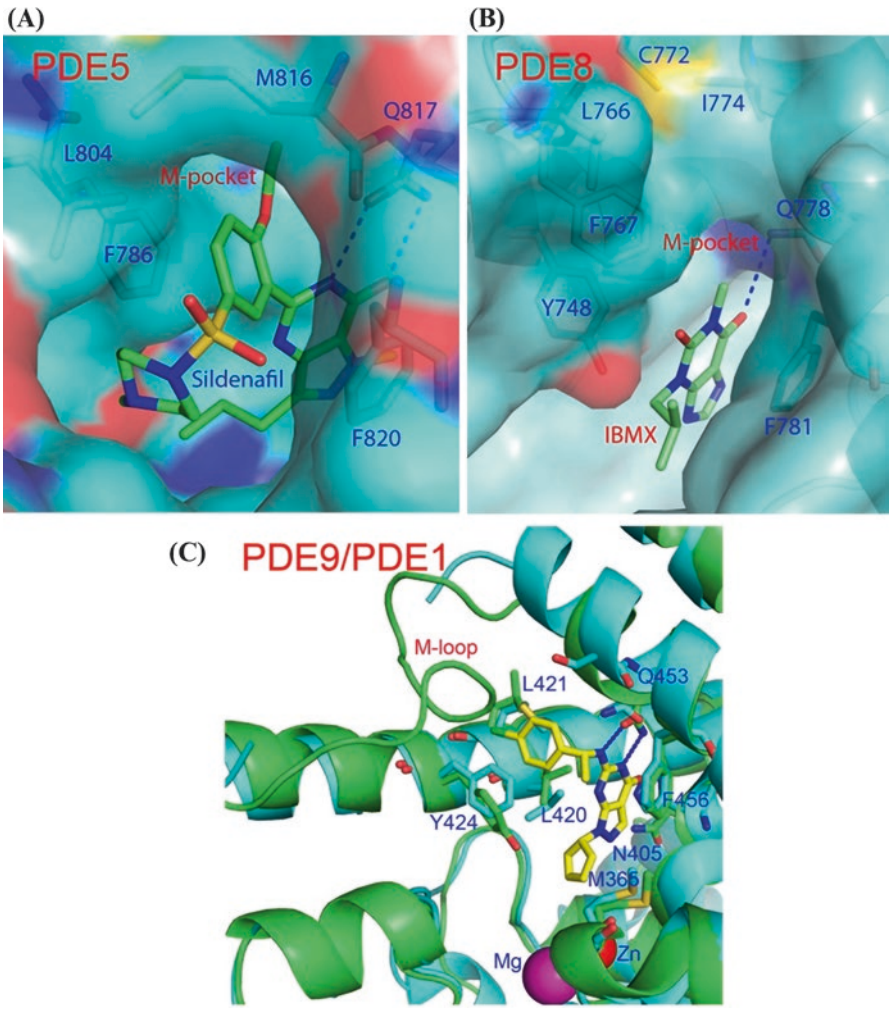


**Fig. 17.5** The M-pockets in some PDE families. (**a**) Surface presentation of the M-pocket of PDE5. (**b**) Surface presentation of the M-pocket of PDE8A. IBMX is a non-selective inhibitor of PDEs. (**c**) Ribbon presentation of the superposition of PDE9 (*green*) over PDE1B (*cyan*). The M-loop of PDE1B is partially disordered. The corresponding residues between PDE9A2 and PDE1B are: M365/M336, N405/H373, L420/L388, L421/M389, Y424/F392, Q453/Q421, and F456/F424

PDE9, as shown by correspondence of Leu388, Met389, and Phe392 of PDE1B to Leu420, Leu421, and Y424 of PDE9, respectively. Since part of the M-pocket is disordered in PDE1, it might reasonably predict a poor selectivity of PDE9 inhibitors against PDE1, as observed in the PDE9 structure (Wunder et al. 2005).

## 17.4 Conclusive Remarks

We believe that the M-pocket may serve as a selectivity determinant for inhibitors binding and is useful for improvement of inhibitor affinity and selectivity.

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

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