

Chapter 2

Current Understanding of PDE10A in the Modulation of Basal Ganglia Circuitry

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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 pedunculo-pontine 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 globus 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 ($G_{\alpha s}/G_{\alpha olf}$), whereas the D2Rs are coupled to inhibitory $G_{\alpha i}$ 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 be considered as the *reward* and the indirect pathway as the *punishment* pathway.

What are the activities 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; Spiwoxs-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

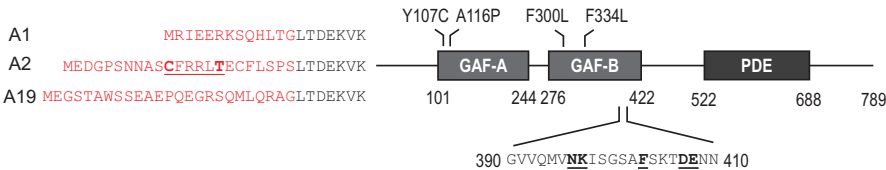


Fig. 2.1 Schematic representation of PDE10A. The N-terminal sequence variation in the different isoforms PDE10A1, PDE10A2 and the novel, primate specific isoform PDE10A19 (MacMullen et al. 2016) are highlighted in red. Numbering of the amino acid residues refers to the human PDE10A2 isoform (Uniprot 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-antagonist 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 pre-synaptic 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 (TrkB) 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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