PDE10A mutations help to unwrap the neurobiology of hyperkinetic disorders

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Abstract

The dual-specific cAMP/cGMP phosphodiesterase PDE10A is exclusively localised to regions of the brain and specific cell types that control crucial brain circuits and behaviours. The downside to this expression pattern is that PDE10A is also positioned to be a key player in pathology when its function is perturbed. The last decade of research has seen a clear role emerge for PDE10A inhibition in modifying behaviours in animal models of psychosis and Huntington's disease. Unfortunately, this has not translated to the human diseases as expected. More recently, a series of families with hyperkinetic movement disorders have been identified with mutations altering the PDE10A protein sequence. As these mutations have been analysed and characterised in other model systems, we are beginning to learn more about PDE10A function and perhaps catch a glimpse into how PDE10A activity could be modified for therapeutic benefit.

Keywords: PDE10A, hyperkinetic movement disorders, striatum, GAF domain, cAMP, Huntington's disease

1. Introduction

Hyperkinetic movement disorders are a heterogeneous group of clinical diagnoses, defined as uncontrolled excess movement that manifests in a number of ways, including but not limited to tremor, dystonia, tics and chorea, which for the context of this PDE10A review is best exemplified by Huntington's Disease (HD) [1, 2]. They are as frequent as hypokinetic movement disorders, best illustrated by Parkinson's Disease (PD). The basal ganglia are a group of subcortical nuclei primarily responsible for the selection and synchronisation of movement cues from cortical outputs, but which also influence motivation and cognitive behaviour [3, 4]. The striatum is the main input region of the basal ganglia and is comprised of principally GABAergic medium spiny neurons (MSNs), the main neuronal cell type lost in HD, and cholinergic interneurons [5, 6]. MSNs can be classified into two distinct groups based on the destination of their axonal projections and molecular expression patterns, especially the G-protein coupled receptors (GPCRs) responsible for relaying the dopamine signal [7-9]. In particular, the MSNs of the

so-called ‘direct’ or ‘striatonigral’ pathway, which promote movement and express the dopamine D1 receptor (D1R), project to the substantia nigra and the internal globus pallidus. While the ‘indirect’ or ‘striatopallidal’ pathway MSNs, which express the dopamine D2 receptor (D2R) and inhibit movement, project to the pallidum [10, 11].

The activation of these distinct pathways within the striatum rely on the tuning of the intracellular second messenger molecules, cyclic 3’ 5’-adenosine monophosphate (cAMP) and cyclic 3’ 5’-guanosine monophosphate (cGMP). cAMP is generated in response to extracellular cues to orchestrate numerous physiological processes by activation of a range of effector proteins [12]. Production of this cyclic nucleotide is well exemplified in the dopamine system, with binding of dopamine to the D1R resulting in activation of the coupled stimulatory Gαs protein, which in turn activates adenylyl cyclases (ACs) and triggers cAMP synthesis and neuronal excitability through modulation of cAMP effector protein activity. In contrast, ligand binding to D2Rs results in inactivation of ACs through release of the inhibitory Gαi protein and suppresses membrane depolarisation [13]. In light of these opposing forces, orchestration of the temporal/spatial synthesis and degradation of cAMP is crucial to illicit the appropriate receptor-specific physiological effect. cGMP is generated by guanylyl cyclases (GCs), which in the brain can be membrane-bound particulate GC or soluble GC, and are activated by a number of different extracellular ligands or nitric oxide respectively [14, 15]. Shaping of cAMP and cGMP gradients in three dimensions occurs via the action of cyclic nucleotide phosphodiesterases (PDEs) that degrade cAMP and/or cGMP by hydrolysing the 3’ cyclic phosphodiester bond to produce inactive 5’-AMP and 5’-GMP respectively. To date there have been 11 PDE families described, yet it is believed over 100 mRNA products exist through alternative splicing and transcriptional initiation sites [16, 17]. PDE families can either specifically hydrolyse cAMP, cGMP or possess dual specificity to hydrolyse both substrates. The functions of specific PDEs are uniquely influenced by their cellular localisation, often directed by targeting sequences within the enzyme’s N-terminal domain [18]. The identification of region-specific expression of different PDEs within the brain highlighted that an intricate crosstalk between families exists in order to

strictly regulate cyclic nucleotide concentration, in a cell type specific manner which drives specific brain circuitry and subsequent behaviour [19-21]. Consequently, understanding of the various PDE isoforms, their expression within different brain regions and the interplay between one another is fundamental in the future development of therapeutics targeting PDEs in the CNS.

Across PDE families, phosphodiesterase 10A (PDE10A) is almost exclusively detected in the brain, although it is also present in the testis [22, 23] and overexpressed in several tumours [24-26]. In the brain, it is enriched in the MSNs of the striatum, although reduced levels of the enzyme are also present in the cerebellum, hippocampus and cortex [27]. PDE10A is expressed in both D1R-containing striatonigral and D2R-containing striatopallidal MSNs [28-31]. The high expression of PDE10A in the dendritic post-synaptic region helps PDE10A to orchestrate incoming cortical glutaminergic and midbrain dopaminergic signals, regulating MSN sensitivity [32-34] and critically influencing various behaviours generated through engagement of downstream circuitry including movement and motivation. Simply through its distribution and implied function, somewhat mimicking a D2R antagonist, PDE10A inhibition has been considered as a therapeutic target for schizophrenia [35], and then more elegant human biology and brain circuitry studies have implicated this enzyme in movement disorders such as HD and PD [36-38]. This review article will focus on the complicity of striatal PDE10A malfunction and the defective cyclic nucleotide signalling in genetic hyperkinetic movement disorders.

2. Structure, localisation and regulation of PDE10A within the brain

PDE10A is an 89 kDa dual-specific phosphodiesterase, able to hydrolyse both cAMP and cGMP with K_m values 0.26 μM and 7.2 μM respectively [39-41], although with a lower V_{max} for cAMP compared with cGMP [41]. On a cellular level, PDE10A protein is present throughout the cell soma, dendrites and axons of MSNs with mRNA expression largely confined to the cell body [27, 42]. Outside the striatum, brain regions such as the hippocampus, cerebellum, brainstem and cortex, exhibited exclusive nuclear localisation of PDE10A [42]. Alternative splicing gives rise to over 30 transcripts in the human brain of which around half are human-

specific, characterised by unique N-terminal and C-terminal regions [16, 32, 43]. Humans possess two prevalent splice variants of PDE10A, PDE10A1 and the more abundant PDE10A2, whereas in rats PDE10A2 and PDE10A3 are more prominent (Kotera *et al.*, 2004). A further primate-specific isoform, PDE10A19, has also more recently been described [16, 43] and other variants are expected to be found as some novel PDE10A transcripts have been predicted to express proteins with specific N-termini [16]. Conserved between the human variants are a carboxyl terminal catalytic region, and two regulatory **GAF** (c**G**MP-stimulated PDEs, *Anabaena* **a**denylyl cyclases, and the *Escherichia Coli* transcription factor **F**hlaA) domains (Figure 1). GAF domains provide allosteric regulation sites on PDEs [44] and are structurally different to cAMP binding sites of the PDE catalytic domain and cAMP effector proteins. For the purpose of this review, the structure and function of the GAF domains are highly germane.

PDE10A is unique in regard to its GAF domains, as it is the only PDE regulated by cAMP rather than cGMP [23]. Low concentrations of cAMP (or a synthetic GAF domain agonist) stimulate degradation of both cAMP and cGMP when bound to the GAF-B domain of PDE10A (EC_{50} ~ 0.1 μM), however the enzyme is completely inactivated at further elevated levels of cAMP [23]. Inhibition of cAMP degradation has only been found at very high levels of cGMP, with an IC_{50} of 14 μM [39]. This suggests a preference of the catalytic domain for cAMP when cAMP is bound to the GAF-B domain, which is in agreement with previous studies showing a higher affinity of PDE10A for cAMP [39, 41]. In contrast, the GAF-A domain of PDE10A does not contain the typical cyclic nucleotide binding consensus motif, $N[KR]X_{(5-14)}FX_{(3)}DE$ or 'NKFDE', associated with cyclic nucleotide binding. Interestingly, not all GAF domains are found to bind cyclic nucleotides, and reports have also implicated GAF domains in protein-protein interactions, such as the homodimerisation of PDE2 and PDE5 [45-48]. This infers another means of regulation for PDE activity, through enzyme stability or subcellular localisation. The differential roles of the PDE10A GAF-A and -B domains will be discussed later in this review, as human mutations in the two domains have a distinct clinical presentation.

As mentioned above, PDE localisation in cellular micro-domains often depends on targeting sequences within the N-terminal region and PDE10A is no different in this regard. Differential encoding of the extreme amino terminal regions account for distinct cellular localisation, with PDE10A1 and PDE10A19 situated in the cytosol whereas PDE10A2 is predominantly membrane-bound [32, 33, 40, 43]. PDE10A2 is post-translationally and irreversibly palmitoylated at Cys11 in its unique N-terminal region, facilitating its membrane association and trafficking to dendrites (Figures 1 and 2). Protein acyltransferases ZDHHC-7 and ZDHHC-19, zinc-finger containing DHHC-containing proteins, we previously reported to mediate this palmitoylation [34]. Critically, PDE10A2 contains an exclusive cAMP-dependent protein kinase (PKA) phosphorylation site at Thr16, which when phosphorylated interferes with post-translational palmitoylation at Cys11 and prevents membrane translocation of the enzyme (Figure 1 and 2) [32, 34]. Therefore, the intracellular cAMP concentration at the time of protein synthesis tightly controls PDE10A2 localisation, and thus the compartmentalisation of the cAMP gradient through PKA activation. Interestingly, overexpression of PDE10A19 has also been reported to interfere with membrane association of PDE10A2 through a direct interaction between the isoforms [43].

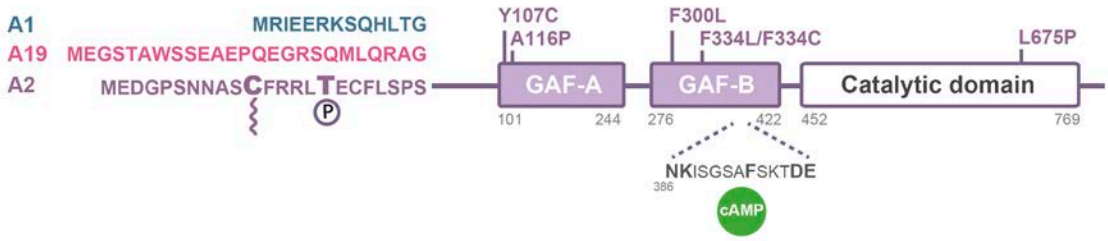

Figure 1. Schematic representation of human PDE10A isoforms. The three major PDE10A splice variants differ in their N-terminal amino acid sequence, which is responsible for the distinctive subcellular localisation pattern. Residues implicated in PDE10A2 trafficking to the plasma membrane are highlighted in bold: palmitoylated Cys11 that serves as an anchor for membrane insertion and phospho-residue Thr16 that can prevent palmitoylation. PDE10A functional domains, cyclic nucleotide binding consensus motif and mutations in PDE10A GAF domains that cause hyperkinetic movement disorders are indicated. Amino acid numbering refers to human PDE10A2.

Fine control of localised cyclic nucleotide gradients in neurons depends on the unique localisation of PDE10A2. Specifically, it has been reported that PDE10A is a member of a signalling complex bound to synaptic membranes, including *N*-methyl-D-aspartate (NMDA) receptor subunits and scaffolding proteins AKAP150 and PSD95

(Figure 2). Phosphorylation by PKA results in PDE10A2 dissociating from this complex and diffusing horizontally through the plasma membrane [49]. Therefore, when production of cAMP overrides the degradation capacity of PDE10A, the enzyme dissociates from the synaptic complex and allows triggering of downstream signalling cascades.

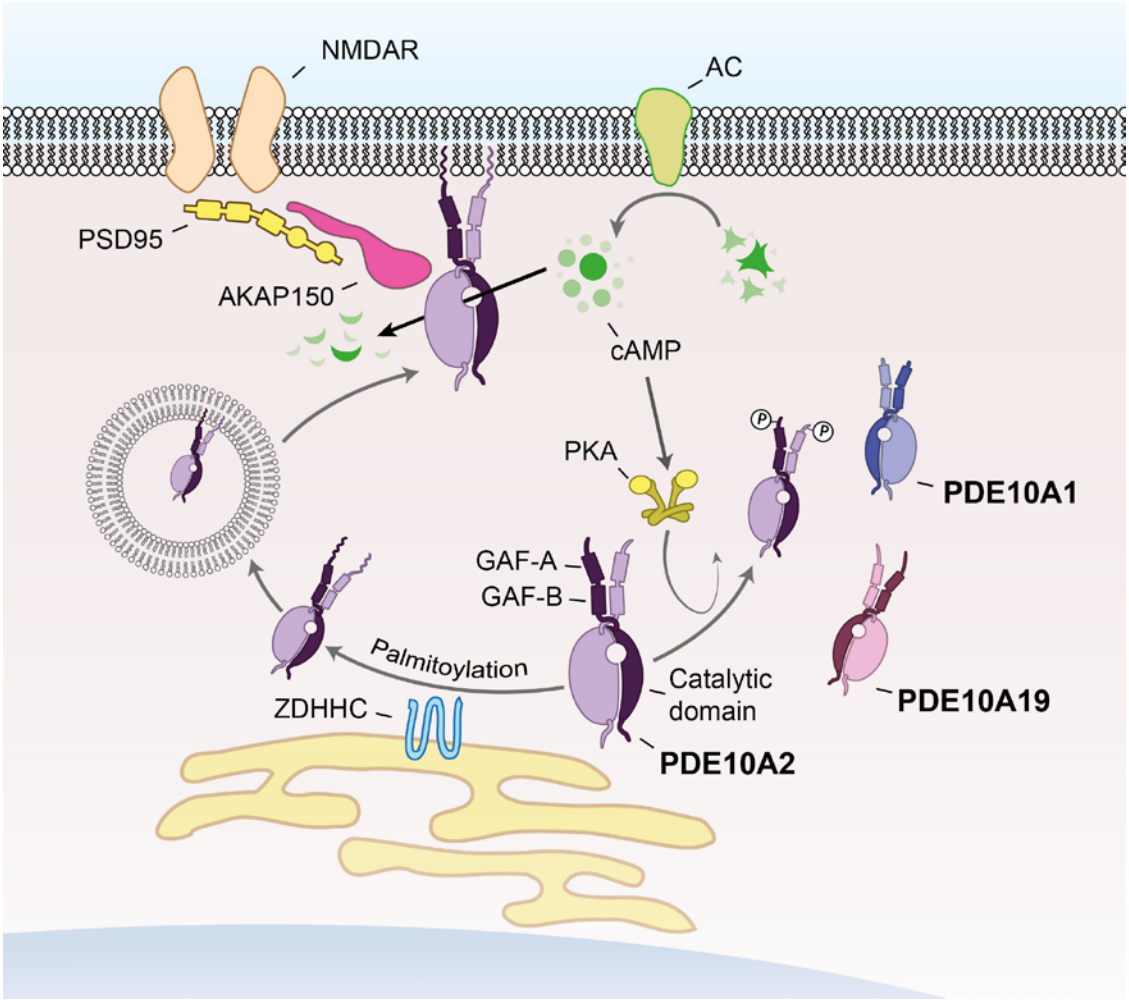

Figure 2. Post-translational regulation and trafficking of PDE10A. PDE10A2 palmitoylation at Cys11 facilitates its trafficking and insertion into the plasma membrane of MSNs where it associates with synaptic proteins such as PSD95, AKAP150 and NMDA receptor subunits. High local cAMP levels can increase PKA activation which results in the phosphorylation of PDE10A2 at Thr16 preventing the action of the palmitoyl acyltransferase (zDHHC). Phosphorylated PDE10A2 accumulates in the cytosol in line with the other two major isoforms PDE10A1 and PDE10A19.

3. PDE10A-related hyperkinetic movement disorders

Alterations in cyclic nucleotide levels have been extensively associated with HD, which results from a trinucleotide CAG repeat expansion in the first exon of the

huntingtin (htt) gene and is characterised by uncontrolled choreiform movements related to selective vulnerability of MSNs. In early stage HD, degeneration of the inhibitory D2R-containing striatopallidal MSNs is observed, in contrast to the seemingly more resilient D1R expressing cells [50]. D1R containing MSNs degenerate as disease progresses, which results in hypokinetic movement consistent with the direct pathway becoming compromised [51]. Reduced levels of cAMP have been measured in both post mortem brain samples from HD patients and mouse models [52]. Interestingly, PDE10A mRNA and protein levels are downregulated in HD patients and animal models of HD prior to the onset of motor symptoms, with this reduction correlating to disease progression and severity [36, 37, 53-56]. Furthermore, the rate of transcriptional initiation of PDE10A was shown to be reduced in the striatum of R6/1 and R6/2 HD mice, indicating the possibility of PDE10A transcriptional repression by the mutant htt protein [37]. Conversely, PDE10A inhibitors have been reported to rescue transcriptional, cellular and behavioural deficits in HD mouse models [57, 58]. If PDE10A levels are actively downregulated during the progression of HD, this raises the question as to why inhibition of PDE10A would be therapeutically beneficial. It has been hypothesised that PDE10A reduction is a compensatory mechanism to restore HD-related cyclic nucleotides decline, which should occur very early in the course of the disease. This happens via the activation of the transcription factor cAMP-response element binding protein (CREB), increased brain derived neurotrophic factor (BDNF) expression and in turn, cell survival [59-61]. Such speculation was at least partially quashed by the results of a Phase II clinical trial led by Pfizer in HD with the PDE10A inhibitor PF-02545920 (ClinicalTrials.gov, NCT02197130). Unfortunately, PF-02545920 was shown to have no advantage versus placebo on the primary endpoint of the Unified-Huntington's-Disease-Rating-Scale Total-Motor-Score (UHDRS-TMS) [62].

In addition to HD, a series of familial mutations have recently emerged implicating PDE10A in child-onset non-progressive hyperkinetic movement disorders, with symptoms reminiscent of early-stage HD [63-67]. A total of five separate mutations in the *PDE10A* gene on chromosome 6 have been reported in 14 individuals, with all mutations creating a single amino acid substitution in either the GAF-A or GAF-B domain of the protein (Table 1). Our own study [63] identified

two familial biallelic mutations within the GAF-A domain of PDE10A. The first family was consanguineous and of Pakistani descent. Affected family members were shown to be homozygous for a c.320A>G mutation causing a cysteine to replace a tyrosine at amino acid position 107 (p.Y107C). Clinical manifestations include axial hypotonia with dyskinesia of the face, trunk and limbs, however cognition was unimpaired. Notably, the degree of severity differed within the family, the oldest member being least affected [63]. The second mutation (c.346G>C; p.A116P) was identified in a Finnish family, who again presented with global dyskinesia and profound axial hypotonia. Cognitive impairment was greater compared to the p.Y107C mutation, with language and movement milestones significantly delayed. The youngest of the p.A116P family presented with a more severe movement disorder, focal epilepsy and was required to feed by a gastrostomy tube [63]. Interestingly, patients with these mutations in the GAF-A domain presented with normal MRI scans. We also made a knock-in mouse model of the p.Y107C mutation (p.Y97C in mice). Mice homozygous for the mutation displayed a range of motoric phenotypes compared to mice heterozygous for the mutation and wild-type. This included reduced locomotor activity in an open-field, reduced motor coordination on a rotarod and also a modified gait. To understand why the mutation had these effects, we investigated PDE10A at the transcript and protein level. Our detailed analyses of the mouse showed a marked reduction in the levels of striatal PDE10A mRNA and protein. We strongly believe that the mutation has an impact on transcript production and/or stability and also causes more rapid turnover of produced protein. A functional consequence of the reduction was demonstrated as phosphorylated CREB was not increased upon PF-02545920 administration compared to wild-type animals, indicating a loss of PDE10A activity. We also characterised levels of the PDE10A p.Y107C protein and transcripts after over-expression in HEK293 cells and we again identified a decrease in both PDE10A protein and mRNA levels compared to wild-type PDE10A expression. Interestingly, in this same overexpression system the p.A116P was shown to be even less stable than p.Y107C which might be connected to the more severe clinical presentation of the p.A116P family [63]. Amazingly (but perhaps ‘predictably’ considering the data in mice and HEK293 cells) we were also able to show a decrease in the levels of PDE10A by PET imaging in man, albeit a single

subject who was old enough to participate in such a study. Using a PDE10A-specific radioligand, we demonstrated a 70% reduction in striatal PDE10A signal independent of total striatal volume loss [63]. Such elegant translation across species gives us some belief that elevation of PDE10A levels and/or PDE10A function in these familial hyperkinetic disorder syndromes might have a beneficial clinical effect. The directionality of PDE10A activity we suggest here is of course opposite to the hypothesis around PDE10A inhibition in HD.

De novo mutations in the cAMP-binding GAF-B domain were also identified in four unrelated individuals of European origin [64, 65, 67]. Two of these patients were heterozygous for a c.898T>C mutation (p.F300L), with the other two carrying a c.1000T>C (p.F334L) heterozygous mutation. Additionally, two related individuals of Indian decent have also been identified with a c.1001T>G (p.F334C) substitution [66]. Patients exhibited childhood-onset, non-progressive chorea yet all met cognitive and developmental milestones. Unlike the GAF-A domain mutations, MRI scanning of affected individuals showed bilateral striatal hyperintensities [64-66]. Interestingly, the younger patient (11 years) with the p.F334L mutation showed restricted diffusion and striatal swelling, indicative of an active disease state. Diurnal fluctuations of chorea were reported with the p.F334L mutation, with symptoms most prominent within two hours of awakening, gradual improvement throughout the day and complete absence at night [64]. Adult carriers of the p.F300L mutation conversely presented striatal atrophy and nigrostriatal degeneration comparable to that of HD patients, who exhibit significant striatal volumetric loss throughout the disease correlating to motor impairment [65, 68]. Moreover, these patients also showed a reduction in striatal and globus pallidus PDE10A levels together with substantia nigra dysfunction [67]. On a molecular level, these mutations are located within the deep binding pocket of the GAF-B domain, where cAMP is bound and activates the wild-type enzyme [69]. cAMP has been shown to interact with the GAF-B domain tightly and specifically, therefore affinity or recognition of cAMP as a modulator is likely to be affected in these mutations [70]. Mencacci and colleagues reported that the p.F300L and p.F334L mutations did not affect the basal activity of the enzyme yet inhibited the cAMP-stimulatory effect for cGMP hydrolysis [65]. Therefore, it is

reasonable to assume that the symptomatic effects of these GAF-B mutations may interfere with the strict cAMP-dependent regulation of cyclic nucleotide hydrolysis. At this moment there are no comparisons of 'GAF-A' versus 'GAF-B' mutations across any model systems. These studies need to be conducted to understand how the two classes of mutation drive disease and eventually how they might be treated.

Furthermore, two sisters from an Afghan family displayed hyperkinetic and choreatic movements, motor restlessness and hypotonia as a consequence of biallelic mutations within the catalytic domain of PDE10A (c.2024T>C; p.Leu675Pro) [71]. This would probably affect the ability to hydrolyse cyclic nucleotides as this amino acid forms part of the active site [72, 73]. Similar to mutations in the GAF-A domain, these patients showed an early manifestation of the symptoms and normal MRI scans [71], inferring a common malfunction of the enzyme activity. Finally, a compound heterozygous mutation in PDE10A outside the GAF domains (c.199G>C; p.E67Q and c.1873A>T; p.I625F) has been detected in one individual with childhood onset of paroxysmal non-kynesigenic dyskinesia [67]. In this case, the patient presented a fronto-parietal cortical atrophy without striatal degeneration, suggesting a prominent role of PDE10A not only in the basal ganglia but in the whole motor circuit.

Mutation	PDE10A domain affected	Inheritance/ Dominance (No. cases)	Age at onset	Clinical presentation	PDE10A imaging	Biochemical impact on PDE10A	Mouse models
c.320A>G; p.**Tyr107Cys**	GAF-A	Familial/ Recessive (6)	~ 3 mo	Generalized dyskinesia of face, trunk and limbs	Reduced PDE10A levels in basal ganglia. Normal brain MRI	Reduced PDE10A levels and activity	Deficits in motor control. Reduced striatal PDE10A levels and function
c.346G>C; p.**Ala116Pro**	GAF-A	Familial/ Recessive (2)	~ 4 mo	Generalized dyskinesia of face, trunk and limbs. Development delay	Normal brain MRI	Reduced PDE10A levels	ND
c.898T>C; p.**Phe300Leu**	GAF-B	De novo/ Dominant (2)	~ 7 y	Non-progressive chorea	Reduced striatal PDE10A levels. Bilateral striatocortical atrophy	Normal basal activity Reduced cAMP-stimulatory effect	ND

c.1000T>C; p.**Phe334Leu**	GAF-B	De novo/ Dominant (2)	~ 4 y	Non-progressive chorea	Bilateral striatal hyperintensities	Normal basal activity Reduced cAMP-stimulatory effect	ND
c.1001T>G; p.**Phe334Cys**	GAF-B	Familial/ Dominant (2)	~ 4 y	Non-progressive chorea	Bilateral striatal hyperintensities	ND	ND
c.199G>C; p.**Glu67Gln** c.1873A>T; p.**Ile625Phe**	N-terminal region/ PDEase	Familial/ Recessive (1)	~ 6 y	Paroxysmal dyskinesia	Fronto-parietal cortical atrophy	ND	ND
c.2024T > C; p.**Leu675Pro**	PDEase	Familial/ Recessive (2)	~ 7 mo	Chorea, hyperkinesia and motor restlessness. Development delay	Normal brain MRI	ND	ND

Table 1. Clinical and molecular features of pathogenic PDE10A mutations. ND, Not Done.

4. Defective cAMP signalling as a central mechanism in hyperkinetic movement disorders/chorea

As described above, mutations in PDE10A are a novel cause of hyperkinetic movement disorders, likely highlighting that cyclic nucleotide signalling in MSNs is a central mechanism underlying the pathogenesis of basal ganglia disorders. Intriguingly, many hyperkinetic movement disorders with a genetic origin are caused by the mutation of a protein involved in the regulation of cyclic nucleotides (Figure 3) [74]. As we have seen, PDE10A is a major degradative enzyme for cAMP and cGMP in the striatum [49], but also expressed within MSNs are PDE1B, PDE4B, PDE2A, PDE7B and PDE8B [19] which together play an important role in the maintenance of cyclic nucleotide levels and compartmentalisation [75].

PDE	Disease	Organism	Biochemical impact on PDEs	Reference
PDE1B	HD	R6/1, R6/2 and Q175 mice	Decline in mRNA levels in the striatum	[36, 58]

PDE2A (recessive mutation in c.1439A>G; p.Asp480Gly)	Childhood-onset choreo-dystonia	Human (Spanish male patient)	Severe decreased enzymatic activity	[76, 77]
PDE4B	HD	R6/2 mice	Increased enzymatic activity in the cortex and striatum	[78]
PDE4AX	HD	R6/1 mice	Reduction in protein levels in the hippocampus	[79]
PDE4D1	HD	R6/1 mice	Reduction in protein levels in the hippocampus	[79]
PDE4D3	HD	R6/1 mice	Reduction in protein levels in the hippocampus	[79]

Table 2. Other PDEs in hyperkinetic movement disorders.

To reinforce the convergence on these specific pathways, a patient presenting with childhood-onset chorea associated with dystonia (not dissimilar to the PDE10A families) was shown to carry a homozygous missense mutation in PDE2A [76]. The mutation is within the cyclic nucleotide binding pocket of the GAF-B domain (p.D480G) and results in a severe decrease in the dual enzymatic activity of PDE2A, which in the striatum has a strong preference for cGMP [80]. There is clearly much more functional characterisation that needs to be done with this mutation. It will also be important to understand how a mutation in different GAF domains in different PDEs leads to differences in phenotypic clinical presentation. At the moment, some progress has been made with the underlying mechanisms of mutations in the GAF domains of PDE6 that lead to retinitis pigmentosa, achromatopsia and autosomal dominant congenital stationary night blindness. Thus, mutations R104W located in the GAF-A domain and Y323N and P391L in GAF-B cause an increase proteolytic degradation of PDE6C [81] while H262N mutant disrupts the inhibitory interaction of Pγ reducing its ability to promote the enzyme folding [82]. Moreover, these changes could be the result of an aberrant compartmentalisation, as the GAF-A domain of PDE6 contains a rod outer segment

localization signal [83].Three different mutations in PDE8B, found in German, Japanese and Portuguese families, produce a truncated enzyme with loss of all functional domains and complete ablation of cAMP hydrolysing ability [84-86]. As a result, these patients present with autosomal dominant striatal degeneration, a neurologic disorder characterized by bradykinesia, dysarthria and muscle rigidity. On the contrary, PDE8B knockout mice exhibit improved motor performance and a reduced age-induced decline in motor coordination [87], which could be due to a different striatal expression pattern than in humans or suggestive of an independent gain of function phenotype for the truncated form of PDE8B.

An aberrant increase of cAMP related to pathogenesis can also occur from dysfunctional adenylyl cyclase 5 (ADCY5), the most abundant AC isoform in MSNs, responsible for integrating signals from multiple receptors including D1 and D2 striatal dopamine receptors [88]. Thus, several mutations in ADCY5 have been identified as an important cause of a wide range of mixed hyperkinetic abnormal movements such as dystonia, childhood-onset chorea and myoclonus [89]. The most recurrent missense mutation occurs at Arg418, located in the first cytoplasmic domain close to the Gαi binding site [90] and causes a moderate to severe movement disorder, whilst a mutation in the catalytic ATP–binding pocket (p.A726T) is associated with a milder phenotype [91]. These mutations have no effect on the expression of ADCY5 but they increase its catalytic activity to synthesize cAMP [92]. Moreover, ADCY5-null mice with a reduction in cAMP levels exhibit hypokinetic motor dysfunction with abnormal coordination, bradykinesia and locomotor impairment accompanied by a reduction in the expression of the D1Rand $G_s\alpha$ [93]. Finally, ADCY5 haploinsufficiency by a splice site mutation that leads to RNA instability also causes chorea and dystonia in patients [94]. The similar pathological manifestations caused by both ADCY5 loss- and gain-of-function mutations emphasise the complexity of cAMP signalling which orchestrates a multitude of different pathways and the necessity to fine tune its concentration in the striatum.

Alterations in the activity of ADCY5 by mutations in its interacting G proteins have also been reported to cause hyperkinetic movement disorders. GNAO1 encodes the most commonly expressed G α-subunit ($G_o\alpha$) in the central nervous

system which couples to GPCRs including adenosine A1 and D2 receptors [95] and functions as an inhibitor of ADCYs [96]. There are three mutation hotspots in GNAO1 associated with an early-onset chorea and dystonia phenotype (Gly203, Arg209 and Glu246), which are important for the stabilisation of the Gα-containing complexes, mainly in GTP-bound active states, and locate in the region that partially overlaps with the putative ADCY binding site [97]. The resultant mutant proteins exhibit a higher activity, inhibiting ADCY and therefore decrease cAMP levels [98]. Dystonia has also been reported to be caused by mutations in GNAL which encodes the α-subunit of G_{olf} [99, 100]. $G\alpha_{olf}$ is strongly expressed in the striatum, specifically in the striosome compartment [101], where it may couple to adenosine A2A and D1 receptors to activate ADCY5 [102]. Pathological mutations of GNAL lead to unstable truncated proteins, complete deficiency of functional activity [100] or impaired binding to D1Rs [103]. Furthermore, some mutations in the G-protein ß subunit encoded by GNB1 have also been associated with dystonia [104]. The mutations in GNB1 are primarily located in exons 6 and 7 which express the binding surface for interactions with Gα and several downstream effectors, potentially affecting the production of cAMP [105, 106]. Likewise, at the receptor level, a mutation resulting in GPR88 truncation has been identified in a family with early-onset chorea [107]. This orphan GPCR is abundantly expressed both in D1R- and D2R- containing MSNs [108] and acts through a G_i protein to inhibit cAMP accumulation [109]. Similarly, *Gpr88*$^{-/-}$ mice show hyperactivity and motor coordination deficits [110, 111]. The intimate relationship between hyperkinetic movement disorders and aberrant cyclic nucleotide signalling unveil that cAMP/cGMP pathways could have a more prominent role in other neurological diseases with coordinated movement alterations. For instance, the cAMP cascade is impaired in Lesch-Nyhan syndrome (LSN), a neurological disease with mental retardation, dystonia and chorea caused by mutations in the gene encoding the hypoxanthine-guanine phosphoribosyltransferase (HPRT) [112]. LSN patients exhibit a marked loss of striatal dopaminergic fibres without MSNs degeneration [113]. Accordingly, the HPRT knockout mice also display abnormal striatal architecture [114] that could be related to the increased expression of PDE10A and reduction of other cAMP effectors such as DARPP-32 [115].

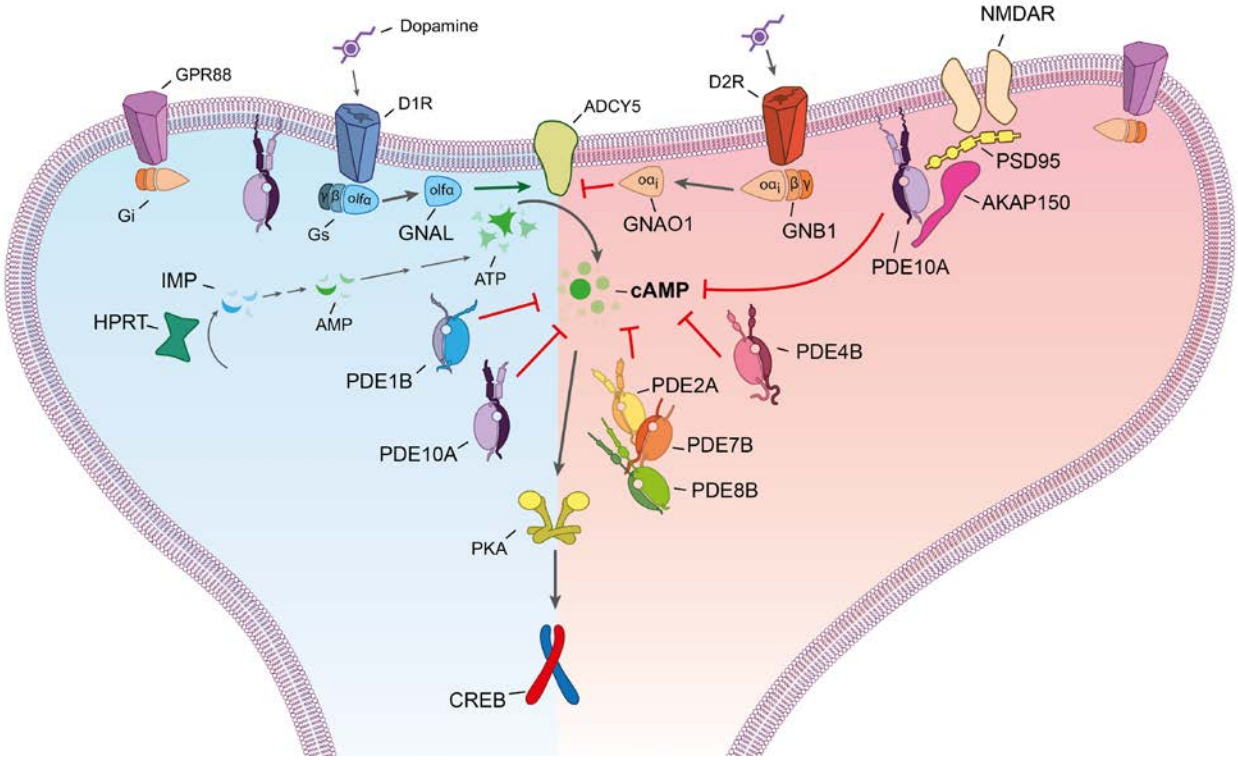

Figure 3. Dysregulation of cAMP signalling in MSNs is associated with hyperkinetic movement disorders. Mutations in genes implicated in the synthesis, degradation or distribution of cyclic nucleotides in the striatonigral and striatopallidal neurons (represented in blue and red respectively) have been identified as causes of hyperkinetic movement disorders. cAMP synthesis in the striatum is primarily controlled by ADCY5, which has been associated with hereditary chorea. Regulation of this enzyme by G proteins is also critical in coordinated movement. Thus, mutations in GNAO1 that expresses the inhibitory Goα, in GNAL that encodes the Golfα subunit or in the β subunit-encoding gene GNB1, can lead to a dystonia phenotype. Furthermore, an aberrant function of the purine metabolic enzyme HPRT or the GPCR GPR88 are also related to a genetic origin of chorea. The compartmentalisation of PDEs shapes the local gradients of cyclic nucleotides in the striatum, which are essential for the regulation of the motor function. Accordingly, several pathogenic substitutions in these cAMP degradative enzymes induce movement disorders, such as PDE2A, PDE8B and especially PDE10A.

5. Concluding remarks

A convergence of human mutations driving hyperkinetic clinical disorders on components of the cAMP and cGMP regulatory networks highlights the importance of tuning levels and maintaining the correct cellular compartmentalisation of these critical second messenger molecules. This article has considered the consequences of mutations in the regulatory GAF domains of PDE10A. The GAF-A and GAF-B grouped mutations present quite similarly, but the GAF-A biallelic mutations seem more severe including an earlier age of onset and in general a more complex set of

clinical issues. Interestingly however, they have no obvious structural deficits as shown by MRI while the GAF-B patients show abnormal bilateral hyperintense lesions. There have been no studies to date, in cell systems or model organisms, where the two classes of mutations have been compared. These types of studies will be essential in the future to decipher how these mutations relate to disease pathology and to the understanding of how they manifest in patients. Comprehension of the molecular mechanisms within the cAMP and cGMP signalling systems perturbed by PDE10A GAF mutations may also inform the direction of novel therapeutic strategies that may alleviate symptoms in patients affected by the movement disorders described above. One concept that may be germane in this context is the development of PDE10A activators. This could be achieved pharmacologically using compounds that potentially lock PDE10A in an active conformation akin to that when cAMP is bound to the GAF domain. Bacterial, viral and plant GAF domains are known to associate with a varied collection of small molecules that are structurally unrelated to cyclic nucleotides [116] and PDEs are the only family of mammalian proteins that contain such domains [117]. The divergence between mammalian PDE GAF domains should also allow specificity of action if GAF activator compounds can be discovered. Another possible route to PDE10A enhancement would be viral transfer of PDE genes to specific brain regions. This strategy is currently being used for PDE6 in the retina where a loss of PDE6 activity promotes retinal degeneration [118]. Delivery of PDE6 using AAV8 stabilised retinal cGMP concentrations in canine retinas and served to protect rods and cones from deterioration. If optogenetic delivery of PDE10A virus could be achieved, the spatial and temporal control of cyclic nucleotide levels in distinct brain regions could be orchestrated by restricted light emission and this would certainly guard against side-effects associated with systemic administration of pharmaceuticals that concomitantly change the activity of a PDE family in all organs, tissues and cellular locations.

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