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# Phosphodiesterase type 4 inhibition in CNS diseases

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## Abstract

Phosphodiesterases (PDEs) have been an interesting drug target for many diseases. Although a vast amount of mainly preclinical studies demonstrate beneficial effects of PDE inhibitors for CNS diseases, no drugs are available for CNS indications yet. In this review, the rationale of PDE4 inhibitors for different CNS diseases is discussed: memory impairments, striatal disorders, multiple sclerosis, and acquired brain injury. However, clinical development has been problematic due to mechanism-based side effects of these drugs in humans. Our increased understanding of factors influencing the conformational state of the PDE4 enzyme, and how to influence binding affinity of PDE4 subtype inhibitors, holds great promise for the successful development of novel selective PDE4 inhibitors with higher efficacy and less side effects.

## Key words (2-6)

memory, corticostriatal system, neuroinflammation, brain damage, neuronal plasticity

## Highlights

There is a vast amount of preclinical studies showing positive effects of PDE inhibitors in CNS disease models. However, there are no clinically approved PDE inhibitors for CNS indications. For PDE4, approval has been hampered mainly because of side effects.

PDE4 inhibition appears to be effective for many CNS indications, as it shows beneficial effects on memory in old healthy volunteers and schizophrenia patients.

PDE4 inhibition can lead to improved neuronal plasticity and promote anti-neuroinflammatory effects. These effects underlie restorative effects in models of memory impairment, schizophrenia, ADHD, multiple sclerosis, and acquired brain injury.

Understanding the factors influencing the conformational state of the PDE4 enzyme, and how to influence binding affinity of PDE4 subtype inhibitors, holds great promise for the successful development of novel selective PDE4 inhibitors with a desirable therapeutical window.

## Outstanding questions

How can we dissociate the positive clinical and adverse effects of PDE4 inhibitors?

Do we need isoform-selective PDE4 inhibitors to treat different CNS diseases?

The highly conserved catalytic domain of the four PDE4 isotypes makes it difficult to develop highly selective inhibitors. Will the current structural biology approach be successful in finding selective ligands?

How can we target specific pathways underlying different CNS diseases with PDE4 inhibitors?

How can we translate knowledge about the compartmentalization of PDE4 isoforms into drug discovery?

Which other therapeutic strategies can be used, beyond direct PDE4 inhibition, to increase cAMP?

Could specific PDE4 inhibition also be effective in modulating neuroinflammatory diseases such as Alzheimer's disease?

## **PDE inhibitors: basic properties and current status**

Phosphodiesterases (PDEs) are known for about 50 years and have attracted much attention in various research fields [1]. Based on their regulation of intracellular cAMP and cGMP levels, PDEs have a pivotal role in cellular functions. Not surprisingly, there has been a great interest in how PDEs can regulate cell function and whether their activity can be modulated to treat diseases. One of the first studies reporting the role of PDEs in the regulation of intracellular cAMP signaling in the kidney was published in 1968 [2]. Some years later, a paper described the effects of xanthine derivatives as inhibitors of PDE enzyme activity in fat cells [3]. In 1972, the first evidence was found for two different types of PDE in amoebas. Since then many more subtypes have been described and currently we distinguish 11 different mammalian PDE families (PDE1 to PDE11) [1, 4].

These different families are categorized based on features such as, mechanisms of regulation, subcellular distributions, and enzymatic and kinetic properties. In addition, each of these families contains multiple subtypes/genes (e.g., PDE1A, PDE4B), which can encode several transcript variants (e.g., PDE4D1-PDE4D9). Currently, this adds up to more than 100 different PDE types, sometimes referred to as the PDE superfamily. The PDEs can be found in many different cell types throughout the body and exert their functions by regulating the cyclic nucleotides cGMP and cAMP. Of note, PDE families differ in their ability to bind and degrade substrate, which can be cAMP-selective, cGMP-selective, or both [for a recent overview see 1].

Next to differences in substrate selectivity, PDE gene families are expressed in an organ-specific manner [e.g. 5]. The distribution on PDEs in the body and brain has been essential for selecting new drug targets for PDE inhibitors for different diseases [6]. For example, the localization of PDE4 in inflammatory cells (keratinocytes, neutrophils, T cells) has led to the development of PDE inhibitors for clinical use in chronic obstructive pulmonary disease (COPD), atopic dermatitis and psoriasis [e.g. 7, 8]. Also, selective PDE inhibitors have been developed and approved for treating cardiovascular and intermittent claudication [e.g. 9, 10]. These applications indicate that PDE inhibitors have a clear clinical potential. Although there has been a great effort to develop PDE inhibitors for CNS disorders, there are no PDE drugs approved for clinical use in this field yet [e.g.

11]. Various reasons have been offered for the failures in the clinical development of selective PDE inhibitors in CNS diseases [see 12, 13].

The issues regarding clinical efficacy of PDE inhibitors in CNS diseases may also be related to a lack of knowledge regarding their precise role in intracellular signaling pathways. Although PDE inhibitors are generally known to degrade cGMP and cAMP, the actual effects of PDEs and inhibitors on overall cell physiology appear to be more complex [e.g. 1, 14-16]. For example, inhibition of PDE1 in striatal medium spiny neurons (MSNs) decreased the level of surface AMPA receptors that are regulated by allosteric activation of PDE2 [17]. This complex interactive regulation of cellular processes is related to the compartmentalization of the specific PDEs. In light of this complex regulation of intracellular signaling by PDEs, and the apparent unique profile and function of the different gene families and isoforms indicates that a good understanding of these processes is required in order to successfully develop selective drugs.

The expression of different PDEs in the brain is relevant for selecting PDE targets for specific brain diseases. However, the expression of PDEs can be delineated at different levels. A first level is the expression pattern on the gene level in different brain structures. In an extensive study by Lakics et al [5] it was shown that the expression of PDE gene families in the brain and periphery was heterogeneous. These data may give a hint towards PDE subtypes that could make good targets for drugs to treat CNS diseases based on expression in disease-relevant brain structures. However, these data merely show a rather global expression level. There is only a relative limited number of studies using PDE-selective antibodies that investigated the subcellular localization in neurons and their role in signaling pathways [e.g. 18], and single cell RNA sequencing studies usually do not distinguish between transcripts encoding different PDE isoforms [e.g., 19]. This limits our understanding of the cellular functions of PDEs [e.g. 20], and how compartmentalized PDE signaling may lead to altered brain function.

Although more research is needed to understand the complex regulation of cellular processes by PDEs, a vast amount of animal studies show beneficial effects of selective PDE inhibitors in preclinical models of CNS diseases. Table 1 provides a global overview of these studies listing different disease categories in relation with a PDE subtype [for a general overview see 1]. Here it

can be seen that there is support for PDE1 inhibitors for Alzheimer's disease and schizophrenia [21-24]. PDE2 inhibitors were shown to be active in animal models for memory dysfunction [23] and some studies hinted at an antidepressant effect [25]. For PDE3 inhibition there is strong evidence that it could have beneficial effects in stroke [26, 27], and to a less extent in animal models for memory dysfunction [11, 28]. PDE4 inhibitors have been shown to be effective in different disease areas such as stroke [26, 29], animal models of Alzheimer's disease [11, 30], models of schizophrenia [21, 31], multiple sclerosis [32, 33], and different developmental disorders [34-37]. Some studies showed antidepressant effects after PDE4 inhibition [30, 38]. Interestingly, studies in humans have shown positive effects of PDE4 inhibition on cognition in healthy old subjects [39] and schizophrenic patients [40]. For PDE5 there is some preclinical evidence for a role in cognition models [23] and in stroke [23, 26]. Human studies showed memory enhancing effects [41] and no effects on cognition [42].

For PDE7 inhibitors some effects on cognition have been found, but most promising data have been shown in models of multiple sclerosis [33]. Some preclinical studies showed some promising effects of PDE9 inhibitors in cognition models [11, 43], but failed to improve cognitive performance in schizophrenia [44]. PDE10 inhibitors have been developed for treating corticostriatal disorders including schizophrenia [45-47], but clinical studies in schizophrenia have been disappointing (see [clinicaltrials.gov](http://clinicaltrials.gov)). For PDE11 only a few studies show relevance for improved social memory [48].

This overview strongly supports the notion that PDE inhibition is beneficial for treating different CNS diseases. PDE4 seems to be an attractive molecular target for several reasons. Firstly, PDE4 is strongly expressed in brain regions and neurons/cells related to these different disorders [e.g. 5, 49]. Secondly, preclinical data with PDE4 inhibitors show positive effects in many different disease areas (see Table 1). Thirdly, beneficial effects of PDE4 inhibition can be linked to signaling pathways underlying neuroplasticity and inflammation [49, 50]. Fourthly, some clinical studies showed positive effects on memory in healthy subjects and in schizophrenic patients [39, 40]. Therefore, this review aims to highlight the potential of PDE4 inhibitors in different CNS diseases.

## **PDE4 and memory**

There is strong evidence for a role of PDE4 in memory formation, largely based on the seminal work of Eric Kandel on the molecular mechanisms of memory [50]. In this framework, cAMP is an essential second messenger that leads to activation of protein kinase A (PKA) and subsequently the phosphorylation of cAMP response element binding protein (CREB → pCREB; see Figure 1). PKA activation also leads to insertion of AMPA receptors into the membrane [51]. pCREB is responsible for the transcription of neuronal plasticity genes including AMPA receptors and brain derived neurotrophic factor (BDNF) [29, 52]. Linked to this, cAMP has been found to play a pivotal role in the induction and maintenance of long-term potentiation (LTP) [50]. Since PDE4 is located in hippocampal neurons and shows specificity towards cAMP, inhibition of PDE4 can elevate cAMP levels and improve LTP [53]. Consequently, PDE4 is important for hippocampal functions via: 1) presynaptically enhancing glutamate (and also acetylcholine) synthesis and release; 2) postsynaptically by stimulating the neurotransmitter(s) receptor signaling. In line with these notions, the non-selective PDE4 inhibitor rolipram was shown to improve memory in rodents in 1982. This finding has been replicated in many other animal models and with more (subtype) selective PDE4 inhibitors [54].

Although PDE4 inhibition appears to be a very straightforward scientific rationale, and different drug discovery programs were aimed to develop a PDE4 inhibitor to treat memory disorders [11]. One of the main issues with developing PDE4 inhibitors are the adverse side effects, mainly emesis. This has been related to the expression of PDE4D in regions related to the emetic response [55]. Recently, PDE4 subtype-selective inhibitors have been developed to maximize the therapeutic window and minimize adverse effects. These data suggest that PDE4D may be more relevant for cognition enhancement [e.g. 56]. On the other hand, recent studies show that a non-selective PDE4 inhibitor (roflumilast) has beneficial effects on memory in humans without having clear adverse side effects [39, 40]. Understanding this beneficial effect of roflumilast may open new avenues in developing PDE4 inhibitors to improve memory performance.

## **PDE4 and corticostriatal functions**

The striatum mostly contains neurons with similar morphology, referred to as medium spiny neurons (MSNs). MSNs receive glutamatergic projections from the cortex, but their plasticity is dependent on dopaminergic signaling [57]. They are the only projection neurons of the striatum, integrating all input to this brain region [e.g. 13]. The vast majority of information arriving at striatal MSNs is for a large part conveyed onto cyclic nucleotide pathways, with a major role for cAMP (see Figure 2). The signal compartmentalization is achieved through the generation of cyclic nucleotide compartments by PDEs with a prominent role for PDE4 [4, 13, 58]. In analogy to the hippocampal functions, PDE4 is important for corticostriatal functions by two mechanisms of action: 1) presynaptically enhancing dopamine synthesis, release, and metabolism, as well as dopamine D1 receptor signaling, and 2) postsynaptically stimulating/inhibiting dopamine receptor signaling. Both functions independently constitute rationales for how PDE4 can regulate corticostriatal functions.

Regarding presynaptic effects, PDE4 is expressed at dopaminergic terminals in neurons of the substantia nigra pars compacta (SNc), where its inhibition leads to enhanced dopamine release [59]. By increasing the levels of dopamine at corticostriatal synapses, PDE4 inhibitors could have therapeutic potential for disorders characterized by corticostriatal hypodopaminergia, including attention deficit hyperactivity disorder (ADHD) and Parkinson's disease. Additionally, it has been described that PDE4B is localized at DARPP-32-expressing neurons in the mouse frontal cortex [31]. Here, rolipram enhanced dopamine D1 receptor-induced phosphorylation of DARPP-32. This presynaptic regulation of dopamine release and enhancement of dopamine receptor signaling provides an interesting scientific rationale for PDE4 as a molecular target for novel therapeutics of disorders that involve corticostriatal hypodopaminergia (See Figure 3).

The second main rationale for PDE4 in corticostriatal functions is linked to its regulation of postsynaptic dopamine receptor signaling in MSNs of both the direct and indirect pathway, as shown in striatal slices and *in vivo* [31]. Downstream of cAMP, the PDE4 inhibitor rolipram increased the phosphorylation of DARPP-32 and enhanced adenosine A<sub>2a</sub> receptor-mediated phosphorylation of DARPP-32 (representative of indirect pathway activation). Conversely, rolipram did not affect dopamine D1 receptor-mediated phosphorylation of DARPP-32 (representative of direct pathway activation). These findings suggest that PDE4 is exclusively



expressed in indirect pathway MSNs. Immunohistochemical analysis of striatal slices revealed that PDE4B expression can be found in both pathways but with a higher expression in MSNs of the indirect pathway [31]. Due to a main indirect pathway activation, PDE4 inhibitors are considered as a symptomatic treatment for hyperkinetic movement disorders (e.g., Huntington's disease). This is further supported by data in Huntington's disease mouse models that show increased expression of PDE4B in striatum and cortex [60]. This increase in PDE4 activity appears to be driven by mutant Huntingtin sequestering DISC1, a protein that would normally bind to and inhibit PDE4B.

Activation of the inhibitory indirect pathway by PDE4 inhibitors also mimics the action of dopamine D2 receptor antagonists, known for their antipsychotic potential. As a result, PDE4 inhibitors are investigated as a treatment for positive symptoms in schizophrenia. Additionally, PDE4 inhibition has proven to benefit cognitive function in clinical studies and preclinical models of schizophrenia [13]. The involvement of PDE4 in schizophrenia is further supported by interaction of PDE4B with DISC1, as a chromosomal translocation of this gene increases susceptibility for schizophrenia by disrupting binding of DISC1 to PDE4B [31]. Recent studies in humans did indeed support the notion that PDE4 inhibition could have beneficial effects in schizophrenia [40].

Although not extensively investigated, PDE4 might prove a therapeutic target in different diseases related to other disturbed corticostriatal functions (see Figure 3). Addiction and obsessive compulsive disorder (OCD) are some examples where PDE inhibition could be effective. Using behavioral sensitization, conditioned place preference and drug self-administration as behavioral models, various studies have shown that local or systemic administration of PDE4 inhibitors reduces drug intake and/or drug seeking for psychostimulants, alcohol, and opioids in rats or mice [61]. In OCD patients, activation of the indirect pathway may result in similar behavioral inhibition.

### **PDE4 and multiple sclerosis**

As mentioned in the Introduction, PDE4 inhibitors are being used to treat inflammatory diseases such as COPD and psoriasis. The fact that neuroinflammation is a hallmark of multiple sclerosis (MS) provides a good rationale to explore the therapeutic potential of selective PDE inhibitors [62]. The cellular pathogenesis of MS is driven by perivenular infiltration of auto-reactive lymphocytes that creates a pro-inflammatory microenvironment triggering phagocyte-induced CNS damage.

cAMP has three important functions in inflammation, it 1) it decreases endothelial junctional permeability at the level of the BBB and diminishes trans-endothelial transport of inflammatory mediators [26], 2) drives the development of regulatory T cells to maintain immunological homeostasis [63], and 3) differentiates phagocytes into an anti-inflammatory, repair-inducing phenotype [64]. The role of PDE4 has been studied for all three mechanisms. First, PDE4 (and PDE7) inhibitors reduced the cerebrovascular endothelial permeability in experimental autoimmune encephalomyelitis (EAE), a neuroinflammatory animal model for MS [62]. Second, inhibition of PDE4 decreased T cell proliferation and reduced the secretion of pro-inflammatory cytokines (TNF- $\alpha$  and IL-17) while increasing the release of anti-inflammatory cytokines (IL-10) in EAE mice [62]. Interestingly, upon anti-CD3/CD28 stimulation of primary human CD4<sup>+</sup> naive or memory T cells, the enzymatic activities of PDE4A and PDE4D alone were upregulated, although mRNA levels of PDE4A, PDE4B and PDE4D were increased [65]. Furthermore, knockdown of all PDE4 subtypes in these activated human CD4<sup>+</sup> T cells with siRNA reduced their proliferation rate and inhibited the secretion of IFN- $\gamma$  yielding a primary role for PDE4D [62]. Based on these findings, cAMP-specific PDE inhibition in T cells can lower the inflammatory cytokine production by acting directly on Th1 and Th17 cells or by regulating the immune response through activation of Treg cells.

A third role of cAMP to control the inflammatory process in MS involves modulating phagocyte function in the CNS. CNS infiltrating and resident phagocytes contribute to the inflammatory response by producing pro-inflammatory cytokines and chemokines while effectuating demyelination [66]. Increasing cAMP skews phagocytes rather towards an anti-inflammatory phenotype characterized by high levels of arginase 1 (Arg1), thereby hampering phagocytosis

[67]. In line with this, inhibition of PDE4 has been found to shift the inflammatory response in different models towards an anti-inflammatory response [e.g., 68].

Although promising results were obtained in preclinical studies, no definitive positive clinical proof of concept data with PDE4 inhibitors in MS patients are available yet. Results from a recent clinical trial with the non-selective PDE4 inhibitor ibudilast look more promising. This drug did not reduced focal inflammatory activity in relapsing MS but attenuated MS-related brain atrophy [69, 70]. These findings indicate that PDE4 inhibition may not be relevant for relapsing MS, but may be a suitable treatment of progressive MS phenotypes. For future research, identification of the key PDE4 genes and isoforms involved in specific disease phases and processes may lead to the development of more effective and better tolerated PDE4 isoform-selective inhibitors for the treatment of MS.

### **PDE4 in acquired brain damage**

Acute brain trauma (non-traumatic, such as stroke, and traumatic, such as accidents) causes ruptured microvessels which lead to secondary pathophysiological processes, including inflammation, cellular stress and activation of apoptotic cascades. These in turn can result in a myriad of subacute and chronic effects at the molecular, cellular, subcellular, and brain function level as depicted in Figure 4. Certain changes occur quite rapidly, whereas others may last for many months after the lesion [71]. The blood-brain barrier (BBB) plays a central role in the pathophysiology of acquired brain injury (ABI). The sustained increase in BBB permeability and the subsequent leakage of inflammatory cells and humoral factors can lead to long-lasting impairments in BBB integrity [72]

The role of cAMP and PDE4 during this post-injury increase in permeability of the endothelial cells of the BBB is well documented [73]. Some studies showed an upregulation of the PDE4 enzyme after ABI [74, 75]. Much less is known about their role in cytoskeletal (CSK) function and their effects on cell adhesion molecules (CAMs). As the CSK and CAMs are important for BBB function, there is a need to bridge this gap in our understanding. Initial findings indicating that PDE4 can mediate CAMs in peripheral cells may inform further mechanistic studies in endothelial

cells of the BBB and potentially in neurons (see Figure 4). Another effect of ABI is an upregulation of PDE expression that compromises the effects of cAMP in cell functioning as shown in different ABI models [26, 76]

Together, these findings support the notion that PDE4 inhibitors can restore brain function at early *and* later ABI disease stages by a dual mechanism (see Figure 4; [77, 78]) involving their anti-inflammatory effects following modulation of different inflammation pathways [49]. In line with the previous section on inflammation, an increase in cAMP levels by PDE4 inhibition could lead to a shift towards an anti-inflammatory state in various cells [68]. More specifically, PDE4B, but not PDE4D, seems to have a critical role in the LPS-induced inflammatory response [79, 80], indicating that this PDE4 subtype has a critical role in microglia activation. This could be interesting for early as well as later stages of ABI. A second mechanism for restoration of brain function after ABI is enhancement of neuroplasticity following modulation of the (cAMP/PKA/CREB) plasticity pathway [50]. This may be most relevant at later stages of ABI.

With respect to effects of PDE4 inhibitors on neuronal plasticity, including increased BDNF levels [29] and AMPA receptor upregulation [81], various studies suggest that enhanced neuronal plasticity contributes to the positive effects of different PDE4 inhibitors in different ABI models on different cognitive functions. Interestingly, this has been shown for PDE4B inhibitors [e.g. 82] as well as for PDE4D inhibitors [e.g., 83]. The relative contribution of anti-inflammatory effects and enhanced neuroplasticity to the effects of PDE4 inhibitors on cognition is not fully understood. However, there is substantial evidence that microglia function is directly related to neuroplasticity, and that these may go hand in hand during different phases of brain damage [84].

In conclusion, PDE4 inhibitors may consist of a novel class of drugs for the treatment of residual symptoms in ABI attenuating the pathophysiological consequences by their anti-inflammatory effects and their positive effects on neuroplasticity. Several animal studies have shown promising effects of PDE4 inhibitors on the functional outcome after ABI (see Figure 4). The finding that PDE4 inhibition was still effective when treatment started 3 months after the induction of brain

trauma appears promising for clinical applications [85]. Clinical studies are indicated to demonstrate the potential of PDE4 inhibitors after stroke and brain trauma.

### **Miscellaneous diseases**

There are other CNS indications for which PDE4 may be relevant. There are some neurodevelopment diseases where PDE4 inhibition has positive effects, such as fragile X [34], Rubinstein-Taybi syndrome [35], juvenile Batten disease [36], and Rett syndrome [37]. In these studies, applying genetic animal models, PDE4 inhibitors improved brain-related parameters that were typical for each disease. Also, PDE4 inhibition restored cognitive functions in these different disease models. These studies suggested that the effects could be related to restoring cAMP functions in development and could also be linked to the increased neuroplasticity after PDE4 inhibition. There is also good support for the notion that PDE4B (but not PDE4D) could be relevant for the treatment of depression [56]. These various studies show a pleiotropic effect of PDE4 inhibitors. It can be speculated that this may be related to the central role of cAMP in different critical cell functions and that PDE4 inhibitors can regulate these disturbed processes in different disease states.

### **Strategies towards safer and more selective PDE4 inhibition**

As mentioned earlier, clinical development of PDE4 inhibitors has been hampered by severe adverse side effects including nausea, emesis and diarrhea. Selective inhibition of PDE4 subtypes (e.g. PDE4B) or isoforms (e.g. PDE4D1) could provide a more promising strategy to obtain a larger window between adverse and therapeutic effects. Although this may be quite a challenge since all PDE4 genes show large homology, especially PDE4B and PDE4D, and produce highly similar catalytic domains. Nevertheless, the PDE4 subtypes exhibit subtle differences in protein structure, which enabled the development of PDE4 subtype-specific inhibitors (see Figure 5; [86, 87]). Although subtype-specific inhibition is possible through interactions with non-conserved residues, adverse side effects may still arise. Notably, expression and gene deletion studies

implicate that PDE4D is the main mediator of emetic effects [88], suggesting that inhibition of other PDE4 subtypes provides safer pharmacological profiles. Although this may be interesting for indications where PDE4B appears to be relevant, this poses a challenge for the generation of procognitive effects which appears to be related to PDE4D [56].

In addition to protein sequence differences among PDE4 subtypes that can confer inhibitor selectivity, PDE4 naturally exhibits different conformations showing distinct affinities for its prototypic inhibitor rolipram; the high-affinity rolipram binding site (HARBS) and low-affinity rolipram binding site (LARBS) [89]. Although the exact nature of HARBS and LARBS is unknown, prior studies indicated that specific cellular functions are regulated by either HARBS or LARBS conformers [90]. As HARBS occupancy correlates with emetic responses [91], it is hypothesized that inhibition of LARBS could reduce emetic effects [92]. HARBS depends on interactions with the UCR2 domain, and dimerized (i.e. long) isoforms stabilize the enzyme in the HARBS conformation [93]. However, neither dimerization nor the presence of UCR1 are requirements for HARBS, suggesting that also short isoforms, which do not dimerize, can exhibit HARBS [89]. Post-translational modifications (e.g., PKA phosphorylation) and interactions with partner proteins (e.g., XAP2), which have divergent effects on enzyme activity, can all increase rolipram sensitivity [e.g. 94]. Similarly, the affinity of the UCR2-interacting PDE4D inhibitor BPN14770 is increased in PDE4D constructs with mutations mimicking PKA phosphorylation [52]. It is proposed that PKA phosphorylation disrupts the UCR1-UCR2 module and that in dimers the UCR2 of one molecule can be '*trans*-capped' onto the catalytic domain of the other providing additional UCR2-inhibitor interactions [95, 96]. This implies that PKA activation 'liberates' the UCR module to facilitate both cAMP hydrolysis and inhibitor binding, reflected by enzyme activation and increased inhibitor affinity respectively. Interestingly, the PDE4D-selective and UCR2-interacting inhibitors PMNPQ and RS25344 [95] and those from the GEBR family shows similar affinities towards short and long PDE4D forms [97, 98], suggesting that UCR2-inhibitor interactions may also occur in monomeric PDE4. Additionally, interactions of the C-terminal with UCR2 and PMNPQ have been observed which may provide additional effects on the binding affinity of UCR2-interacting inhibitors [95]. On the other hand, subtype-specific residues in the C-terminus enable selective inhibition of PDE4B (e.g. A-33 [99] and a tetrahydrothiophene inhibitor [100]).

These findings indicate that HARBS and LARBS cannot be fully attributed to differences between long and short isoforms but are rather resulting from complex interplay of dimerization, protein-protein interactions and post-translational modifications generating multiple conformations with different affinities [for a recent review on these interactions see 1]. Accordingly, inhibitors may preferentially bind isoforms bound to a partner protein or those that are post-translationally modified. PDE4 can be post-translationally modified in many ways [101] and, mainly through common UCR2 and C-terminus domains, can bind multiple partner proteins [102]. These effects can even be isoform-specific via unique N-terminal domains (e.g., inhibition of PDE4D7 upon PKA phosphorylation [103] and preferential binding of  $\beta$ -arrestin to PDE4D5 [104]). Thus, although the regulation of the conformational state of PDE4 is complex, it can yield distinct inhibitor affinities, thereby offering the opportunity to target PDE4 isoforms or conformations specifically. Alternatively, PDE4 activity can be modulated using protein-protein interaction disruptors or compounds that act allosterically [105, 106].

Taken together, many factors influence the conformational state of PDE4 and thus inhibitor affinity. Prior studies already showed that different modes of inhibition (i.e. solely through interactions with the catalytic domain or additional binding with UCR2) produce different cellular effects [e.g. 107]. Therefore, future studies should indicate what PDE4 subtypes or isoforms, in what configuration, are involved in processes leading to adverse side effects. Subsequently, inhibitors showing low affinity to these isoforms or configurations would produce safer pharmacological profiles. Vice versa, elucidating what isoforms, in what configuration, are involved in the processes leading to therapeutic activity will facilitate the development of more efficacious PDE4 inhibitors.

### **Concluding remarks and future perspectives**

The current overview provides a strong case for PDE4 as a potential target for different CNS diseases. Although the adverse effects of PDE4 inhibitors are a major issue, there are alternative ways emerging to increase the therapeutic window for PDE4 inhibitors (see Outstanding Questions). A first approach may be linked to the properties of roflumilast. This is a non-selective

PDE4 inhibitor that has been shown to improve memory without any clear adverse side effects [39, 40]. Even for COPD, where 3-5 times higher doses are required, adverse effects are modest [7]. Further studies that look into the binding properties of roflumilast at the PDE4 enzyme may reveal some interesting characteristics and opportunities to improve the therapeutic window of PDE4 inhibitors. A second approach is to design PDE4 subtype- or isoform-selective inhibitors that have a more favorable therapeutic window. Linked to this, a better understanding of how the conformational state of PDE4 subtypes and isoforms is affected by different modulators, e.g. protein-protein interaction and phosphorylation, could further improve the clinical potential of these drugs.

Inhibition of PDE4 restores compromised cAMP functioning and reverses functional deficits in animal models of CNS disorders. It seems that neuroplasticity and anti-inflammatory effects are the key properties by which the effects of PDE4 inhibitors are mediated. These effects could also work synergistically (e.g., brain injury). Based on the effects of PDE4 inhibitors in animal models and humans on brain functions, and our current knowledge of the molecular biology of PDE4 subtypes, we believe that it is feasible to find more efficacious and safe PDE4 inhibitors for the treatment of CNS diseases.



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