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Phosphodiesterase Inhibition to Target the Synaptic Dysfunction in Alzheimer's Disease

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Abstract Alzheimer's Disease (AD) is a disease of synaptic dysfunction that ultimately proceeds to neuronal death. There is a wealth of evidence that indicates the final common mediator of this neurotoxic process is the formation and actions on synaptotoxic b-amyloid (Aβ). The premise in this review is that synaptic dysfunction may also be an initiating factor in for AD and promote synaptotoxic Aβ formation. This latter hypothesis is consistent with the fact that the most common risk factors for AD, apolipoprotein E (ApoE) allele status, age, education, and fitness, encompass suboptimal synaptic function. Thus, the synaptic dysfunction in AD may be both *cause* and *effect*, and remediating synaptic dysfunction in AD may have acute effects on the symptoms present at the initiation of therapy and also slow disease progression. The cyclic nucleotide (cAMP and cGMP) signaling systems are intimately involved in the regulation of synaptic homeostasis. The phosphodiesterases (PDEs) are a superfamily of enzymes that critically regulate spatial and temporal aspects of cyclic nucleotide signaling through metabolic inactivation of cAMP and cGMP. Thus, targeting the PDEs to promote improved synaptic function, or 'synaptic resilience', may be an effective and facile approach to new symptomatic and disease modifying therapies for AD. There continues to be a significant drug discovery effort aimed at discovering PDE inhibitors to treat a variety of neuropsychiatric disorders. Here we review the current status of those efforts as they relate to potential new therapies for AD.

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Contents

1 Introduction

Alzheimer's disease (AD) is the most common form of chronic neurodegeneration, affecting as many as 5.3 million people in the USA alone. The major risk factor for AD is aging. Consequently, as the USA and most other countries continue to enjoy increased longevity, the prevalence of AD is projected to increase dramatically. AD is portended by deficits in short-term memory. Mild cognitive impairment (MCI), greater than expected cognitive deficiency in the elderly, is believed to be the earliest antecedent of this aspect of the disease, with those suffering from the amnesic variant of MCI having a high conversion to AD [1]. Progression of AD is accompanied by greater impairment in both declarative and nondeclarative memory domains, along with disruption of reasoning, abstraction, and language, and the emergence of disturbing behavioral problems including anxiety and excessive emotionality, aggression, and wandering [2]. These pervasive cognitive and behavioral symptoms are devastating to patients and place a tremendous burden on caregivers in the home and in care giving institutions. Thus, there is an aggressive effort to develop therapies that may alleviate the symptoms of AD. Two such therapies are currently available, the acetylcholinesterase inhibitors [3] and the NMDA receptor antagonist memantine [4]. These therapies offer symptomatic relief and slow down clinical progression;

however, they have little or no effect on disease modification and so have only a limited window of therapeutic benefit within the long-term course of the disease. Thus, the goal is discovery of new therapies that both alleviate symptoms and substantially slow or halt the progression of AD.

The symptoms of AD are the result of a progressive loss of neuronal function, beginning in the temporal lobes and then spreading to a widening network of interconnected cortical regions. The key to identifying approaches to slow disease progression is to understand the underlying cause of this neuronal dysfunction and the reason for the characteristic pattern of progressive pathology. A seminal finding was the discovery in 1991 that mutation in the gene encoding for the amyloid precursor protein (APP) results in autosomal dominant inheritance of AD [5, 6]. APP is metabolized to a family of small peptides, the β-amyloids (Aβ), which form the core of one of the hall mark pathological markers in the AD brain, the amyloid plaque. These observations focused attention on the Aβ peptides as somehow being the key causal toxic agent in AD. However, the putative causal role of Aβ, including the underlying toxic mechanism and primary target of toxicity, has yet to be definitively established. The hypotheses are many: compelling arguments have been made that Aβ, in either soluble form or as higher order aggregates, in various intra- and extracellular compartments, is directly toxic to neurons, is disruptive to the cerebral vasculature, and/or induces a deleterious inflammatory response. There are a myriad of corresponding therapeutic strategies currently under development that target these putative toxic mechanisms. Although it may seem chaotic, rigorous testing of these multiple hypotheses is precisely what is needed to reach a definitive understanding of the role of Aβ toxicity in AD.

A second seminal set of findings is that AD is a disease of synaptic failure [7]. A striking feature of the end stage AD brain is the tremendous loss of neurons. Consequently, there has been considerable focus on identifying therapies to prevent neuronal death in AD. However, it is becoming increasingly recognized that synaptic dysfunction is the more proximal pathological event. Synaptic pathology is responsible for the cognitive decline characteristic of the earliest phases of the disease. In addition, it is highly likely that the loss of synaptic interconnectivity contributes significantly or is directly responsible for the ultimate death of neurons in AD.

The novel premise from which we are working is that synaptic dysfunction may also be an initiating factor in AD in that it may promote synaptotoxic Aβ formation [8]. This latter hypothesis is particularly intriguing in that it may account for the neuroanatomical progression of AD pathology as well as the most common risk factors: apolipoprotein E (apoE) allele status, age, education, and fitness. Thus, the synaptic dysfunction in AD may encompass both *cause* and *effect*. Given this premise, remediating synaptic dysfunction in AD may be predicted to have acute effects on the symptoms present at the initiation of therapy and, significantly, may also slow disease progression.

The mechanistic approach we are pursuing to remediate the synaptic pathology of AD is the use of cyclic nucleotide phosphodiesterase (PDE) inhibitors. The

cAMP and cGMP signaling systems are intimately involved in the regulation of synaptic homeostasis. The PDEs are the enzymes responsible for the metabolic inactivation of cAMP and cGMP and, as such, are critical regulators of cyclic nucleotide signaling [9]. Furthermore, among all of the classes of molecular targets in the cyclic nucleotide signaling cascades, the PDEs are the most highly amenable to pharmaceutical development. Thus, targeting the PDEs to promote "synaptic resilience" may be an effective and facile approach to new symptomatic and disease-modifying therapies for AD. We briefly provide additional context for this therapeutic approach and then present an analysis of the potential uses of inhibitors of PDE2A, PDE4A, B and D, PDE5A, PDE7A and B, PDE8B, and PDE9A to treat the synaptic dysfunction of AD.

2 AD as a Disease of Synaptic Dysfunction

2.1 Synapse Loss in AD

Synapse loss has been established as the strongest correlate of cognitive dysfunction in MCI and early AD [10, 11] and is apparent as a decreased synapse density in ultrastructural studies as well as decreased expression of synaptic proteins [12]. The significant reduction in the number of presynaptic boutons precedes frank pyramidal neuron loss. An illuminating finding has been that many of these synaptic changes also precede development of the diagnostic pathologies of the disease, parenchymal amyloid deposition, and intraneuronal neurofibrillary tangles (NFT) [13].

In individuals exhibiting the behavioral symptoms of AD, the diagnosis is formally confirmed at autopsy by the presence of two neuropathological features: the presence within brain of parenchymal plaques containing aggregated Aβ, and intraneuronal NFT arising from hyperphosphorylated fibrils of the microtubule-associated protein tau [14]. Much of modern AD research has focused on divining the underlying cause of the disease from these pathological markers. Neurofibrillary tau pathology in AD begins in the entorhinal cortex and spreads in a hierarchical manner into the hippocampus proper and cortex. Tau pathology increases as memory impairments become more severe and other cognitive and behavioral symptoms develop [15, 16]. In fact, the hierarchical progression of tau pathology "maps" the progressive deterioration of cortical systems reflected in the progression of symptoms. Given the importance of microtubules in intraneuronal transport, axonal growth, and maintenance of dendritic architecture, it is reasonable to suspect a role for tau dysregulation in the synaptic dysfunction of AD. While tau pathology may be an *effector* for synaptic toxicity, there is no compelling evidence to suggest that tau hyperphosphorylation and aggregation is the principal *causative* factor in the disease. In contrast, there is strong genetic evidence to suggest such a causative role for Aβ.

2.2 Aβ and Synapse Function

The term Aβ encompasses a small family of 39–43 amino acid peptides derived from the intramembranous cleavage of the APP by the sequential action of β- and γ-secretases [17]. Inheritance of the rare autosomal dominant early onset forms of AD (EOAD) is caused by mutations within the APP or presenilin genes. The latter encode for proteins that, together with three other proteins, form the γ-secretase complex. Various mutations in these different genes all result in an increase in the ratio of formation of Aβ42:Aβ40 [18]. Thus, this genetic evidence strongly suggests that aberrant over-production and/or mis-metabolism of APP/Aβ causes EOAD. The much more common form of AD is late in onset (LOAD) and of idiopathic etiology. Significantly, the clinical presentation, disease course, and neuropathology are nearly identical between EOAD and LOAD. This suggests a common underlying pathological mechanism and, thus, implicates a causal role for Aβ in LOAD as well [19]. Indeed, imaging studies using a ligand that binds to amyloid (thioflavin S β-pleated sheet material composed of deposited Aβ) have now documented an increase in brain amyloid burden in asymptomatic EOAD patients as well as patients diagnosed with "probable" LOAD [20]. Based on this compelling data, candidate compounds that inhibit the production or enhance clearance of Aβ are now entering late stages of clinical testing.

2.3 Synapse Loss as Both Cause and Effect in AD

The findings reviewed above beg a critical question – what causes aberrant over-production and/or mis-metabolism of APP to Aβ in the common, idiopathic form of AD? A plausible explanation linking APP processing to the cause of AD was proposed in 1993 based on two considerations [8]. First, the entorhinal cortex, the area of brain that demonstrates the earliest neurofibrillary pathology [15, 16], also has the highest levels of APP in brain [8, 21]. Second, this region undergoes an adaptive upregulation of APP turnover late in life in response to a life-long progressive loss of synaptic connectivity [8]. In some individuals, this response is hypothesized to cross a threshold resulting in the formation of neuropathological toxic products [8]. Thus, instead of promoting compensatory synaptic connectivity, the increased APP turnover results in synaptic toxicity. This synaptic toxicity disconnects the entorhinal projection from its postsynaptic targets [22], decreasing excitatory drive on the targets and thereby setting up a recurrent cycle of synaptic disconnection/APP upregulation/toxicity [8]. This cycle cascades in a hierarchical progression that is marked by NFT formation within a neuronal circuitry that mediates normal learning and memory processes in the anatomical progression of hyperphosphorylated tangle pathology defined by Braak and Braak [15, 16]. The earliest enunciation of such an "amyloid cascade hypothesis" of AD pathogenesis posited that accumulation of Aβ-containing plaques was causative to disease

pathogenesis [19]. However, individuals who, in life, experienced no pathological memory impairment may be found, at post mortem, to have fulfilled the neuropathological criteria for amyloid plaque burden [23]. This implies that plaques per se are not directly causative in disease onset and/or progression. Instead, evidence is converging on soluble forms of Aβ (i.e., Aβ that is not sequestered in plaque) as the "synaptotoxic" agent [7].

Since the discovery that mutations in the gene encoding for APP result in autosomal dominant inheritance of AD, there has been considerable research into the physiological functions of APP and related proteins [24]. This research is consistent with the above hypothesis in indicating that APP is regulated by, and involved in the regulation of, synaptic activity at multiple levels. Evidence suggests a role for the APP holoprotein in axonal transport and extracellular cell/cell interactions and adhesion [24]. Furthermore, proteolytic fragments of APP are suggested to have distinct signaling functions. Processing by α- or β-secretase releases soluble N-terminal fragments (the sAPPα or sAPPβ) into the extracellular space, where these peptides appear to have neurotrophin-like signaling properties. The C-terminal fragment released following cleavage by γ-secretase is suggested to be transported to the nucleus, where it functions to regulate transcription. However, the most enigmatic aspect of APP processing is the minor (<10%) component comprising the sequential action of β- and γ-secretases to form Aβ. Initially, the Aβ peptides were considered as simple by-products of the formation of the other signaling fragments of APP. Instead, it is becoming increasingly clear that Aβ, too, has distinct roles in regulating synaptic function. Aβ formation is regulated by neuronal activity [25, 26]. In some studies, very low (pM) levels of soluble, cell-derived Aβ were found to reduce synaptic potentials and spine density when applied to primary neuronal cultures [27, 28]. When these same soluble Aβ species were administered intrathecally to rats, cognition was impaired [29]. However, in other experimental systems, synthetic Aβ42 positively modulated synaptic plasticity and enhanced hippocampal-dependent memory [30], and Aβ monomers were found to be neuroprotective [31]. Recently, Tampellini et al. provide evidence to suggest that there are two pools of Aβ, intra- and extracellular, that interact to impact synaptic function in different ways [25]. Taken together, these data suggest that Aβ peptides are formed in response to synaptic activity to impact normal neurophysiological function.

The key point of understanding is exactly why, in some individuals, formation of Aβ at excitatory synapses crosses from physiological to pathological. Our premise is that this is related to properties intrinsic to the synaptic physiology of these at risk individuals. All of the major risk factors for idiopathic AD are associated with reduced synaptic function. In addition to age, the major environmental risk factors for idiopathic LOAD are lower native intelligence (operationally defined as education level) and reduced overall physical health. Each of these factors has a negative impact on synaptic function. Particularly illuminating may be the emerging data suggesting that synaptic function is also impacted by apolipoprotein E4 (apoE4) status, the most significant genetic risk factor for AD [32, 33]. The E4 allele of the apoE gene is a well-characterized risk factor for AD, with E4 carriers having an

increased probability of suffering AD, at an earlier age of onset [34]. In humans, E4 carriers exhibit reduced cognitive capacity, reductions in resting brain glucose metabolism, and a distinct pattern of brain activity that is observed well before onset of AD symptoms [35–37]. Furthermore, the apoE4 allele is positively linked to subclinical epileptiform activity, which is remarkable in light of the recent compelling evidence showing that aberrant excitatory neuronal activity is a primary upstream mechanism for cognitive decline in AD [38–41]. In mice that express human apoE4, dendritic architecture, spine number, and electrophysiological parameters are significantly reduced when compared to age- and background-matched mice expressing human apoE3 [42]. These findings suggest that the E4 allele may reduce overall synaptic function and that this occurs well before frank neurodegeneration.

Taking into account all of the factors discussed above, we hypothesize that reduced synaptic function is the key "initiating" factor in LOAD. This reduced synaptic function is hypothesized to be responsible for the susceptibility to a change in Aβ processing from physiological to pathological and/or an increase in susceptibility to Aβ toxicity. Reduced synaptic function is also hypothesized to be a key facilitatory factor in the progression of synaptic disconnection that initiates in entorhinal cortex and progresses throughout interconnected cortical networks. "Synaptic resilience" is the inverse of this reduced synaptic function. Thus, therapies that promote synaptic resilience may reduce the risk and/or slow AD progression; that is, such therapies may have a true disease-modifying effect.

Unfortunately, at present we do not have a complete understanding of the molecular underpinnings of the reduced synaptic function that is hypothesized to be causal to AD. There is, however, a tremendously expanding understanding of fundamental processes that mediate physiological synaptic function and plasticity. It seems reasonable to assume that we will want to manipulate some of these fundamental processes to get at the synaptic dysfunction of AD. Thus, this body of knowledge serves as the logical starting point to explore such therapies. The basis for our interest in the potential of PDE inhibitors in this regard is outlined below.

3 Cyclic Nucleotides and Synaptic Plasticity

Synaptic plasticity is a term that encompasses a wide range of complex processes. At the level of the individual synapse, synaptic plasticity is the process by which the architecture and complement of signaling molecules are adjusted in response to recent activity, in preparation for future activity. In the simplest terms, the past predicts the future, and so recent activity increases synaptic strength, whereas lack of activity leads to synapse deconstruction. A critical modulator of this general rule is the coordination (i.e., timing) of events between the pre- and postsynaptic sides of individual synapses. At the level of the neuron and neuronal circuit, synaptic plasticity is the means of encoding information. That is, changes in individual

synaptic strengths are integrated and reflected in changes in the way a neuron interconnects with neuronal networks. It is the change in pattern of activities in large networks of neurons that read out as "behavior" and "cognition." Thus, when we seek to modulate synaptic plasticity to slow disease progression, we are concerned with modulating the biochemistry of individual synapses, whereas when we seek to modulate synaptic plasticity to improve cognition in AD, we are concerned with modulating network activity. It remains to be proved whether both of these goals can be accomplished through a single molecular mechanism. There are a myriad of such mechanisms that may be targeted to impact synaptic plasticity. To paraphrase an earlier statement, rigorous testing of multiple mechanism-based hypotheses is precisely what is needed to reach an understanding of the utility of targeting synaptic plasticity in AD. The cyclic nucleotide PDEs may be particularly advantageous to target in this regard. These enzymes are intimately involved in the regulation of cyclic nucleotide signaling, and these signaling cascades are intimately involved in the regulation of synaptic plasticity, as briefly described below.

cAMP and cGMP signaling is ubiquitous in mammals. A wide variety of intercellular communicative, hormonal, and metabolic events trigger the activation of adenylyl and/or guanylyl cyclases to catalyze the formation of cAMP and cGMP from ATP and GTP, respectively. cAMP and cGMP subsequently bind to a variety of effectors including their cognate protein kinases [43], ion channels [44], Epacs [45], and other PDEs [9], resulting in both acute- and long-term changes in cellular function. Both cAMP and cGMP signaling mechanisms are implicated in the regulation of synaptic plasticity at multiple levels [46, 47].

3.1 cAMP

The canonical role of the cAMP/PKA signaling cascade is in the regulation of postsynaptic, protein synthesis-dependent long-term potentiation (L-LTP) [48], widely believed to be an in vitro model of learning and memory [49]. There are considerable data indicating that the cAMP/PKA signaling cascades are also involved in regulation of earlier stages of LTP in the postsynaptic compartment. This includes potentiation of Cam KII induction by PKA-mediated inactivation of protein phosphatases that are responsible for dephosphorylation of Cam KII [50], and PKA phosphorylation of the GluR1 subunit of AMPA receptors to drive insertion of this subunit into the postsynaptic active zone [51] and increase AMPA receptor open channel probability [52]. The cAMP/PKA signaling cascade is also implicated in the regulation of plasticity in the presynaptic compartment. The clearest example is the presynaptic form of LTP characterized at mossy fiber synapses in the dentate gyrus of the hippocampus [53]. Mossy fiber LTP is critically dependent on activation of a calcium/calmodulin-dependent adenylyl cyclase, leading to an increase in presynaptic cAMP and activation of PKA [54] and phosphorylation of the synaptic vesicle-associated protein

Rim1a [55]. This form of PKA-dependent presynaptic plasticity is also observed in cerebellum and at corticothalamic and corticostriatal synapses [53]. Synaptic plasticity also involves adaptive decreases in synaptic strength [56]. Of these, the archetype is NMDA receptor-dependent long-term depression (LTD) in the hippocampus [57], which appears to be critically dependent on the dephosphorylation of PKA substrates. Of particular significance is the selective dephosphorylation of the PKA site Ser845 on GluR1 which decreases the probability of AMPA receptor channel opening and increases AMPA receptor endocytosis [58].

3.2 cGMP

Although less extensively studied, there is a body of evidence implicating cGMP signaling cascades as important pathways for many forms of synaptic plasticity [59]. The canonical role of cGMP in synaptic plasticity is as mediator of the retrograde messenger nitric oxide (NO) at glutamatergic synapses [60–63]. It is also now clear that cGMP signaling cascades participate at several additional levels of regulation that influence hippocampal LTP, including postsynaptic protein synthesis-dependent mechanisms [64]. These distinct presynaptic and postsynaptic functions are perhaps most clearly demonstrated in studies of LTP in visual cortex, where the two guanylyl cycles isoforms are differentially localized to pre- and postsynaptic compartments, and genetic deletions of either isoform have demonstrated separable effects on LTP [65]. Compartmentalization is further indicated by the finding that the source of NO is also an important determinant [66]. Finally, there is evidence indicating a role for cGMP signaling cascades in the depression of synaptic activity [67–69].

4 The Phosphodiesterases

4.1 Enzyme Structure and Function

The PDEs are the family of enzymes that terminate through metabolic inactivation signaling by cAMP and cGMP. Thus, these enzymes are intimately involved in the regulation of cyclic nucleotide signaling throughout the body, including those cyclic nucleotide pathways involved in the regulation of synaptic plasticity. The PDEs are encoded by 21 genes that are functionally separated into 11 families [9, 70]. Further physiological diversity stems from differential mRNA splicing and, to date, more than 60 PDE isoforms have been identified. There is a rapidly expanding body of knowledge about the physiology of these enzymes, from the atomic and structural level to the role in specific signaling processes. This

information has both garnered interest in and facilitated drug discovery efforts. Below, we first touch on the current knowledge of the structural features of these enzymes, particularly with regard to drug discovery. We then turn to biological functions and highlight a number of the enzyme families that may be particularly relevant to the treatment of AD.

The PDEs are modular enzymes in which the catalytic domain in the C-terminal portion of the protein is coupled to regulatory elements that reside in the N-terminal region. The 11 PDE families differ most significantly from one another within the unique N-terminal regulatory domains. On the other hand, the C-terminal catalytic domains are highly conserved with respect to specific invariant amino acids, three-dimensional structure, and catalytic mechanism [71]. Nonetheless, subtle differences within the catalytic core impart important family-specific characteristics [72]. To date, essentially all of the pharmaceutical developments around the PDEs have been toward the discovery of catalytic site inhibitors. Structural information from single crystal X-ray crystallography has played an important role in elucidating the important functional differentiating features within the catalytic domains of the 11 gene families that allow for the development of family-specific inhibitors. Indeed, current lead optimization projects without the use of some form of structure-based drug design are becoming practically unthinkable. This area of knowledge is summarized below.

Structures of the catalytic domains of all but two PDE families (PDE6 and PDE11) have been solved. Since the field was last reviewed in 2007 [73], two new PDE families have been added to the list of solved structures, namely PDE8 in its unliganded form as well as in complex with IBMX [74] and PDE10A with various ligands [75, 76]. Characteristics of all PDE structures solved so far are the following features which are also important for the design of new inhibitors:

- The active site contains a glutamine residue that contributes to the binding of the natural substrate cAMP or cGMP through a dual hydrogen bond. The "Glutamine Switch" mechanism [77] suggests that hydrogen-bonding residues surrounding the glutamine serve to either lock it in a fixed conformation (cAMP or cGMP selective PDEs) or allow it to change conformation (PDE1, 2, 3, 10 and 11). Although very elegant in its simplicity, the glutamine switch hypothesis remains somewhat controversial [78, 79]. The glutamine is also nearly invariably involved in hydrogen bonding to PDE inhibitors, although not necessarily through two hydrogen bonds (see [80] and references cited therein).
- A phenylalanine, situated just below the plane of the bound substrate/inhibitor, participates in the substrate binding by π–π interactions. This hydrophobic region, usually referred to as the "Clamp" region, explains why many PDEs appear to have a preference for flat and π-electron-rich inhibitors of the sildenafil type [81].
- The metal ions in the active site may also be targeted for inhibitor binding; however, this approach is not usually addressed by design elements in PDE inhibitors intended for central nervous system (CNS) indications. Specifically,

a good ligand for the metal ion is by its very nature rather polar, thereby adding to the overall polar surface area of the inhibitor to such a degree that transport across the blood–brain barrier becomes exceedingly difficult.

4.2 Compartmentalization of PDE Signaling

The desire for inhibitors selective for different PDE families (and individual isozymes, see below) stems from the fact that cyclic nucleotide signaling is highly compartmentalized within individual cells [82, 83]. Thus, PDE isozymes have distinct signaling roles in individual cell types and there appears to be little or no overlap in function. Compartmentalization is the result of physical localization of signaling pathways to discreet areas of cells and, further, the physical association of the different components of a signal cascade mediated by adaptor and scaffolding proteins. Thus, a scaffold may bring a cyclase, an effector kinase, and a specific PDE isoform together with a cell surface receptor to affect a very localized signaling event. Compartmentalization of PDE-regulated signaling has been most clearly elucidated for the PDE4 family [84]. For example, physical compartmentalization allows only PDE4B to regulate Toll-like receptor signaling in mouse peritoneal macrophages despite the fact that these cells also express PDE4A and PDE4D [85].

The complexity and compartmentalization of PDE-regulated signaling are particularly evident in the CNS [86] and are crucial to the analysis of the different PDEs that may be targeted to impact synaptic plasticity. As noted above, cAMP and cGMP signaling cascades are implicated in the regulation of plasticity in numerous temporally and spatially distinct compartments. It is reasonable to conjecture that different PDE families and isoforms service these distinct signaling compartments, at the level of the individual synapse, neuronal subtype, and brain region. Thus, the challenge is twofold. The first is to determine the role of individual PDE isoforms in the regulation of different aspects of synaptic plasticity at the synaptic and sub-synaptic levels. The second is to relate the effects of manipulating the PDEs at the synaptic level to the impact that may have on neuronal circuits and networks. Fortunately, the localization of the different PDE isoforms throughout the brain continues to be investigated, and the pharmacological tools needed to accomplish these types of analyses are becoming available to allow investigation of function. With regard to AD, the potential targets include PDE2A, PDE4A, 4B, and 4D, PDE5A, PDE7A and 7B, PDE 8B, and PDE9A [86]. We review the current state of knowledge regarding these enzymes with regard to localization, the availability of pharmacological inhibitors and the knowledge to date on the effects of these inhibitors on behavior that may be relevant to AD therapy. We start with PDE4, the most highly pursued drug target among the PDEs, followed by PDE8B and PDE2A as additional cAMP signaling-specific targets. We then turn to PDE5, the most commercially successful PDE target, and finish with PDE2A and PDE9 as the new targets generating the most interest.

4.3 PDE4

PDE4 is the largest and most complex of the PDE gene families and is the major cAMP-regulating enzyme in the body [84]. The PDE4s are encoded by four genes, PDE4A–D, with PDE4A, B, and D expressed appreciably in the CNS. Furthermore, mRNA transcribed from each gene is subjected to alternative N-terminal splicing to yield three major variants. The long variants contain two conserved N-terminal domains, UCR1 and UCR2, with a conserved PKA phosphorylation site at the N-terminal end of UCR1. Phosphorylation at the PKA site stimulates activity and is a key element in the regulation of these variants. The short variants are truncated and lack UCR1 and the PKA site, whereas the supershort variant is further truncated to lack both UCR1 and the N-terminal portion of UCR2. Accounting for genes and splice variants, over 20 PDE4 isoforms have been identified. Thus, the PDE4s provide a rich repertoire for fine tuning cAMP signaling.

PDE4 has been heavily pursued as a therapeutic target. Initial interest in the 1980s was as a CNS target, stemmed from the finding that rolipram, the prototypical PDE4 inhibitor, had clinical antidepressant activity. However, it is the potential to treat inflammatory airway disease that has sustained the most interest. Recently, CNS interest has re-emerged in the potential for PDE4 inhibitors to treat cognitive dysfunction [87], particularly in AD. This latter interest derives from the seminal finding that PDE4 is a key element in the cAMP/PKA signaling cascade involved in protein synthesis-dependent L-LTP in hippocampus and that rolipram potentiates L-LTP in hippocampal slice preparations [88].

The hippocampus has a broad range of functions, but is particularly implicated in the formation of long-term memories. L-LTP putatively represents the molecular mechanism that supports this function [48]. Thus, the robust finding that PDE4 inhibition augments LTP in hippocampus implies that PDE4 inhibitors should facilitate long-term memory formation in vivo. There is ample experimental support for this hypothesis. Administration of rolipram robustly improves the performance of both rodents and nonhuman primates in various long-term memory tasks, under conditions where performance is disrupted by a variety of pharmacological or other manipulations [87, 89]. Rolipram is competitive at the cAMP-binding site of PDE4. The potency at the high affinity site that predominates in brain is approximately 2 nM. The dose of rolipram most often reported as efficacious in rodent cognition assays is 0.1 mg/kg, which yields an estimated free brain concentration of 2–3 nM (unpublished observation calculated from data in the literature).

Studies with rolipram also suggest that PDE4 inhibition may specifically reverse deficits in synaptic function caused by Aβ [90]. Direct application of Aβ to hippocampal slices or in vivo impairs LTP in some systems [91–93]. LTP deficits are also observed in slices prepared from transgenic mice that overexpress Aβ [90]. Significantly, acute rolipram administration to transgenic mice reduced deficits in LTP in slices prepared from these mice, and this beneficial effect was maintained for at least 2 months beyond the end of treatment [90, 93].

Unfortunately, despite more than 30 years of pharmaceutical research, no PDE4 inhibitor has been approved for any indication. The primary obstacle has been severe side effects, notably emesis, nausea, and vasculitis, at exposures that are within the range where therapeutic benefit may begin to be realized. This obstacle led to abandonment of rolipram for the treatment of depression. These side effects of PDE4 inhibitors have also, to date, prevented a thorough exploration of the dose range for efficacy for inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD) [94]. It remains to be determined whether there is a sufficient therapeutic index for PDE4 inhibition for the treatment of cognitive dysfunction in AD. The available preclinical data suggest that this may be a challenge. As stated above, the estimated level of PDE4 inhibition associated with improved cognition in rodent models, extracted from the data with rolipram mentioned above, indicates that a significant fractional inhibition may be required. However, it is not possible directly determine the TI in rodents, since rats and mice lack an emetic response. Furthermore, the ferret model of emesis has proved not to be predictive of the emetic potential of PDE4 inhibitors in humans [95]. There are limited data on the effects of rolipram on cognition in nonhuman primates, where it is possible to gage a therapeutic index. Rutten et al. reported positive effects of rolipram in an object retrieval paradigm in cynomolgus monkeys with maximal efficacy at 0.03 mg/kg; however, the next highest dose of 0.1 mg/kg was not tolerated due to emesis [96]. In an earlier study, Ramos et al. found no effect of rolipram in a delayed match to position paradigm in rhesus monkeys at doses up to the maximum tolerated dose of 0.01 mg/kg; a dose of 0.05 mg/kg was not tolerated [97]. Thus, if there is efficacy, the therapeutic index for the treatment of cognitive dysfunction may be similarly low as for the treatment of inflammatory airway disease. However, given the severity of the AD and the fact that the neurodegenerative process may have altered the sensitivity to potential therapeutic and/or adverse effects of PDE4 inhibitors, it is still of interest to investigate PDE4 as a target for the treatment of AD. In fact, Merck & Co. recently completed a Phase II proof of concept study in patients with AD with the PDE4 inhibitor, MK0925, although no results have been published and the compound is no longer listed in the company pipeline. Thus, alternative strategies are worth considering as we await this clinical feedback, as discussed below.

To date, the vast majority of research into the procognitive potential of PDE4 inhibition has relied on the use of inhibitors such as rolipram that are competitive at the catalytic site. These compounds inhibit each of the PDE4 gene families, consistent with the very high homology in the cAMP-binding pocket. There is also no evidence to suggest that such compounds distinguish among the major N-terminal splice variants. An approach for overcoming the narrow therapeutic index of such pan-PDE4 inhibitors may be the development of compounds that interact with specific PDE4 subtypes to capture therapeutic effects while avoiding those subtype(s) that mediate emesis and nausea. The question is, which subtype to target?

Cherry and Davis [98] mapped by immunohistochemistry the distribution of PDE4A, B, and D in mouse brain to many regions relevant to higher order cognitive functions. All three isoforms are expressed in neocortex, albeit with distinctive laminar distributions. PDE4D is most highly expressed in the hippocampus proper, and genetic deletion of PDE4D also potentiates LTP to subthreshold stimuli in hippocampal slices [99], although this was accompanied by poorer performance of the animals in behavioral tasks that measured cognition. PDE4B is most highly expressed in the striatal complex and the dentate gyrus. Nonetheless, in hippocampus, PDE4B expression and subcellular localization respond to the induction of LTP, suggesting a specific role for the 4B isozymes in this form of plasticity [100]. This finding takes on added significance in light of the fact that PDE4B disruption [101] and genetic variation [102, 103] are associated with neuropsychiatric disease.

Targeting specific PDE4 isozymes must also take into consideration particular isozymes that may be involved in the side effects associated with pan-PDE4 inhibition. Based on studies with PDE4 knockout mice in an innovative behavioral approach, Robichard et al. have put forward the hypothesis that PDE4D is specifically involved in the emetic response [104]. Significantly, an inhibitor with ~100-fold selectivity for PDE4D over other family members has been identified and found to cause emesis in early clinical studies in humans [105]. Taken together, these data suggest that inhibitors selective for PDE4A/B over PDE4D may be of particular interest for the treatment of cognitive dysfunction while obviating tolerability issues. The challenge now is to identify compounds with sufficient PDE4B selectivity with which to test this hypothesis.

Compounds with significant PDE4D selectivity have been identified [105]; however, it is unclear how this selectivity is achieved and, therefore, how to utilize the structure–activity relationships around these selective compounds to generate compounds that are selective for other PDE4 isozymes. Recently, Asahi Kasei Pharma (**1**, Fig. 1) and GSK (**2**, Fig. 1) have independently reported on two series of compounds with selectivity for PDE4B over PDE4D [106, 107]. Importantly, the GSK group is beginning to determine the molecular requirements that accompany this selectivity.

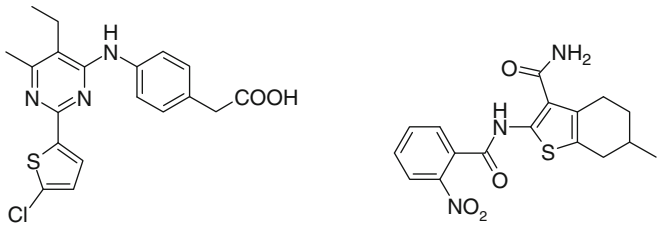


Fig. 1 PDE4 inhibitiors selective for the PDE4B isoform

Another very interesting advance is the recent disclosure by deCODE Genetics in a series of patents of a new class PDE4 inhibitors that are noncompetitive with respect to the cAMP-binding site [US Patents 12275152, 12275164, and 12275165]. This suggests that deCODE has identified a binding site on PDE4 outside of the substrate-binding pocket through which PDE4 enzymatic activity can be modulated. Although the deCODE compounds are selective for PDE4D over PDE4B, it is possible that knowledge about the PDE4D selectivity mechanism may allow for the development of other classes of compounds selective for the other PDE4 isozymes.

Finally, an area of PDE4 medicinal chemistry that has not yet been explored is the possibility of developing compounds selective for long versus short splice variants. Such an undertaking would be greatly facilitated by crystal structures of PDE4 that include regions of the protein beyond the catalytic domain.

4.4 PDE7 and PDE8B

While PDE4 is the major cAMP metabolizing enzyme, there are two other PDEs that are also selective for cAMP, PDE7, and PDE8. Thus, it is reasonable to investigate whether these PDE families may also play a role in regulating one or more of the many cAMP signaling pathways involved in synaptic plasticity. The physiology and pharmacology of these two enzymes are beginning to be investigated in depth, as reviewed below.

The PDE7 family is composed of two members, PDE7A and PDE7B, which demonstrate high affinity for cAMP but that are insensitive to rolipram [108, 109]. Unlike most other PDEs, PDE7 does not contain defined N-terminal regulatory domains although a consensus site for PKA phosphorylation does exist. While the protein expression profile of PDE7A and 7B is largely unknown, the mRNA levels for both isoforms reveal abundant expression in the CNS. PDE7A mRNA is expressed in the olfactory bulb and tubercle, hippocampus (dentate granule cells), and brain stem nuclei, while the highest level of PDE7B mRNA is localized to the cerebellum, striatum, dentate gyrus, and thalamic nuclei. Moreover, in humans there are three known splice variants for PDE7A that contain unique N- and C-terminal mRNA modifications that likely influence intracellular localization as well as interactions with other proteins. Promoter variants have also been reported for PDE7A, offering additional subtleties with respect to cAMP-responsiveness. PDE7A1 appears to encode a protein that contains peptide sequences in the N-terminal region that directly inhibit PKA catalytic activity [110]. Thus, this splice variant of PDE7A may regulate PKA activity in two ways, through regulation of cAMP levels and through a direct interaction with the PKA catalytic subunit.

The highest level of interest in PDE7 remains as a target to treat inflammatory disease, whereas interest in neurological diseases is just beginning to develop. There is a growing patent and medicinal chemistry literature developing around

inhibitors that will serve as useful tools to explore these areas [111]. Omeros Corporation has disclosed in a patent application that in the MPTP mouse model of Parkinson's disease, PDE7 inhibitors restore stride length to prelesioned level when administered alone and also potentiate the activity of L-DOPA [112]. Thus, these apparently potent, brain-penetrant PDE7 inhibitors can serve as much needed tools to investigate the role of PDE7 in brain.

The PDE8 family is encoded by two genes, PDE8A and PDE8B, located on chromosomes 15 and 5, respectively [113]. In vitro, the catalytic properties for both PDE8A and 8B isoforms have been assessed and demonstrate very high affinity (40–60 nM) and specificity for cAMP. Both of the PDE8 mRNAs code for putative N-terminal regulatory elements within their protein structure, although their exact function is still unknown. Each of the putative regulatory domains found in PDE8 (the "REC" domain and "PAS" domain) are unique to this particular PDE family and share homology with highly conserved regulatory domains found in bacteria and mammalian several proteins. In lower organisms, the REC domain has been characterized as a sequence responsible for receiving signals from a particular sensor protein. As yet, it is unclear whether the REC domain in PDE8 plays a similar role in mammals. The PAS domain has been identified in several proteins involved with regulation of circadian rhythms as well as to be a potential site for ligand binding that may influence protein interactions. Alternative splicing of PDE8A results in several isoforms that lack the PAS domain. In addition to alternative start sites within the PDE8 promoter, additional variants are produced from modifications of primary transcripts (for instance, PDE8A2 is a splice variant from PDE8A1).

PDE8A mRNA has been localized to several tissues in the periphery, while the expression of PDE8B is highest in brain, thyroid, and testes. In addition, the PDE8B1 variant appears to be expressed only in the brain, while an equivalent level of expression of the PDE8B3 variant has been reported to occur in brain and thyroid.

The understanding of the role of both PDE7 and PDE8 in the CNS has been hampered by the lack of selective pharmacological tools and the lack of neuronal phenotypes in knockout animals. Nonetheless, there is some information to suggest a specific interest in these two enzymes for the treatment of AD. Recently, the levels of PDE7A, PDE7B, PDE8A, and PDE8B were investigated using specific oligonucleotide probes and in situ hybridization to postmortem brain samples from control and AD patients. Both PDE7 isoforms and PDE8B mRNA were found to be widely distributed in human brain, while PDE8A was not detected. In AD brain samples, the level of PDE7A mRNA was positively correlated with disease stage such that PDE7A mRNA levels decreased in the dentate gyrus with advancing disease progression (Braak stage III–VI). The levels of PDE7B mRNA remained unchanged. PDE8B mRNA levels were the highest in the pyramidal cell layer with advanced AD (Braak stage III–VI) and were also positively correlated with increasing age. This suggests a compensatory relationship between age and cAMP signaling that is enhanced with AD progression.

4.5 PDE5A

PDE5 inhibitors, such as sildenafil **3**, Vardenafil **4**, and Tadalafil **5** (Fig. 2), for the treatment of male erectile dysfunction are the first commercially successful "blockbusters" to arise from pharmaceutical development around the PDE superfamily. This success has generated tremendous interest in PDE5 as a therapeutic target for other disorders [114] and has provided the excellent pharmacological tools that so greatly facilitate such investigation. PDE5 is cGMP-specific. Given the extensive literature on the role of NO/cGMP signaling in synaptic plasticity, there has been considerable interest in PDE5 inhibitors to treat cognitive disorders. However, the effect of PDE5A inhibitors in preclinical models of cognition is enigmatic.

PDE5A inhibitors are robustly active in rodent assays of novel object recognition ([115, 116] and unpublished observation). PDE5A inhibition also attenuates spatial learning impairment in the 14-unit T-maze induced by cholinergic blockade, inhibition of nitric oxide synthase, or in aged rats [117]. Rutten et al. have recently reported that the PDE5 inhibitor sildenafil (Viagra) improves object retrieval performance in nonhuman primates [96]. However, sildenafil failed to effect cognitive deficits in humans suffering from schizophrenia [118]. PDE5 inhibitors also robustly facilitate functional recovery of sensorimotor function after stroke in the rat [119–122]. In these studies, PDE5 inhibitors were administered days after the stroke and had no effect on the infarct volume. Thus, it is argued that the effect of the compounds on sensorimotor recovery is through facilitating the ability of the brain to reorganize after damage; that is, through an effect on plasticity. Recently, Puzzo et al. reported effects of PDE5 inhibition that may be directly relevant to AD. This group found dramatic improvements caused by the PDE5 inhibitor sildenafil on hippocampal LTP measured in vitro in slices and on performance in cognitive tasks in a mouse model of AD, the APP/PS1 mice [123]. These effects were accompanied by an upregulation of CREB phosphorylation and a reduction in the levels of Aβ.

The data reviewed above, from various laboratories and in various model systems, indicate a potentially significant beneficial effect of PDE5 inhibition on brain function in general and synaptic plasticity in particular. The enigma stems from the fact that the expression of PDE5A in forebrain neuronal populations relevant to these effects is very limited. In rat forebrain, PDE5A mRNA was found only in isolated, phenotypically unidentified neurons in one report [124]

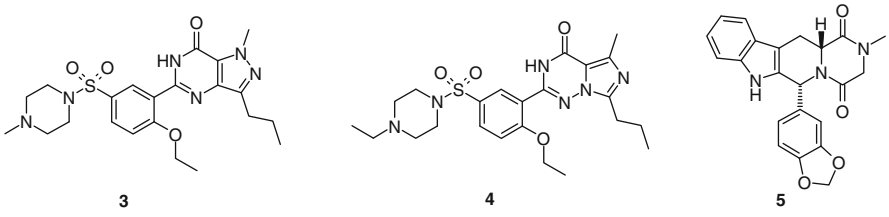


Fig. 2 PDE5A inhibitors approved for clinical uses

and was found not at all in another [125]. In addition, PDE5 protein was not detected [125] or only rarely detected [119] in rat forebrain in studies in which two different antibodies were used. PDE5A mRNA was also not detected in postmortem samples of forebrain from patients suffering from AD [126]. In contrast, PDE5 message and protein are robustly expressed cerebellar Purkinje neurons, some brain stem neurons, and spinal cord [119, 125, 127], as well as the cerebrovasculature [119]. However, it is difficult to reconcile the distribution of the enzyme in these latter neuronal populations with the various effects of the PDE5A inhibitors on brain function that have been reported. Thus, although PDE5A inhibitors are clinically available and are very well tolerated, a better understanding of the mechanisms underlying the effects on brain is warranted to provide meaningful clinical context.

4.6 PDE9A

Of the newly emerging PDE targets, the most interest is being generated around PDE9A and PDE2A, both of which regulate cGMP signaling in the brain. These are reviewed in the final two sections.

PDE9A is a high affinity, cGMP-specific enzyme that is expressed widely throughout the brain, albeit at apparently low levels [124, 126, 128]. PDE9A is the only isoform of this family but exhibits a complex pattern of gene transcripts yielding a total of 20 human splice variants [129, 130]. All splice variants use the same transcriptional start, but generate unique changes in the 5′ region of the mRNA, possibly allowing tissue-specific expression patterns [130, 131]. Functional changes mediated by these variations remain unclear as both the C-terminal catalytic domain and the main part of the N-terminal domain remain unaltered. The primary structure of PDE9A does not contain recognized regulatory domains, such as GAF domains, and the C-terminal homology compared to other PDEs is low, resulting in insensitivity of the enzyme to most known PDE inhibitors [132, 133]. Nonetheless, PDE9A is thought to be key player in regulating cGMP levels as it has the lowest K_m among the PDEs for this nucleotide [132, 133].

Only little is known about the protein expression and localization pattern of PDE9A. Two variants have been examined to date, PDE9A1, which was found in the nucleus, and PDE9A5, which is located in the cytoplasm [131]. A recent immunohistochemical analysis of PDE9A in the trigeminal ganglion confirmed neuronal localization of the protein in the cytoplasm [134]. Significantly more information is available on the expression of PDE9A mRNA, which has been detected in many tissues, reaching peak levels in kidney, brain, spleen, gastrointestinal tissues, and prostate [129, 130, 132]. In the brain, PDE9A mRNA is widely but very moderately expressed [124, 128]. It reaches peak levels in cerebellar Purkinje cells and is furthermore easily detectable in olfactory bulb, hippocampus, and cortical layer V [124]. Here, expression is considered primarily neuronal, but signals have been detected in astrocytes and Schwann cells as well [124, 134]. In human postmortem brain tissue of healthy elderly people and Alzheimer patients,

PDE9A mRNA was detected in cortex, hippocampus, and cerebellum in a pattern comparable to the rodent [126]. No differences in expression were observed in the Alzheimer patients.

Considerable interest in this enzyme was engendered following characterization of BAY 73-6691 (see **10**, Fig. 5, below), the first PDE9A-specific inhibitor [135]. This compound selectively inhibits human PDE9A with an in vitro IC_{50} of 55 nM and a minimum 25-fold window to other PDEs. In a broad pharmacological assessment, BAY 73-6691 enhanced early LTP after weak tetanic stimulation in hippocampal slices prepared from young adult Wistar rats and old, but not young, Fischer 344 X Brown Norway (FBNF1) rats [136]. Significantly, BAY 73-6691 enhanced acquisition, consolidation, and retention of long-term memory in a number of preclinical behavioral paradigms, including a social recognition task, a scopolamine-disrupted passive avoidance task, and a MK-801-induced short-term memory deficit in a T-maze alternation task [136]. Subsequently, it was reported that LTP is enhanced in hippocampal slices prepared from PDE9A knockout mice, and that this effect is mimicked by a PDE9A inhibitor in slices prepared from the rat hippocampus [137]. These latter inhibitors robustly facilitated object recognition memory in both mice and rats and increased baseline cGMP levels in hippocampus, cortex, and striatum [137, 138]. These observations further underline the central role of PDE9A in regulating cGMP levels in the CNS. Taken together, these data suggest that PDE9A inhibition may provide AD patients with some therapeutic benefit. Based on this data, Pfizer Inc. has advanced a PDE9A inhibitor into clinical development for AD. This compound enhanced cGMP levels in the CSF of healthy volunteers, providing proof-of-mechanism to the concept of PDE9A inhibition in humans [139].

In summary, initial doubt around the potential of PDE9A as an effective target for the symptomatic treatment of dementia predicated on the overall modest expression pattern of the gene in the CNS has been superseded by the positive data achieved with selective PDE9A inhibitors outlined above. Two central biological questions still remain to be answered: (1) the nature of a PDE9A sensitive cGMP pool and (2) the subcellular localization pattern of PDE9A in terms of temporal and spatial resolution. Nonetheless, the available data fuel several extensive medicinal chemistry efforts that are summarized below.

Sequence analysis and X-ray crystallographic evidence reveal a number of fundamental differences between PDE9A and other PDEs, but from a chemogenomic perspective the low affinity of PDE9A to IBMX is a clear indicator that PDE9A inhibitors must fulfill other structural requirements than inhibitors of most other PDE isoforms [140]. Full-length PDE9A is inhibited by IBMX with an IC_{50} value of around 230 μM which is significantly lower than for all other PDEs except PDE8 [141]. Nevertheless, a crystal structure of IBMX bound to the PDE9A2 catalytic domain has been obtained by crystallizing the protein with a large excess of IBMX. The X-ray crystal structure reveals a single hydrogen bond between the xanthine N-7 of IBMX and the glutamine 453 of PDE9A, rather than the double hydrogen bond usually observed in complexes of IBMX with other PDEs. A subsequent study of PDE9A crystallized with its natural ligand at low temperature has provided important information about the catalytic mechanism [78].

Although initially elusive, the search for selective PDE9A inhibitors has yielded a number of interesting compounds from various classes. It appears that PDE9A has a very pronounced preference for compounds displaying variations of the purinone scaffold, i.e., flat, aromatic heterobicyclic compounds capable of forming the characteristic double hydrogen bond to the active site glutamine as observed in structures of many other PDE inhibitors such as sildenafil and vardenafil [142]. These structural characteristics are also recognizable in the chemical classes that have resulted from the four major discovery efforts disclosed so far; these chemical classes are discussed below.

Pfizer. Pfizer appears to have been involved in at least two distinct discovery programs centered on PDE9A pharmacology, namely programs in the indications diabetes and cardiovascular disease, as well as neurology. While the peripheral and central indications may have differing requirements of the inhibitor in terms of selectivity profile, pharmacokinetics, and organ distribution, it is interesting to see both programs in comparison.

The starting point for Pfizer's first published PDE9A projects [US20040220186] was compound **6** (Fig. 3), which had been identified by screening a library of PDE inhibitors from previous projects on other PDE isoforms [143]. The compound **6** is a potent inhibitor of PDE9A (IC_{50} = 10 nM) but essentially nonselective with activity on PDE1A-C and PDE5A in the same range and is notably similar to sildenafil. Structural optimization over several iterations led to selective compounds such as **7** (Fig. 3), a 41 nM PDE9 inhibitor with a selectivity factor of 30 or better toward PDE1A-C and PDE5A. The compound **7** is active in vivo in mice (glucose lowering) after oral dosing of 100 mg/kg and above. Other compounds with promising in vitro profiles had no in vivo effect, probably due to poor absorption as a result of relatively high polar surface areas.

Other compounds with carboxylic acid substituents were identified as very potent and reasonably selective, but such compounds almost certainly have very low CNS exposure. Thus, Pfizer's second and currently most advanced PDE9A program aimed at identifying a PDE9A inhibitor for the treatment of cognitive deficits in AD and other neuropsychiatric disorders, and so sought compounds with properties that improve brain penetration. Although relatively little has been disclosed about the in vitro and in vivo profile so far, it is clear that Pfizer's scientists have identified a very potent compound class: numerous compounds in the patent application have IC_{50} values in the single-digit nanomolar region or even below [WO2008139293]. Pfizer has completed Phase I with a PDE9A inhibitor from this

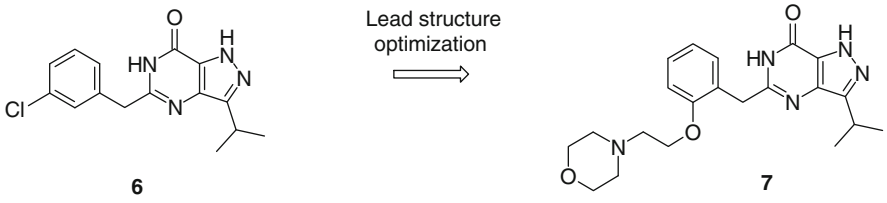


Fig. 3 Lead optimization of PDE9A inhibtors disclosed by Pfizer

series [139], but the structure of the compound has not been disclosed. A couple of examples from the patent application are shown below (**8** and **9**, Fig. 4). Some of the most potent compounds are characterized by a high polar surface, and so the clinical candidate likely to be a compound in which a good in vitro activity and good overall PK and pharmaceutical properties are unified in one molecule.

Bayer. Bayer, the first company to publish detailed pharmacological data for a selective PDE9A inhibitor [135], has also been involved in several compound classes and indications. BAY 736691 (**10**, Fig. 5) [WO2004099211, WO 2004099210, WO 2004018474] belongs to the class of pyrazolopyrimidinones, but several published patent applications describe a second chemical class, the cyanopyrimidinones [WO2004113306, WO2005068436, WO2006125554] exemplified by compound **11** (Fig. 5). Bayer has never disclosed the structure of a clinical candidate from this series, but it appears that there is a high degree of similarity between the SAR in the two series.

ASKA. ASKA Pharmaceutical Co. Ltd of Tokyo has been involved in PDE9A discovery projects for years; the first patent application was filed in 2006 and until now a total of four patent applications on PDE9A inhibitors have been made public [WO2006135080, WO2008018306, WO2008072778, and WO2008072779]. Two distinct compound classes have been disclosed: the first is represented by **12a** and **12b** (Fig. 6) and a class of heterotricyclic compounds (**13**, Fig. 6).

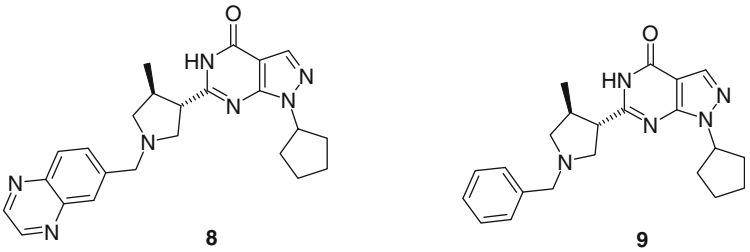


Fig. 4 Examples of PDE9A inhibitors optimized to improve brain penetration

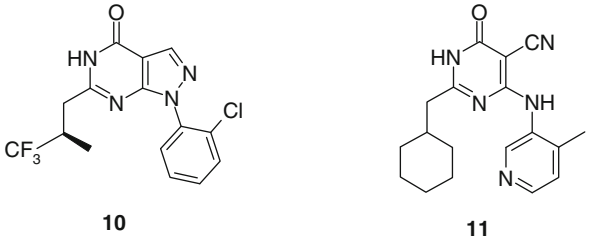


Fig. 5 Pyrazolo- and cyano-pyrimidinone PDE9A inhibitors disclosed by Bayer

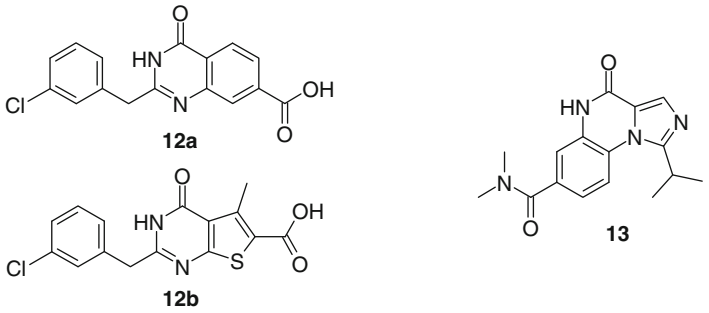


Fig. 6 PDE9A inhibitors disclosed by ASKA. The carboxylic acid group of 12a and 12b is replaced in 13 to improve brain penetration

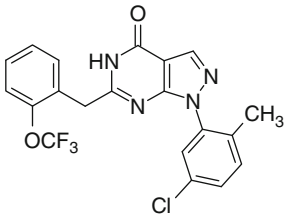
Although various indications have been claimed for both classes (including CNS indications such as AD and general neuropathy), the carboxylic acid makes any high CNS exposure rather unlikely, and it would appear that these compounds are targeted for peripheral indications such as prostate disease, incontinence, or pulmonary hypertension, although no in vivo data have been published to support these claims. The tricyclic systems, on the other hand, seem more promising in that respect, but the general SAR appears to overlap with that of the Bayer and Pfizer programs, so there is reason to believe that the binding mode of this compound is essentially the same (as is the case for the other ASKA compounds). No structural data have been published so far though. It is unclear whether ASKA is still actively involved in PDE9A-related research and development: there is no mention of the project on the company homepage although the most recent patent application was published in 2008.

Boehringer Ingelheim. The most recent player to enter the increasingly competitive field of PDE9A research is Boehringer Ingelheim with a patent application detailing inhibitors of the pyrazolopyrimidinone type [WO2009068617]. Although no detailed biological data have been disclosed, this focused compound class seems to be quite selective vs. PDE1 and generally rather potent on PDE9A (14, Fig. 7, published with IC50 value as a range as shown).

The similarity to BAY736691 (**10**, Fig. 5) is noticeable, and interestingly one of the original Bayer inventors appears on the Boehringer Ingelheim patent application which seems to indicate that the Boehringer Ingelheim program is based on intellectual property acquired from Bayer. One would expect to see more patent applications from this source in the future.

4.7 PDE2A

PDE2A belongs to the dual substrate PDEs hydrolyzing both cAMP and cGMP [144]. PDE2A is a single gene family with three known splice variants (PDE2A1-3) that differ with respect to their N-terminus [145–147]. It is unclear whether all



14
IC_{50} between 10-500 nM
Selectivity vs PDE1: 271-fold

Fig. 7 PDE9A inhibitor disclosed by Boehringer Ingelheim

splice variants are shared across species. PDE2A2 and PDE2A3 are the predominant splice variants expressed in the brain where they are associated with membranes [148]. This localization is partially due to N-terminal palmitoylation [149], but was recently shown for PDE2A3 to be mainly mediated through N-terminal acetylation [148]. PDE2A1 is found in soluble fractions and lacks the most N-terminal region present in PDE2A2/3.

PDE2A is unique in that it is allosterically activated by physiological concentrations of cGMP binding to the N-terminal GAF domains, which triggers the degradation of cAMP [146]. This positive cooperativity constitutes a mechanism of crosstalk between distinct cAMP and cGMP-regulated signaling pathways. It also indicates that, although PDE2A remains silent under baseline conditions, it is selectively activated upon neuronal stimulation that causes an increase in cGMP. In primary cultures of forebrain neurons, PDE2A preferentially metabolized cGMP [150], suggesting that in the CNS this enzyme may also serve as an inhibitory feedback regulator of cGMP signaling. As for all other PDEs, it is of central interest to reveal the subcellular localization of PDE2A to understand the impact of this selective activation on cellular function. Several studies show PDE2A expression in various tissues reaching highest levels in the CNS and particularly the limbic system [145, 151, 152]. A recent immunoreactivity study by Stephenson and colleagues substantiates earlier findings on PDE2A expression in the neuronal dendrites and axons, suggesting compartmentalization of the enzyme directly at the input and output region of neurons. Interestingly, a fine punctuate pattern in neurites is pronounced in areas known to be involved in learning and memory formation and affected in AD pathology, like the hippocampus, striatum, and cortex. Here, neuropil localization is accompanied by a lack of PDE2A immunoreactivity in cell bodies. In further studies, PDE2A was detected in membrane rafts [149] and synaptosomal membranes [148], substantiating evidence for a localization at the immediate site of synaptic contacts and thus in a suitable position to hydrolyze the second messengers cAMP and cGMP immediately at the synapse. Inhibition of PDE2A therefore appears attractive as it might offer a selective prolongation of cAMP and cGMP levels directly related to synaptic activation. In fact, one of the highest levels of PDE2A expression in brain appears to be the mossy

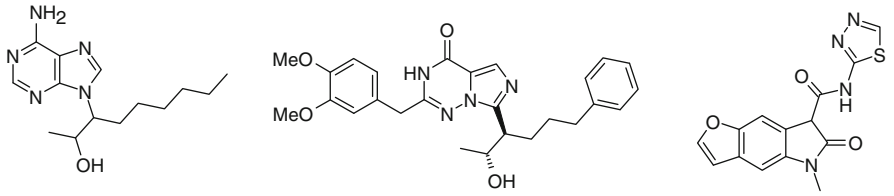


Fig. 8 Prototype PDE2A inhibitors

fibers emanating from the hippocampal dentate granule cells and receiving input from the entorhinal cortex, one of the first brain regions showing morphological signs of pathology in AD [152]. This raises the intriguing possibility that PDE2A is involved in regulating presynaptic forms of synaptic plasticity. Perhaps, PDE2A is one of the mediators of retrograde NO signaling in the presynaptic terminal, either through regulating cGMP directly or by regulating cAMP levels in response to cGMP binding to the GAF domain. Interestingly, in a few brain regions like the medial habenula and neuronal subsets in the cortex, substantia nigra pars compacta or raphe nuclei show somatic staining. This heterogeneous localization pattern within different neuronal populations indicates divergent roles of PDE2A in different cell populations. The CNS expression pattern of PDE2A is preserved in mammals, including humans [126], and remains unaltered in postmortem brain of Alzheimer patients [117].

Recently, a highly potent and selective PDE2A inhibitor, BAY 60-7550 (**16**, Fig. 8), with an IC_{50} for human recombinant PDE2A of 4.7 nM has been shown to enhance LTP at the CA3/CA1 synapse in hippocampal slices [153]. Systemic administration of BAY 60-7550 to rodents has also been shown to attenuate natural forgetting in young rats and improve age-related impairment on old rats in behavioral tasks addressing episodic short- and long-term memory in rats [116, 153, 154]. The compound also reverses working memory deficits in mice induced by a time decay or acute treatment with the NMDA receptor antagonist MK-801 [153]. The various temporal stages of memory consolidation, reaching from working to short-term and long-term memory, have been suggested to be differentially regulated by cAMP and cGMP in either pre- or postsynaptic terminals [89]. It was therefore speculated that interference with a dual substrate PDE that is localized both at the pre- and postsynaptic site should have a broad impact on different temporal stages of memory processing. The promnemonic effects achieved with BAY 60-7550 are in line with this hypothesis [89]. It should be noted that BAY 60-7550 penetrates into the CNS very poorly (authors' personal observations); thus, generalization regarding the effect of PDE2A inhibition on cognitive function awaits confirmatory studies with other compounds. Moreover, a PDE2A constitutive knockout mouse line are not available for behavioral studies as genetic deletion of PDE2A is reported to be embryonically lethal.

Based on the CNS expression pattern, positive cooperative kinetics between cAMP and cGMP, and synaptic association of PDE2A, the enzyme is believed to be a very attractive target to support signaling pathways involved in synaptic plasticity and learning and memory. However, to date a clear link from PDE2A to AD is missing. It should also be noted that PDE2A is widely expressed in peripheral tissues as well, including heart, liver, lung, and kidney, where PDE2A inhibitors have various functional effects [155]. With the identification of more brain-penetrating PDE2A inhibitors, it will therefore be important to identify pharmacological windows between centrally mediated effects on cognition and those in the other organs. Toward this end, PDE2 inhibitors have been pursued by a number of research groups for various indications. So far, discovery of potent and selective PDE2 inhibitors with good CNS exposure has proven to be a real challenge; progress is reviewed below.

The main tool compounds available for mechanistic research at present are EHNA **15** (sub-micromolar inhibitor of PDE2A, the first selective inhibitor of PDE2A described in the literature [156]), BAY 607550 **16** (depending on the construct a nanomolar to sub-nanomolar inhibitor of PDE2A, structurally related to EHNA [153]), and the chemically distinct oxindole **17** (double-digit nanomolar inhibitor of PDE2A with good selectivity [105]) as shown in Fig. 8.

All three compounds are unlikely to advance beyond the tool compound level; EHNA is not potent enough to qualify as a development candidate, whereas BAY 607550 has rather poor pharmacokinetic properties and the oxindole has negligible CNS penetration. Still, all three have been immensely useful as mechanistic probes of the PDE2A enzyme and for studying non-CNS pharmacology models.

Bayer. Bayer has been pursuing the structural class around BAY 607550 as documented by various patent applications for CNS and cardiovascular indications [WO2008043461, WO02068423, WO00250078, WO00209713, WO00012504, and WO09840384] although apparently without identifying a clinical candidate.

Pfizer. Pfizer has pursued the oxindole class as well as a class of azaquinazolines [WO2005061497], but again the current development stage remains unclear if this project is uncertain.

Altana Pharma. Altana Pharma (now a part of Nycomed) has been addressing PDE2A through two distinct chemical classes: a BAY 607550-like class **18** [WO2005021037, WO-2004089953] using the EHNA-scaffold and another class of triazolophthalazines **19** [WO2006024640, WO2006072612, WO2006072615] (Fig. 9). No data have been disclosed for individual compounds, but some are reported to inhibit PDE2A in the low nanomolar region. It appears that COPD and inflammation have been the relevant indications for these compounds rather than CNS indications, although the general physicochemical profile might also be compatible with CNS exposure. It is unclear whether Nycomed is actively developing either of these classes of PDE2A inhibitors.

Cell Pathways. The PDE2A inhibitor research program at Cell Pathways has been largely based on substituted indenes (compound **20**) of the type shown in Fig. 10 [EP01749824, US06465494, WO02067936]. Information about pharmacological properties are scarce (the best example reported in the patent literature is a

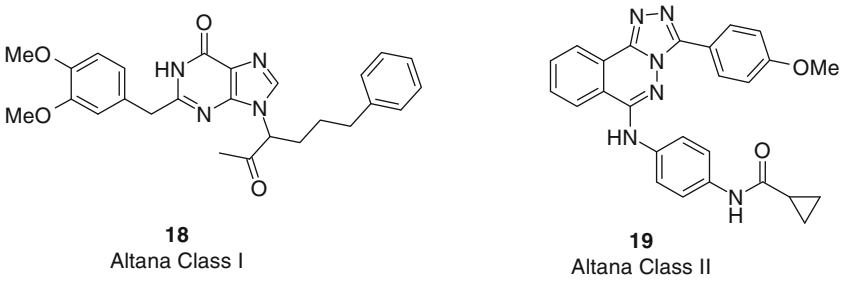


Fig. 9 Representatives of two classes of PDE2A inhibitors disclosed by Altana (Nycomed)

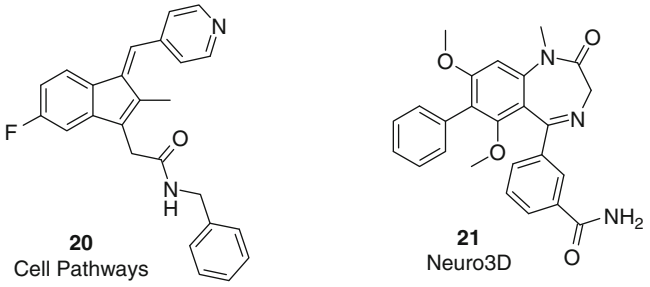


Fig. 10 PDE2A inhibitors disclosed by Cell Pathways and Neuro3D

0.68 μM PDE2A inhibitor), but it seems clear that these compounds are meant for non-CNS indications such as inflammatory bowel disease.

Neuro3D. Finally, Neuro3D (now acquired by Evotec AG) have been involved in PDE2A research with a class of benzodiazepinones **21** (Fig. 10) [EP01548011, WO2004041258] that are reported to be selective although not especially potent inhibitors of PDE2A.

5 Perspective

The suggestion that PDE inhibitors should be explored as a novel approach for the treatment of AD is based on several premises such as follows: (1) AD is principally a disease of synaptic dysfunction, and targeting this dysfunction is a means to impact both the symptoms and the progression of the disease; (2) the cyclic nucleotide signaling cascades offer a molecular entry point for such therapeutic approaches, given the significant roles played in the regulation of synaptic function; and (3) manipulation of PDE activity is a physiologically relevant and pharmaceutically facile way to manipulate cyclic nucleotide signaling. Indeed, these premises form the basis for the development and ongoing clinical trials with both PDE4 and PDE9A inhibitors for the treatment of AD.

While we await important feedback from these clinical trials, there are several points that bear further consideration and investigation. The most important of these is the nature of the synaptic defect that underlies the propensity of an individual to develop AD and that serves as a target for a PDE inhibitor therapy. The "ante" for many therapeutic approaches that target cognition/synaptic dysfunction has been potentiation of LTP at the CA3/CA1 synapse in the hippocampus. This is clearly the case for the PDE inhibitors. There is a wealth of data suggesting that this particular form of synaptic plasticity mediates the long-term memory function in the hippocampus. Given that a deficit in hippocampal-mediated memory function is a hallmark of AD, mechanisms that facilitate hippocampal LTP are certainly reasonable targets to consider for treating those memory deficits. However, even this simple premise must be qualified, given the observation with the PDE4D knockout mice, where increases in hippocampal LTP in slice preparations are associated with deficits in cognitive behavioral tasks. Furthermore, PDE2A, PDE4, PDE5A, and PDE9A inhibitors have all been shown to facilitate hippocampal LTP. As stated above, cAMP and cGMP signaling is intimately involved in the regulation of synaptic function along the entire spatial and temporal continuum of plasticity. Given that PDE function is highly compartmentalized, it is a near certainty that each step in this continuum involves a distinct PDE isozyme. Thus, PDE2A, PDE4, PDE5A, and PDE9A inhibitors may all potentiate hippocampal LTP, but which specifically targets a signaling defect relevant to the synaptic pathology in AD? As a next step to address this issue, it would be very informative to conduct a comparative analysis of the effects of the relevant PDE inhibitors on plasticity at the CA3/CA1 synapse to establish the role and position of each of the cognate enzymes in the complex cascade of events mediating this type of plasticity. These inhibitors then become tools to investigate these specific steps in relevant disease models to determine whether a particular step represents a therapeutically relevant end point. Such an iterative approach should yield interesting insights into the disease and a better focus on the best new therapeutic opportunities.

Finally, the PDEs may be considered more broadly as potential therapeutic targets to treat a range of neuropsychiatric diseases that have as a fundamental pathology synaptic dysfunction. The most obvious examples of these are schizophrenia and autism. However, synaptic dysfunction may also play a principal, though underappreciated, role in neurodegenerative conditions beyond AD. In Parkinson's disease, the loss of dopamine nerve terminals in the striatum appears to precede loss of dopamine neurons in the substantia nigra. The loss of dopamine terminals is also significantly greater than that of cell bodies as the disease progresses. This suggests that therapeutic strategies that preserve dopamine terminals may have a significant impact on both the symptoms of Parkinson's disease and, perhaps, on disease progression. Similar arguments can be made in the treatment of Huntington's disease, where disruption of corticostriatal synapses may be, at least in part, responsible for the loss of cortical BDNF delivery to the vulnerable striatal medium spiny neurons as well as the retrograde transport of BDNF from striatum back to the cortex. Thus, in these cases, maintenance or facilitation of synaptic function through PDE inhibition goes beyond the scope of "cognition

enhancement" toward promoting more fundamental brain functions. This is clearly an area for further investigations.

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