Picking the best isoform

Appendix

Impact Paragraph

Scientific impact

An increasing prevalence of Alzheimer's disease (AD) has greatly stimulated the urge to increase the amount of more and better treatments. However, the progression to the clinic of pharmacological treatments that treat memory problems associated with AD has stagnated since the development of acetylcholinesterase inhibitors (i.e., rivastigmine, galantamine, and donepezil) and memantine. Although the drug aducanumab was recently approved by the Food and Drug Administration (FDA), yet rejected by the European Medicines Agency (EMA), the severe adverse effects associated with this treatment has caused a lot of controversy. Therefore, the efficacy and safety of aducanumab remain to be better defined. In general, the efficacy and safety of current AD medication can be regarded as suboptimal as therapeutic actions are often short lasting, patient-specific and/or concurrent with adverse effects like headaches, nausea, vomiting, and diarrhea. Thus, there is an urgent need for new pharmacological treatment strategies to combat AD-associated memory problems.

As outlined in this thesis, insights into the molecular biology of PDE4 as pharmacological target opens up avenues to improve PDE4-targeting therapies for the treatment of Alzheimer's disease (AD). While the potential of PDE4 inhibition to stimulate memory consolidation has been studied extensively, adverse side effects associated with PDE4 inhibition required this treatment strategy to become more specific. Since the PDE4 enzyme family comprises several subtypes and isoforms (**Chapter 2**), target specification can be performed in order to make PDE4-targeting therapeutic more efficacious and/or safer. From a scientific perspective, the work in this thesis has provided the insight that long PDE4D isoforms in particular are involved in the regulation of neuronal plasticity, also in presence of AD-associated pathology. This insight has implications for the design and development of PDE4 inhibitors, which can eventually translate into pharmacological treatments for memory problems in AD patients. Since long isoforms can adopt distinct conformational states compared to (super)short isoforms and PDE4D expresses

subtype-unique amino acids, PDE4 inhibitors can be developed that exploit these features to obtain long PDE4D isoform binding specificity. Since potential PDE4 inhibitors are often solely screened for their affinity of binding the PDE4 catalytic domain rather than affinity towards specific PDE4 subtype and/or isoforms (**Chapter 2**), many small molecules may have been disregarded as lowly potent inhibitors whilst they may actually be able to selectively bind certain subtype or isoform specific conformations. Thus, since targeting specific subtypes or isoforms may be therapeutically more efficacious or safer, potential subtype-selectively or conformation-selectivity of (disregarded) PDE4 inhibitors may have to be reconsidered.

Accordingly, based on a molecular docking screening approach, the ability of PDE4 inhibitors (n=3013) to bind different PDE4 subtypes and conformations was modeled. In **Figure 1**, the affinity of these inhibitors towards the PDE4D catalytic domain and PDE4D catalytic domain with its C-terminus capped is shown, indicating that certain inhibitors bind with higher affinity to C-terminus capped conformations. Thus, based on the insights from this thesis that certain isoforms provide a more promising therapeutic target than others, PDE4 inhibitors can be selected or designed to selectively bind the isoform of interest. A preliminary selection of inhibitors could be based on *in silico* screenings, as exemplified here, to provide multiple small molecules that can be tested and validated as subtype-specific or isoform-specific PDE4 inhibitor to treat cognitive problems in AD.

In addition to a more precise targeting of PDE4 subtypes and isoforms, this thesis has provided a proof-of-principle set of experiments that highlight the therapeutic potential of the synergistic actions of PDE4 and PDE2 inhibition on memory consolidation processes (**Chapter 6**). Further characterization of the efficacy and safety of this treatment strategy in preclinical disease models and subsequent clinical studies may eventually lead to a clinically safe pharmacological intervention that can alleviate memory problems in patients suffering from AD.

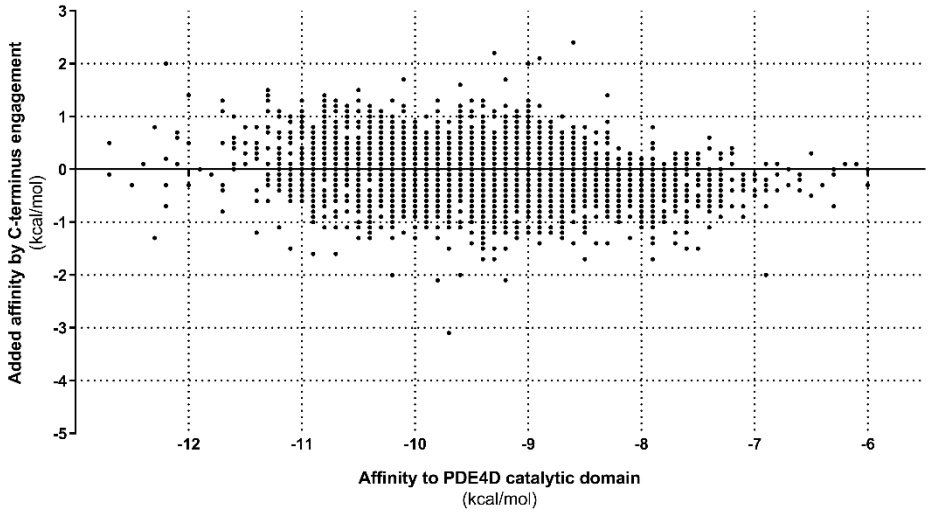

Figure 1. Binding affinities of PDE4 inhibitors (n=3013) for PDE4D catalytic domain and PDE4D catalytic domain with the C-terminus capped based on molecular docking *in silico* screening using crystal structures of different PDE4D conformations available on RCSB Protein Data Bank.

Beyond AD, the principles of subtype/isoform specific PDE4 inhibition and synergistic PDE4 and PDE2 inhibition as therapeutic strategies has also implications for the research into PDE4 as a therapeutic target in other disorders. Since PDE4-mediated cAMP signaling occurs in any cell type, PDE4 enzymes provide interesting targets in a myriad of diseases. Since PDE4 inhibitors, depending on administration route and dose, can be associated with severe adverse effects, target specification towards PDE4 subtypes and/or isoforms is key to optimize efficacy and safety. Thus, similar approaches as taken to optimize PDE4 inhibition to treat AD as delineated in this thesis, could, and perhaps should, be applied to other diseases in which PDE4 inhibition has shown promise. In **Table 1**, several illustrative disorders are listed for which PDE4-targeting treatment strategies could be optimized by means of target specification.

Table 1. Illustrative list of diseases that may benefit from PDE4 target specification.

Type of condition	Disease	Reference
Neurological	Parkinson's disease	(Chen et al., 2022; Dong et al., 2021)
	Multiple Sclerosis	(Schepers et al., 2019)
	(Ischemic) stroke	(Cai et al., 2022; Kaur et al., 2022)
	Fragile X syndrome	(Berry-Kravis et al., 2021)
	Neuropathic pain	(Zhang et al., 2022)
Dermatological	Psoriasis	(Aljefri et al., 2022)
Gastro-intestinal	Inflammatory Bowel Syndrome	(Liu et al., 2022)
Pulmonary	Idiopathic Pulmonary Fibrosis	(Herrmann et al., 2022)
Hepatic	Non-alcoholic fatty liver disease	(Tao et al., 2022; Yu et al., 2021)
Metabolic	Obesity	(Irelan et al., 2022)
Addictive	Methamphetamine reinforcement	(Honeywell et al., 2022)
Cancer	Breast cancer	(Mukherjee et al., 2022)
	Prostate cancer	(Powers et al., 2015; Xie et al., 2021)

Considering the potential of improving therapeutic strategies for these disorders by targeting specific PDE4 isoforms as outlined in this thesis, identifying the PDE4 isoforms involved in each particular disease is crucial. Determining the most promising PDE4 subtype and/or isoform could be determined by testing subtype-specific and isoform-specific PDE4 inhibitors, but also through measuring PDE4 subtype and isoform expression in disease states, insight can be acquired about which subtypes/isoforms could provide the best therapeutic target. The classification and comparison of the different PDE4 subtypes and isoforms has brought the insight that each of the PDE4 genes displays a characteristic exon architecture (**Chapter 2**). Each PDE4 isoform can be distinguished by sequences coming from an isoform-unique exon. As described in **Chapter 3** and **Chapter 4**, disease-associated mRNA expression of PDE4 isoforms can be measured using qPCR primers that amplify sequences stemming from these isoform-specific exons. Since the isoform-specific exons and the associated sequences are known, this information can be used to extract PDE4 isoform-specific expression patterns from (freely accessible) online data repositories of exon array and RNAseq experiments. As these data are available for

many diseases/experimental designs, also those in which PDE4 inhibition may have therapeutic effects, target specification towards PDE4 subtypes and/or isoforms could be explored be re-analyzing these datasets at the isoform level. Exon array datasets are particularly suitable for this approach as the expression of isoform-specific exons will give a direct indication of the expression of the associated isoform. Given the distinct roles of specific PDE4 isoforms, it is crucial to measure expression differences at the isoform level as general PDE4 expression may 'dilute' and mask isoform-specific expression differences. As a proof of principle, several publicly available exon array datasets were re-analyzed to investigate PDE4 isoform expression differences. As an example, in **Figure 2** the PDE4 isoform-specific expression is shown as measured by means of exon array analysis in post-mortem material of the entorhinal cortex of AD patients and healthy controls (based on GEO accession ID: GSE26972, (Berson et al., 2012)).

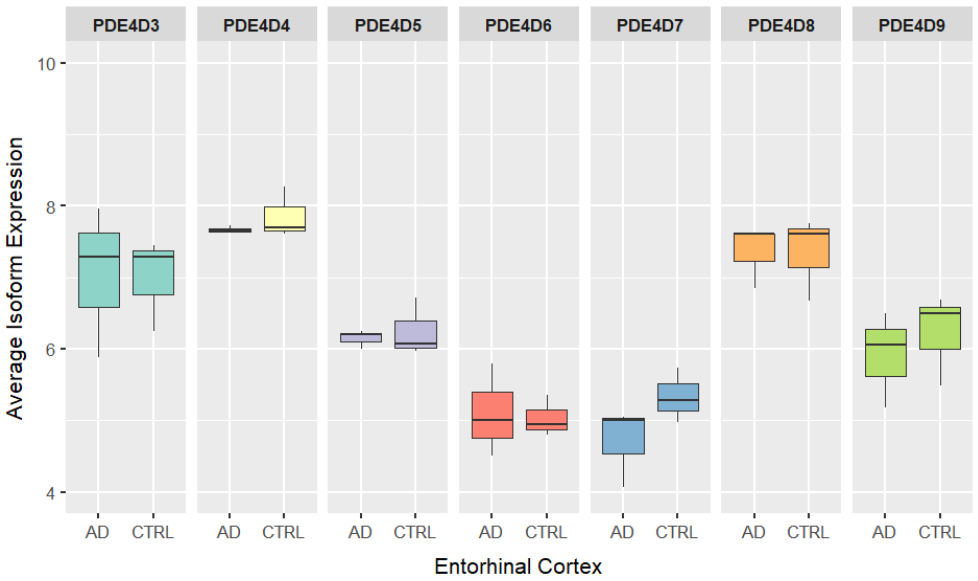

Figure 2. Proof-of-principle indicating that knowledge of PDE4 isoform-specific sequences can be used to determine PDE4 isoform-specific expression by means of re-analyzing publicly available Exon Array datasets. Note that the expression of isoforms PDE4D1 and PDE4D2 cannot be specifically and reliably assessed using the specific exon array probes used in this dataset.

Overall, oriented towards AD, the major scientific implications of the work in this thesis are the validation of new avenues to treat memory problems in AD by means of more specific PDE4 inhibition at the level of PDE4D isoforms or combined treatment with a PDE4 and PDE2 inhibitor. More generally and rather focused on PDE4 as therapeutic targets, this thesis has provided several *in silico*, *in vitro*, and *in vivo* research strategies that can be utilized to optimize PDE4-targeting therapies in a multitude of diseases.

Societal impact

The fundamental insights into the role of specific PDE4D isoforms in the regulation of neuronal plasticity processes will be of use in developing more efficacious and safer therapies to treat memory deficits in AD. Although there is a long trajectory ahead until the treatment strategies explored here are validated in a clinical setting, the societal impact of this thesis will be most evident upon the development of isoform-specific PDE4D inhibitors and/or the validation of synergistic PDE4-PDE2 inhibition. Upon validation, patients suffering from AD-associated memory problems can be effectively treated and by mitigating their symptoms, the quality of life of patients, their family and caretakers will be greatly improved. More generally, society as a whole will benefit from improved PDE4-mediating therapeutics, as AD-associated burden on the healthcare system will be alleviated. Similarly, as highlighted in the previous section, the scientific insights into PDE4's molecular biology can also improve the treatment of other diseases which will benefit many other patient populations and associated families and caretakers.
Despite the fact that it is hard to predict whether the outlined treatment strategies will be validated in a clinical setting, the scientific advancements presented here are sought to provide hope to patients, their families, caretakers and contributors to funding agencies like Alzheimer Nederland, as it shows that new treatment strategies can be created but only await to be proven successful.

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