PDE4 gene inhibition

Summary

To allow proper nervous system functioning, glia cells must structurally and metabolically support electrical signal-conducting neurons. However, neurological disorders such as MS, SCI, stroke, and CMT disease severely impact nervous system functioning, leading to prominent disabilities. However, due to the limited regenerative potential of neurons, combined with the destructive micro-environment upon nervous tissue damage, endogenous repair mechanisms are limited in neurological disorders. Furthermore, despite recent progress being made in the development of new treatment strategies, no effective or curative treatment has been approved, capable of improving the patients quality of life. Therefore, the main aim of this dissertation is to evaluate the therapeutic potential of PDE4 subtype and isoform inhibition as a novel and more targeted approach to treat demyelinating and neurodegenerative disorders, while circumventing typical side effects seen upon on full PDE4 inhibition such as diarrhea, nausea and vomiting.

In the chronic demyelinating disorder MS, immune cell infiltration and subsequently neuroinflammation leads to focal demyelination and loss of myelinating oligodendrocytes. Once the inflammation is resolved, newly formed oligodendrocytes will regenerate myelin membranes, thereby remyelinating the nude axons. At later stages in the disease, remyelination becomes insufficient and less efficient, leading to prominent and persistent demyelination upon disease progression. Current treatment strategies for MS mainly include anti-inflammatory and immunosuppressive drugs. Unfortunately, even though currently available drugs are becoming more effective for treating the initial inflammatory phase of MS, disease progression cannot be halted, nor can repair be induced. As such, there is an urgent need for alternative treatment strategies capable of restoring the remyelination process, thereby inducing repair. Modulating second messenger (cAMP and cGMP) signaling has previously been demonstrated to control both inflammation and CNS repair. Therefore, in chapter 2, an overview is provided regarding the role of PDE inhibition in limiting pathological inflammation and stimulating repair in MS. Subsequently, chapter 3 addresses the therapeutic potential of specifically PDE4B and PDE4D inhibition in different (animal) models of MS. We demonstrated that the full PDE4 inhibitor roflumilast supports neuro-regenerative responses and suppresses neuroinflammation in both the cuprizone and EAE animal model of MS. Importantly, we segregated the **myelination-promoting** role of PDE4 inhibition into a **PDE4D-dependent** process, while selective **PDE4B inhibition** accounted for the **anti-inflammatory effects**. A major drawback in translating PDE4 inhibitors towards clinical applications are the predicted emetic side effects. Importantly, we demonstrated that the subtype specific inhibitors A33 and Gebr32a used in this dissertation, did not showed preclinical signs of emetic-like behavior as determined via patch-clamp and the *in vivo* xylazine/ketamine anesthesia test. Nevertheless, since the predicted emetic side effects are ascribed to be related to *PDE4D* expression in the *area postrema* in the medulla oblongata, we additionally determined the *PDE4D* isoform expression profile specifically in neurons of the human *area postrema*. While short and super-short *PDE4D* isoforms are hardly expressed in the neurons, these isoforms are highly expressed in human OPCs isolated from human MS lesions. These findings render the (super) short PDE4D isoforms an interesting target to safely enhance remyelination.

Since attenuating neuroinflammation and initiating CNS repair are not processes limited and promising for the treatment of only MS, we further explored the therapeutic potential of PDE4 subtype specific inhibitors in other neurodegenerative disorders. Indeed, PDE4 inhibition has already yielded promising results in the context of SCI research due to its broad effects on different injury-related

processes including neuroregeneration and immunomodulation. However, as mentioned above, the translation of full PDE4 inhibitors remains limited due to the dose-limiting emetic side effects, leading to poor tolerability in patients. Therefore, in chapter 4, we demonstrated that especially **PDE4D inhibition** by means of Gebr32a improved **functional recovery** after SCI. Comparable to the full PDE4 inhibitor roflumilast, Gebr32a-mediated PDE4D inhibition led to a reduced SCI lesion size, a reduced demyelinated area, decreased neuronal apoptosis, increased 5-HT serotonergic tract regeneration, and enhanced oligodendrocyte differentiation. Furthermore, using *in vitro* primary mouse neuronal cultures and human iPSC-derived neuronal precursor cell cultures, we demonstrated that the neuroprotective feature of PDE4D subtype inhibition can, at least partially, be attributed to a direct neuronal effect. Finally, using human iPSC-derived neurospheroids, we further demonstrated neuroprotection in a 3D culture model, which was accompanied with increased neuronal differentiation, further supporting the use of the PDE4D inhibitor Gebr32a for the treatment of SCI.

Using a proof-of-concept study, the anti-inflammatory potential of PDE4B inhibition was further elucidated in chapter 5 in an animal model of ischemic stroke. By prophylactically administering the PDE4B inhibitor A33, cerebellar infarct size was significantly reduced 24 hours following experimental dMCAO induced ischemic stroke. The reduced lesion size could be attributed to a decreased neuroinflammation as a reduction in infiltrating neutrophils in the ipsilateral hemisphere, and an increase in $Arg1^{+}$ macrophages throughout the brain was observed. Furthermore, the immunomodulatory properties of PDE4B inhibition were highlighted *in vitro* since human neutrophil activation was significantly reduced upon PDE4B inhibition as demonstrated in a luminol-based ROS assay.

Finally, since demyelinating neurodegenerative disorders are not restricted to the CNS, we further explored the potential of PDE4 subtype inhibition to treat peripheral neuropathies. In the PNS, the myelin-producing glial support is provided by Schwann cells. Besides their myelinating properties, Schwann cells play a crucial role in nerve regeneration following PNS neuropathies as they secrete neurotrophic factors supportive of nerve repair. Interestingly, up to now, the direct effect of pan PDE4 inhibition or PDE4 subtype inhibition on Schwann cells has not been elucidated. In chapter 6, we therefore demonstrated for the first time that PDE4 inhibition, by means of roflumilast, promoted Schwann cell differentiation into a myelinating phenotype in both 2D and 3D culture models. Furthermore, roflumilast-treated Schwann cells promoted axonal outgrowth of human iPSC-derived nociceptive neurons while simultaneously enhancing their myelination capacity, thereby supporting the use of PDE4-inhibitor based treatment strategies for the treatment of peripheral demyelinating neuropathies. Finally, a hereditary peripheral neuropathy animal model for CMT1A disease was used in chapter 7 to evaluate the therapeutic potential of the PDE4D subtype inhibitor Gebr32a to stimulate peripheral remyelination. In line with the myelin regenerative properties observed in CNS pathologies, Gebr32a significantly enhanced sciatic nerve conduction in CMT1A animals, indicating improved myelination. Additional motor functioning phenotyping demonstrated improved motoric coordination, improved sensorimotor functions and increased grip strength upon Gebr32a treatment in CMT1A animals. Finally, post mortem analysis confirmed a remyelination promoting effect of PDE4D inhibition by means of Gebr32a in the sciatic nerve of these animals, indicating the potential of PDE4D inhibition to functionally and molecularly enhance remyelination in the context of CMT1A pathology.

Taken together, the development of new and improved remyelinating enhancing and immunomodulatory therapies may prove beneficial for treating a wide range of neurodegenerative and demyelinating disorders including MS, SCI, stroke, peripheral nerve injury and CMT1A. In this dissertation, we provided an incentive for further developing PDE4 subtype specific inhibitors as a novel and clinically relevant drug-based strategy for treating both CNS and PNS related disorders.