Abstract

The non-selective Na^+ leak channel NALCN allows the flow of Na^+ ions into cells, crucially contributing to their resting membrane potential. It is widely expressed throughout the central nervous system, where it was shown to regulate the excitability of neurons in different brain regions. Dysfunction of this channel leads to distinct forms of rare but severe disease. However, pharmacological measures to manipulate NALCN function are currently very limited. Functional regulation of NALCN by various other proteins has been reported, but the mechanisms underlying these regulations are poorly understood.

We explored the functional regulation of NALCN by co-expressing the NALCN channel complex (NALCN, UNC79, UNC80, FAM155A) with different suggested interacting proteins in *Xenopus laevis* oocytes and evaluating NALCN function using two-electrode voltage-clamp. We found that activation of co-expressed GABA-B receptor, a G protein-coupled receptor, lead to a small reduction in NALCN currents. We next co-expressed NALCN with different G proteins, the main signaling pathway of the GABA-B receptor. While co-expression of $G\beta_1\gamma_2$ had no effect on NALCN, co-expression of $G\alpha_i$ or $G\alpha_o$ strongly reduced NALCN currents. By contrast, NALCN currents were increased by co-expression of $G\alpha_s$, the physiological counterpart of $G\alpha_{i/o}$. Co-expression of $G\alpha_s$ and $G\alpha_o$ also altered the voltage-sensitivity of NALCN.
Pharmacological manipulation of the adenylyl cyclase, the common downstream target of both $G\alpha_s$ and $G\alpha_{i/o}$, did not affect NALCN function, suggesting that it is not involved in the regulation of NALCN by G proteins. Using chimeric constructs in which intracellular regions of NALCN were exchanged to $rNa_v1.4$, we found that the C-terminus of NALCN was necessary to be affected by $G\alpha_s$, but not $G\alpha_o$.

Overall, we provide new insight into the mechanisms underlying the functional regulation of NALCN by G protein-coupled receptors and G proteins. This not only improves our understanding of NALCN physiology, but potentially also opens new avenues to pharmacologically manipulate its function.

As part of a separate project, we also investigated post-translational modifications of the voltage-gated Na^+ channel $Na_v1.5$. This ion channel is the main Na_v isoform in the heart, where its opening allows the fast inflow of Na^+ ions to initiate the cardiac action potential, and dysfunction of $Na_v1.5$

is closely linked to different forms of cardiac arrhythmia. Post-translational modifications, such as phosphorylation, can regulate $Na_v1.5$ function in different ways, but these effects are often not well understood, as the incorporation of post-translational modifications into live cellular model systems remains challenging.

To overcome this problem, we used a semi-synthesis approach based on split inteins, naturally occurring splice-active protein sequences. Using this technique, we assembled functional $Na_v1.5$ protein in live *Xenopus laevis* oocytes including a small synthetic peptide corresponding to parts of the intracellular DI-DII-linker. Different versions of this synthetic peptide contained the phosphorylation sites at serine 571 and threonine 594 as either alanine / valine (non-modifiable) or phosphonoserine / -threonine (stable phosphomimic). This allowed us to stably define the state of these phosphorylation sites in the full-length protein and to characterize their effects on $Na_v1.5$ function using two-electrode voltage-clamp.

While the functional characterization of the targeted phosphorylation sites is still in early stages, we demonstrated successful implementation of the split intein-based semi-synthesis approach to assemble functional $Na_v1.5$ protein with stable modifications in its DI-DII-linker.

In summary, this thesis provides new insights into the regulation of NALCN by G proteins and $Na_v1.5$ by post-translational modifications, expanding our knowledge on these very different but physiologically crucial Na^+ channels.