Cellular models of cardiac channelopathies

Summary

A delicate balance between ion channels and ion-channel associated proteins in cardiomyocytes ensures the generation of action potentials and their propagation as electrical impulses throughout the heart. Mutations in the genes encoding for ion-channel subunits may lead to an impaired action-potential shape and/or duration and potentially to life-threatening arrhythmias. To study basic mechanisms of genetic or drug-induced arrhythmias cellular models have been increasingly used. This doctoral thesis focuses on the study of arrhythmogenic mechanisms using cellular models of cardiac channelopathies, complementing clinical investigations and *in-silico* approaches.

Chapter 1 provides a general overview of the electrical functions of the heart and describes the main characteristics of the action potential in human cardiomyocytes, with a focus on the physiology and pathophysiology of cardiac ion channels, especially the voltage-gated Na^+ channel ($Na_v1.5$). A summary of cellular models used to investigate channelopathies is offered, with an account of their establishment over time.

For the studies of **Chapter 2,** CHO cells were used to investigate an *SCN5A*-founder mutation that co-expresses with a common *SCN5A* variant in a Dutch-German familial cohort. In this so-called "Worm population", divergent ECG phenotypes and a relatively high incidence of cardiac conduction disease and ventricular tachyarrhythmias are found. By combining *in-vitro* and *in-silico* models, the arrhythmogenic consequences of named *SCN5A* variants, in the context of α-β1 subunit interactions of the Na^+ channel, are then described.

As the interplay between Ca^{2+} and Na^+ handling in human cardiomyocytes plays a crucial role in arrhythmogenesis, in **Chapter 3** we investigated the influence of intracellular Ca^{2+} on the function of $Na_v1.5$, comparing two heterologous expression systems, CHO and HEK293 cells. Intrinsic differences owing to the background expression of endogenous proteins in these two cell systems are discussed.

In **Chapter 4,** a characterization of the ionic current and Ca^{2+}-handling components of ventricular-like human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) is presented. Upon I_{Kr} block, beat-to-beat variability of repolarization successfully predicted a proarrhythmic risk better than repolarization prolongation *per se*, both in single hiPSC-CMs and in 2D-monolayers.

General-discussion **Chapter 5** describes how the careful choice of different cellular models, as practiced in this thesis, contributes to an improved understanding of mechanisms underlying cardiac arrhythmias. Furthermore,

the impact of advanced *in-vitro* models on the reduction/refinement of *in-vivo* studies, as well as on the development of mechanism-based and personalized therapies are discussed.

The results of this thesis indicate that the choice and integration of cellular and *in-silico* models are important for a comprehensive investigation of the mechanisms of cardiac channelopathies. Improving quality, reliability and reproducibility of the data obtained by such integrated approaches will enhance the knowledge of arrhythmia mechanisms, will offer more personalized and safer therapies, and a reduction/refinement of the use of animal experiments.