Abstract

HCN channels (*hyperpolarization-activated and cyclic nucleotide-gated channels*) play a crucial role for the generation and regulation of the heartbeat. HCN channels are regulated by cyclic nucleotides and by phosphorylation. The Ca^{2+}-calmodulin-dependent kinase IIδ (CamKIIδ) is mandatory for the excitation-contraction coupling in the heart. In my PhD thesis, I was able to show that the isolated C-terminus of the murine HCN2 channel (mHCN2 CNBD) consisting of the CNBD (*cyclic nucleotide-binding domain*) and the C-linker can be phosphorylated *in vitro* by the CamKIIδ. The affinity for cyclic nucleotides, however, seems not to be affected by the phosphorylation: The exchange of the phosphorylated serine against an aspartate (S641D) to mimic the phosphorylated state does not change the affinity for the cAMP-analog 8-NBD-cAMP. In the full-length mHCN2 channel phosphorylation at S641 was not detected. Electrophysiological studies showed that neither the voltage of half-maximal activation nor the activation kinetics of mHCN2 channel is affected by co-expression with the constitutively active $CamKII\delta_2$. Taken together, these results suggest that the properties of the mHCN2 channel are not modulated by the CamKIIδ.

Although a wealth of information is available on the structure and function of HCN channels, the molecular mechanisms that relay ligand binding to channel opening are poorly understood, because both events are intimately coupled. In my PhD thesis, I could show that the mHCN2 CNBD binds both cAMP and cGMP with high affinity. The K_D-value for both ligands is in the nanomolar range. For the modulation by cyclic nucleotides the formation of a tetrameric gating ring seems to play a crucial role. I was able to show that in the absence of cyclic nucleotides the mHCN2 CNBD is primarily monomeric; the dimeric fraction is small. Tetramers could not be detected under these conditions. In the presence of cyclic nucleotides tetramers are formed. Using the stopped-flow technique, I studied the *on*- and *off*-rates of 8-NBD-cAMP binding to the mHCN2 CNBD. Binding and competition experiments show a biphasic time course. The oligomeric state strongly affects the *off*-rate: 8-NBD-cAMP binding to the tetramer lasts approximately 100-fold longer than to the monomer.