Abstract

Hyperpolarization-activated and cyclic nucleotide-gated (HCN) ion channels belong to a family of ion channels with unusual properties. HCN channels are activated upon hyperpolarization of the membrane potential and the channel activation is modulated upon binding of cyclic adenosine monophosphate. The interplay between the voltage-induced activation and ligand binding is extremely complex: on one hand ligand binding changes the voltage dependence of the channel and on the other hand, the voltage-dependent channel activation changes the binding affinity.

I wanted to investigate this interplay in more detail. My aim was to track binding of individual molecules by a fluorescence optical approach. For this I used a fluorescent analog of cyclic adenosine monophosphate (Atto488cAMP) and characterized its binding properties. With plasma membrane sheets and TIRF-microscopy I examined Atto488cAMP binding of heterologously expressed HCN2 channels as well as of a isolated binding sites both macroscopically and at a single-molecule level. The analysis of the single-molecule data was complicated, as there were non-specific binding events, and as the signal-to-noise ratio was only moderate.

I expanded the experimental set-up, in order to perform electrophysiology and TIRF microscopy at living cells in parallel. With this set-up I established macroscopic binding studies. Based on these results, the binding times of individual ligands at HCN2 channels can now be studied at diffenerent membrane potentials.