Abstract

TRPA1 is a thermosensitive ion channel from the family of TRP (transient receptor potential) receptors. In primary sensory neurons, TRPA1 is an important transducer of painful stimuli, where it contributes to detection of noxious, irritant and inflammatory compounds of endogenous and exogenous origin. The major activation mode of TRPA1 is covalent modification of N-terminal cysteines or lysines by electrophilic compounds. The potency of the electrophilic agonists is increased by voltage dependency of the TRPA1 channel, which contributes substantially during membrane depolarization. To date, the role of several cysteine residues in the N-terminus has been demonstrated. However, the functional role of six cysteines in the transmembrane domain is still unknown.

The first part of the thesis focuses on the functional role of the transmembrane cysteines in the activation of human TRPA1 channel. Our results indicate that these sites do not mediate reactive-electrophile-induced activation but four of the six cysteines substantially contribute to voltage-dependent gating of the channel and two participate in calcium-dependent modulation of TRPA1.

In the second part of this thesis we aim to explore the proximity of two specific charged residues, located in the linker between the fourth and the fifth transmembrane domain. By using the double cysteine mutant E854C/K868C, we demonstrate that inter-subunit interactions between adjacent regions stabilize the conformations associated with chemically and voltage-induced gating of the TRPA1 ion channel.