Štěpána Boukalová

Mechanisms of activation and modulation of vanilloid TRP channels

TRPV1 and TRPV3 are thermosensitive ion channels from the vanilloid subfamily of TRP receptors. TRPV1, which is primarily expressed in nociceptive sensory neurons, is an important transducer of painful stimuli and is also involved in the detection of noxious heat. TRPV3 is expressed mainly in the skin where it regulates proliferation and differentiation of keratinocytes. Similarly to voltage-dependent potassium (K_v) channels, TRP receptors are comprised of four subunits, each with six transmembrane segments (S1–S6).

Using mutational approach, we tried to elucidate the role of S1 in TRPV1 functioning. Our results indicate that the extracellular portion of S1 plays a crucial role in TRPV1 gating. TRPV1 channels with a conservative mutation of positively charged residue in this region (R455K substitution) were overactive. However, they were neither activated nor potentiated by low pH; on the contrary, protons stabilized the closed conformation of this mutant channel. Very similar phenotypic properties were found in other TRPV1 mutants with substitution in S4/S5–S5 region and in the pore helix. In K_v channels, extracelular portion of S1 forms a small contact surface with the pore helix, which allows efficient transmission of conformational changes from the voltage sensor domain to the pore's gate (Lee et al., 2009). We hypothesize that analogous interaction between S1 and the pore helix might be valid for TRPV1 channel gating.

The second part of this thesis focuses on the modulation of the activity of TRPV3 receptors endogenously expressed in immortalized human keratinocyte cell line (HaCaT) induced by epidermal growth factor (EGF). In patch-clamp experiments, we were able to detect TRPV3-mediated currents in HaCaT cells in response to application of TRPV3 agonists. Short-time incubation of cells in the presence of EGF led to marked increase in TRPV3 sensitivity, which was prevented by addition of MAPK (mitogen-activated protein kinase) inhibitors to the culture medium. Our results indicate that the sensitizing effect of EGF on the activity of TRPV3 is mediated by stimulation of MAPK pathway.