Ligand-gated ion channels involved in pain pathways: An automated patch clamp study

A. R. Obergrussberger, N. Becker, M. Rapedius, T. A. Goetze, Maria Giustina Rotordam, N. Brinkwirth, I. Rinke-Weiß, S. Stölzle-Feix, C. Haarmann, M. George, A. Brüggemann, N. Fertig

Nanion Technologies, Munich, Germany

Chronic and neuropathic pain is a significant problem affecting millions of people worldwide each year. A variety of different ion channels play significant roles in pain transmission including TRP, P2X, NMDA, Na_V and, more recently, HCN1 and 2. Among ligand-gated ion channels, NMDA and P2X receptors have been implicated in diverse chronic and neuropathic pain pathways. HCN1 and 2, primarily expressed in dorsal root ganglion neurons, have received some attention recently as pain mediators. The Na^+/K^+ inward current which flows due to activation of HCN channels (Ih), appears to have a role in mediating neuropathic pain, supported by gain-of-function mutations or over-expression of HCN in animal models.

We have systematically tested these diverse ion channels using automated patch clamp. Firstly, we have recorded NMDA receptor combinations NR1/NR2A and NR1/NR2B to investigate both positive and negative modulation. P2X receptors, particularly $P2X_3$ homo- or $P2X_{2/3}$ heteromers, are thought to be involved in pain conditions such as allodynia and hyperalgesia. $P2X_{2/3}$ and $P2X_3$ receptors expressed in CHO or 1321N1 cells were activated by ATP and aß-methylene ATP in a concentration-dependent manner and blocked by suramin or A317491. Secondly, we have focused on the newly identified contribution of Ih to neuropathic pain, by measuring HCN2 currents in HEK cells. We were able to record the current on a high throughput patch clamp instrument. HCN2 was blocked by ivabradine and ZD-7288 in a concentration-dependent manner with an IC_{50} of 5.8 µM (n = 88) and 17.3 µM (n = 93), respectively. Finally, we focused on human induced pluripotent stem cell-derived neurons (hiPSC-neurons) as important models to study neurodegenerative diseases such as motor neuron disease, Alzheimer's and Parkinson's disease. We have successfully recorded Na_V currents and ligand-gated currents mediated by $GABA_A$, AMPA and nACha7 receptors from these neurons using the automated patch clamp approach.

Our results demonstrate that pain pathways can be successfully studied on automated patch clamp systems, facilitating the discovery of novel pain therapeutics.