Project for SUR students in 2023

Identification of pH-sensing residues involved in the gating of human ASIC1a

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Introduction: Acid-sensing ion channels (ASICs) are Na^+-permeable channels of the nervous system that are transiently activated by extracellular acidification. The activation of these channels alters the neuronal excitability. ASICs have physiological roles in neuronal plasticity and pain sensation, and are involved in various pathologies such as neurodegenerative diseases and neuronal cell death following ischemic stroke. Functional ASICs are made of three identical or homologous subunits. Each ASIC subunit comprises a large extracellular domain, two transmembrane helices and intracellular N- and C-termini. Acidification leads to protonation of many extracellular residues, which then induces conformational changes that lead to the opening of the channel gate. To better understand the activation mechanism of ASICs, it would be important to identify the protonation sites and to know the conformational changes that transmit the protonation signal from these sites to the channel gate. To identify new pH-sensing residues of ASIC1a, we have used a computational approach which allowed us to identify a list of candidate pH-sensing residues. We have then started to mutate these candidate residues, and to determine their pH dependence by electrophysiology. Preliminary results identify some residues/areas of the channel as being involved in the activation and/or desensitization of ASIC1a.

Aim of the project: The student will investigate pH sensing in one of the areas of interest that were identified in the initial screening. The choice of the residues to investigate is based on the computational and the functional analysis.

Strategy, experimental approaches: The student will generate new mutants by side-directed mutagenesis, will express these mutants in *Xenopus laevis* oocytes, and carry out electrophysiological analysis of these mutants in order determine the contribution of the chosen residues to pH-sensing. Understanding the activation of these channels is critical for developing new pharmacological strategies to treat the pathologies mentioned above.
The techniques used are:

1. Site-directed mutagenesis in human ASIC1a, in vitro transcription for RNA production
2. Culture of Xenopus oocytes and injection of cRNA into *Xenopus* oocytes
3. Electrophysiological experiments on *Xenopus* oocytes using Two-electrode voltage-clamp
4. Data organization, analysis and statistical analysis

Significance: The identification of the proton binding sites would tremendously improve the understanding of the activation and desensitization mechanisms of ASIC1a and may help to develop new pharmacological strategies to treat some pathologies.

References:

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Vullo, S., and Kellenberger, S. (2020). A molecular view of the function and pharmacology of acid-sensing ion channels. Pharmacol Res 154, 104166. 10.1016/j.phrs.2019.02.005.

Link to the group website:
https://wp.unil.ch/kellenberger-lab