Pharmacology of lysosomal TMEM175

Introduction

TMEM175 is a novel, constitutively active ion channel involved in regulating lysosomal pH and autophagy. Mutations in this gene impair normal lysosomal and mitochondrial function, thereby increasing aggregation of insoluble proteins such as phosphorylated α-synuclein, leading to symptoms typical of Parkinson’s Disease (PD). Consequently, TMEM175 demonstrates significant potential as a key player in the treatment of PD. The lack of specific pharmacological tools has hampered further investigation into the exact role of TMEM175 in normal lysosomal function and pathological processes. Here, lysosomes from a TMEM175 stable cell line were measured on instruments employing solid supported membrane-based electrophysiology (SSME), the SURFER® N1 and SURFER® 9SE.

SSM-based electrophysiology – How it works

SSME relies on the adsorption of any membrane, native, cell culture-derived or artificial, to a lipid coated electrode, i.e. the solid supported membrane, and the direct current read-out caused by the capacitive charging of the membranes when the substrate is applied via fast solution exchange. The SSM itself consists of a lipid monolayer on top of a thiolated gold coated sensor chip. One important advantage compared to patch-clamp is the large sensor size of up to 3 mm. This allows the measurement of about 10 transporters at the same time and a >1000-fold amplification of the currents compared with conventional patch-clamp, allowing for the measurement of low-conducting membrane proteins, such as transporters. The fact that also intracellular membranes can be accessed by SSME means that ion channels and transporters localized in these membranes can be characterized using a more native preparation.

Electrophysiological properties of lysosomal TMEM175

In SSM-based electrophysiology a substrate gradient established by a fast solution exchange is the main driving force. The transport of charged substrates or ions into the liposomes or vesicles generates a membrane potential. This potential can be detected via capacitive coupling between the sample and the SSM on the gold layer of the sensor. In short: The change in membrane potential. This potential can be detected via capacitive coupling between the sample and the SSM on the gold layer of the sensor. In short: The change in membrane potential. This potential can be detected via capacitive coupling between the sample and the SSM on the gold layer of the sensor. This potential is the main driving force for any current passed through the SSM.

Conclusions

- Functional and pharmacological investigations of TMEM175 were performed on lysosomes purified from HeLa cells overexpressing TMEM175, kindly provided by SB Drug Discovery.
- SSM-based electrophysiology enables the characterization of cation and H+ fluxes through TMEM175 and efficient compound screening, compatible with an HTS environment.
- This technology was developed for the characterization of intracellular target proteins which are barely accessible via patch-clamp, but also for transporters, that are too slow for detection via conventional electrophysiology.