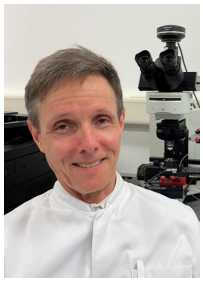
Case study

Targeting TRP channels for the treatment of eye diseases

Tools:
Port-a-Patch

Dr. Stefan Mergler
featured by Nanion Technologies



Stefan Mergler is a researcher and lecturer as well as a group leader at the Charité University Hospital in Berlin, Germany. His research focuses on the role of TRP channels in eye diseases, aiming to develop effective therapies for conditions such as dry eye disease and retinoblastoma.

Eye diseases, ranging from common conditions like cataracts and glaucoma to complex disorders such as macular degeneration, affect millions worldwide, significantly impacting vision and quality of life. These conditions vary in symptoms and severity, with some leading to gradual vision loss and others causing acute blindness.

Ion channels play critical roles in various aspects of eye health and disease. Ion channels in ocular tissues, including the cornea, conjunctiva, lens, retina, and retinal pigment epithelium, are crucial for maintaining corneal transparency, intraocular pressure, and nutrient supply. They also play a pivotal role in the light transduction process in photoreceptors as well as release of pro-inflammatory cytokines.

Dysfunctions in specific ion channels are linked to various eye diseases and therefore, these channels represent potential targets for pharmacological intervention. Cyclic Nucleotide-Gated (CNG) channels have been linked to conditions like retinal degeneration (e.g. retinitis pigmentosa) and achromatopsia in the posterior segment of the eye. L-type Ca^{2+} channels $Ca_V1.4$, crucial for neurotransmitter release, are associated with night blindness. Bestrophin-1 channels, implicated in Best vitelliform macular dystrophy, play a significant role in the retinal pigment epithelium. The CFTR channel, involved in transepithelial transport, along with ClC-2 channels, critical for chloride transport, have been linked to retinal degenerations. In the anterior segment of the eye, a number of temperature-sensitive TRP channels such as TRPV1 and TRPM8 are specifically involved in dry eye disease, which is the most common eye disease in ophthalmology. Notably, TRPV1 inhibitors have also been shown to offer avenues for developing novel treatments for such an ocular condition.

Among a few available methods, the Nobel Prize-awarded patch-clamp technique is most frequently used to investigate the biophysical properties of ion channels. Manual patch-clamping has consistently been the preferred standard for ion channel studies, as it provides a level of detailed data that virtually no other techniques can match.

Nanion's Port-a-Patch platform
is a turn-key miniaturized patch-clamp system enabling the user to rapidly generate high quality data, regardless of experience. Behind its small and compact appearance lies sophisticated technology, producing high-quality measurements with giga-seals and high success rates.

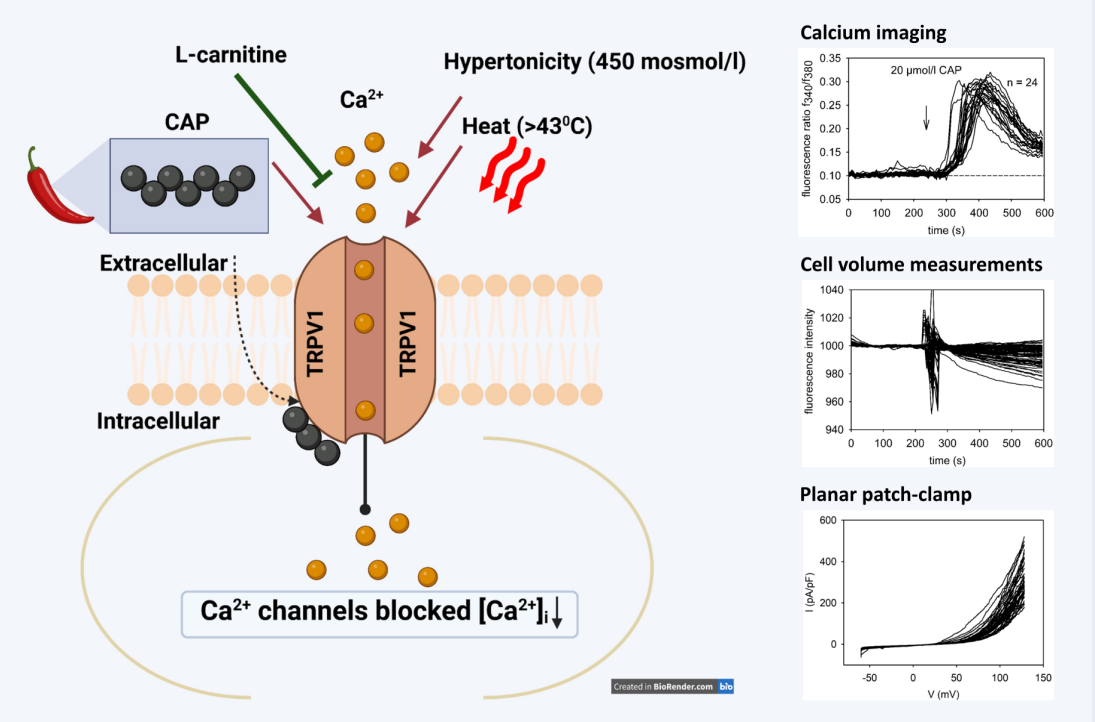
> **"The Port-a-Patch has significantly accelerated our research. Since 2010, we've published more than 20 papers with it. That speaks for itself."**
>
> **Dr. Stefan Mergler,** Charité University Hospital

However, while it's a powerful tool, the conventional patch-clamp technique is often considered to be complicated, time-intensive, laborious, and not conducive to high-throughput experimentation. It necessitates considerable training and technical skills and involves using a complex set of equipment – factors that are regarded as drawbacks or limitations of this technique.

It's encouraging that over the past twenty years, a few companies have made strides in creating an automated version of the patch-clamp technique (APC). APC has addressed many of the challenges inherent in manual patch-clamping. Current APC systems are capable of simultaneously measuring currents from a range of 1 to 384 cells. These advanced systems are user-friendly and often come with automated data analysis features, which considerably reduces the time researchers need to spend on data analysis.

Launched in 2003, the Port-a-Patch instrument has literally revolutionized the field of electrophysiology. This small benchtop semi-automated planar patch-clamp device has enabled scientists to conduct their ion channel research more easily and allows users to rapidly generate high-quality data, regardless of experience. Since its launch, the Port-a-Patch has been widely adopted around the world – over 200 papers have been published using the Port-a-Patch to date. Numerous groups have successfully used the Port-a-Patch to study both voltage- and ligand-gated ion channels in cell lines, primary cells, stem cell-derived cells, and even organelles.

One of these groups, led by Stefan Mergler, has long been studying the role of TRP channels in eye diseases, aiming to develop effective therapies for conditions like dry eye disease, uveal melanoma or retinoblastoma. Since 2008, the Port-a-Patch has been extensively employed in Mergler's lab,



L-carnitine inhibits TRPV1 activation in human corneal epithelial cells. *TRPV1 channel can be selectively activated by capsaicin, hypertonicity, or heat (>43 °C), and blocked by L-carnitine. Three different methods were used to validate the inhibitory effect of L-carnitine, such as single-cell fluorescence calcium imaging, cell volume measurements and planar patch-clamp.*[1]

aiding in the accurate recording of TRP channel activity and complementing techniques like calcium imaging. Dr. Mergler notes that "the Port-a-Patch has significantly accelerated our research. Since 2010, we've published more than 20 papers with it. That speaks for itself."

Over the years, Stefan Mergler's lab used the Port-a-Patch to study thermo-TRPs (mainly TRPV1, TRPV4 and TRPM8 channels) in a variety of cell lines and primary cells, including human corneal endothelium cell line HCEC-12 and epithelial cell line HCE-T, pancreatic neuroendocrine tumor cell line BON-1, human retinoblastoma cell line WERI-Rb1, human conjunctival epithelial cell lines HCjE and IOBA-NHC, human corneal keratocytes HCK, human uveal melanoma cell line 92.1, human pterygial cells hPtEC, and embryonic mouse hypothalamic cell line mHypoE-N41.[1-20]

In their recent study, the group employed the Port-a-Patch to examine the effect of L-carnitine on TRPV1 activation in a human corneal epithelial cell line (HCE-T) in the context of dry eye syndrome, a widespread ocular disorder characterized by a stinging or burning sensation in the eyes.[1] One of the key factors in dry eye is an increase in tear osmolarity, which is known to activate the osmosensitive TRPV1 channel. L-carnitine has previously been demonstrated to be an osmoprotectant against hyperosmotic stress in corneal epithelial cells. The topical use of L-carnitine has been shown to result in rapid and consistent improvements in the symptoms of dry eye patients. By combining Port-a-Patch-based electrophysiology with calcium imaging and cell volume measurements, Dr. Mergler and colleagues have shown that L-carnitine suppresses capsaicin- and hypertonicity-induced TRPV1 activation by blocking cell volume shrinkage. This effect may be of therapeutic value as TRPV1 activation is known to lead to proinflammatory cytokine release and ocular surface inflammation in dry eye. Furthermore, TRPV1 inhibition has been reported to reduce ocular pain as well as tissue fibrosis in dry eye disease.[1]

Previously, the group also showed that L-carnitine, acting as an osmoprotectant, suppressed hypertonicity-induced TRPV1 activation in human conjunctival epithelial cells and human corneal keratocytes (HCK).[2] They demonstrated that L-carnitine inhibited TRPV1 currents in HCK cells, leading to

Dr. Stefan Mergler performs patch-clamp experiments using the Port-a-Patch at Charité University Hospital. *His research focuses on the role of TRP channels in eye diseases, aiming to develop effective therapies for conditions such as dry eye disease and retinoblastoma. Picture by Wiebke Peitz (Charité newspaper).*

"The Port-a-Patch is a very capable system that has been instrumental in our research. Additionally, it is much easier to use than a manual patch-clamp rig, making it an excellent tool not only for research purposes but also for teaching and learning patch-clamp technique."

Dr. Stefan Mergler, Charité University Hospital

reduced keratocyte transdifferentiation into myofibroblasts. These results suggest that blocking TRPV1 in keratocytes with antagonists such as L-carnitine may prove to be a viable approach to suppress corneal opacification in a clinical setting.[2]

Another recent project in Mergler's lab is devoted to the study of retinoblastoma, the most common type of eye cancer in children. There remains a need to develop novel approaches to more effectively treat this disease. Mergler's group proposed that ascorbate may be regarded as a possible supportive agent in anti-cancer therapies and showed that it acts as an oxidant, inducing tumor cell death. The proposed mechanism involves GPCR-mediated increases in intracellular Ca^{2+} influx through TRP channels. The scientists showed that TRP antagonists (CPZ, La^{3+}, NAC) inhibit ascorbate-induced currents in both etoposide-resistant and sensitive retinoblastoma cell lines.[3] Very recently, they described interactions between nerve growth factor (NGF) and cannabinoid receptor 1 (CB1) that modulate control of TRPM8 activation and, in turn, regulate sensitivity for cytostatic drugs such as etoposide in retinoblastoma cells.[21]

Looking ahead, Dr. Mergler plans to continue leveraging the Port-a-Patch in both research and teaching, with exciting projects on the horizon, such as studying pathogens including *Toxoplasma gondii*.

Acknowledging the importance of the planar patch-clamp technique for his research, he's now giving an extra lecture on planar patch-clamp to students in Medical Neuroscience. "The Port-a-Patch is a very capable system that has been instrumental in our research. Additionally, it is much easier to use than a manual patch-clamp rig, making it an excellent tool not only for research purposes but also for teaching and learning patch-clamp technique," says Dr. Mergler.

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