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Application Report

QPatch Compact Opto - Optical modulation of ion channels

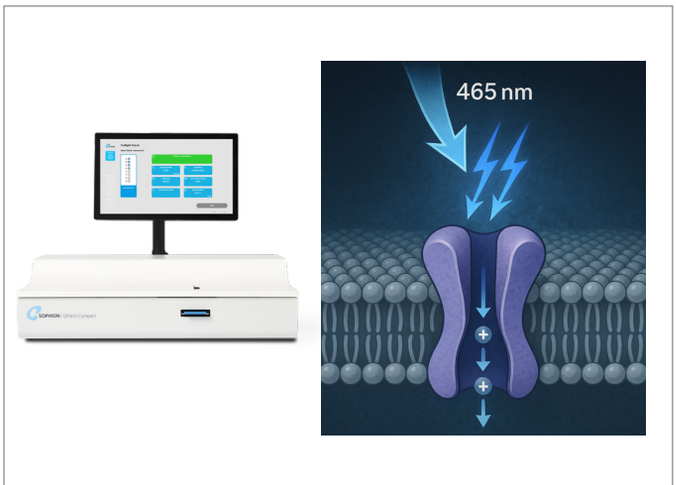
Semi-automated patch clamp combined with optogenetic stimulation of ion channels: directly and through light-activated compounds

Summary

Using the optical features of QPatch Compact Opto, it is possible to evaluate both light-activated ion channels and photoactivated ligands. Here we demonstrate:

Optogenetic experiments:
Channelrhodopsin-2 (ChR2), a blue light-activated ion channel, was expressed and successfully activated using the QPatch Compact Opto system. Sequential increases in light intensity produced corresponding increases in channel-mediated currents. This direct optical control allowed for precise modulation and quantification of ChR2 activity in a semi-automated patch clamp setup.

Photoactivated ligand experiments:
Caged γ-aminobutyric acid (Rubi-GABA) was activated by light, resulting in a $GABA_A$ receptor-mediated current. GABA responses depended on the intensity of light in a dose-dependent manner.



Introduction

Optogenetics combines genetic and optical methods to control ion channels and neuronal activity. Multiple light-activated ion channels, both excitatory and inhibitory, are triggered by various wavelengths. Since the employment of channelrhodopsin (ChR), a photoreceptor type found in green algae, there has been an explosion in the development of new light activated proteins, now including a broad selection of receptors, enzymes, and ion channels (Jiang *et al.*, 2017).

The widely studied ChannelRhodopsin2 (ChR2) is a light-sensitive non-selective cation channel permeable to Na^+, K^+, and Ca^{2+} and, when opened upon illumination, depolarizes the membrane (Volkov *et al.*, 2017). ChR2 and similar channels are important tools in neuroscience for investigating neural mechanisms and developing therapies for neurological disorders.

Photoactivated chemistry uses light to induce chemical reactions, enabling ion channel manipulation and providing insights into their function and therapeutic potential. Combining optogenetics with semi-automated patch clamp techniques enables the pharmacological manipulation of receptors, ion channels, and other proteins with a high degree of temporal control (Zayat *et al.*, 2003). The compound can be a caged compound, which is a large construct cleaved by light, releasing an active ligand, or a photoswitchable ligand, which switches into an active conformation upon light stimulation and reverses conformation to the original state once light is removed.

Methods

ChR2/HEK-293 and $GABA_A(\alpha_5\beta_3\gamma_2)$/HEK-293 cells were cultured according to the supplier's description. ChR2 and $GABA_{AR}$ were stably expressed in the HEK cells. All experiments were carried out at a controlled temperature of 22°C with multi-hole consumables and Sophion standard HEK whole-cell protocol.

Results and discussion

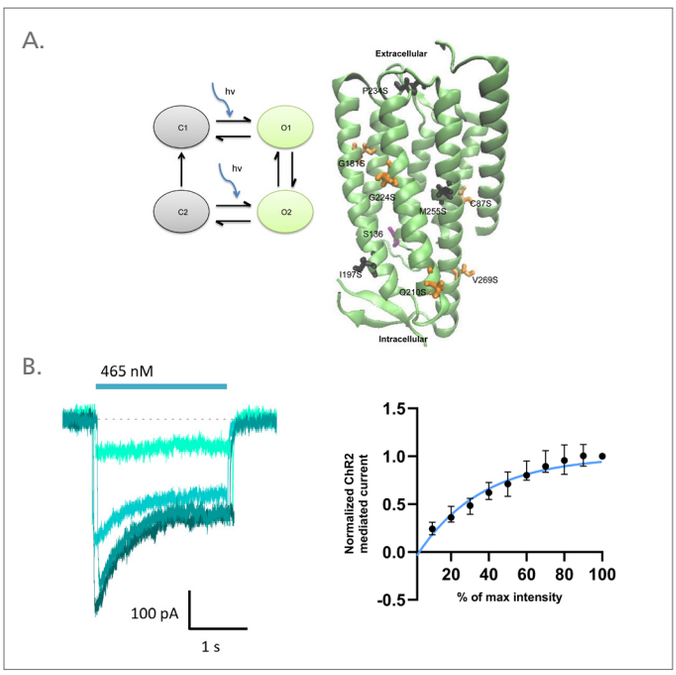


Fig. 1: **Optogenetic stimulation of channelrhodopsin-2**
A) Left: Simple four-state model of the channelrhodopsin-2 (ChR2) photocycle. Blue arrows represent illumination with blue light. From Richards *et al*., 2012. **Right**: Structural model of ChR2 Light intensity-dependent activation. **B) Left:** Representative light-evoked current at LED output intensities of 10,20, 40 and 100% and a holding potential of -90 mV. **Right:** Intensity-response relationship for light from 0 to 100%.

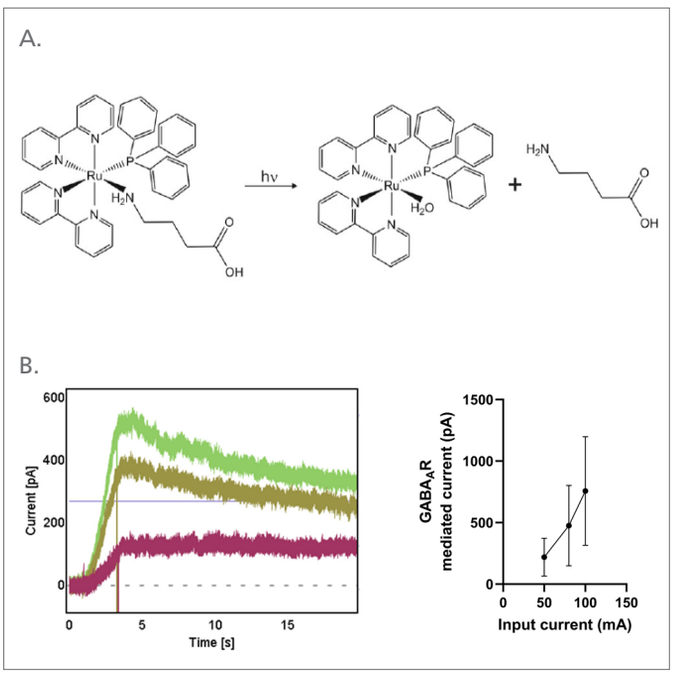


Fig. 2: **Photoactivated chemistry**
A) GABA photorelease from ruthenium-bipyridine-triphenyl-phosphine-GABA (RuBi-GABA). From: Rial Verde et al., 2008 **B) Left:** Optical intensity-response relationship. GABA$_A$ ($\alpha_x\beta_3\gamma_2$) expressing cells were exposed to 100 µM Rubi-GABA and GABA was photoreleased by 3 different intensities of light. **Right:** The duration of the pulses was 1s and the wavelength 465 nm. Intensity-response relationship for Rubi GABA at intensities of 50%, 80%, and 100%, n=6.

Conclusion

In conclusion, the QPatch Compact Opto system effectively combines semi-automated patch clamp techniques with optogenetic stimulation to study ion channels. This innovative approach allows for precise real-time modulation and quantification of light-activated ion channels like Channelrhodopsin-2 and photoactivated ligands such as Rubi-GABA. The results demonstrate the system's capability to provide detailed insights into ion channel behavior and its potential applications in neurological research and drug development. This comprehensive toolkit enhances our understanding of ion channel dynamics and offers promising avenues for future scientific exploration and therapeutic advancements.

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