Optical modulation of ion channels using Qube Opto

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Introduction

Optical modulation of ion channels is traditionally studied using a manual patch clamp system combined with a light source. This approach, however, is limited by very low throughput. In the present work, we show data recorded using a 384-well based automated patch clamp system equipped with 384 integrated light sources (Qube Opto 384).

Optogenetics

In this study we evaluated Channelrhodopsin 2 (ChR2), a light-sensitive non-selective cation channel permeable to Na^+, K^+ and Ca^{2+} opened upon illumination (Berndt et. al., 2012). Furthermore, we employed the chloride-conducting channelrhodopsin (iC^{++}, Govorunova et Al. 2015), which was developed from a non-selective cation conducting channelrhodopsin through a mutational approach. Finally, we used photo-activatable adenylyl cyclase, bPAC, to modulate cellular cAMP levels and thereby alter biophysical properties of the HCN2 channel (Stierl et Al., 2011).

Optopharmacology

Compound activation by light enables the pharmacological manipulation of receptors, ion channels and other proteins with a high degree of temporal control. We used caged GABA (Rubi-GABA, Zayat et. al., 2003) to study the light activation of ligands, in combination with the microfluidic flow channel of the QChip 384, to give a higher degree of experimental control.

Conclusions

With the new optical feature of Qube Opto 384, it was possible to evaluate both light-activated ion channels and photo-activated ligands:

Qube Opto 384 is a Qube with 384 individual, built-in light sources (currently at 475 nm) that can modulate ion channels through optogenetic actuators (e.g. channelrhodopsin) or activate compounds (caged compounds).

Concept System

- Full scale, integrated HTS system
  - Optical stimulation combined with APC – fully integrated
  - 384 format
  - Optogenetics module fully controlled by Qube software
  - Dark cabinet for automated handling of light-sensitive compounds and cells
  - Compatibility with other Qube 384 features such as temperature control
- Overall features of the optical system
  - Addressing 384 sites
  - Individual control of 24 columns
  - Software controlled
    - ◊ Timing, duration and intensity
    - ◊ Ramps and other optical waveforms

Methods

HEK293-HCN2/bPAC (engineered and kindly provided by Axxam S.p.A., Milan), HEK293-ChR2, HEK293- GABAA($\alpha_5\beta_3\gamma_2$) and HEK293- iC++ cells were cultured according to the supplier's description. ChR2, GABAAR and HCN2/bPAC were stably expressed in the HEK cells whereas iC++ was induced 24 h prior to the experiment. All experiments were carried out at ambient temperature using QChip 384X multi-hole consumables and patched using a standard whole cell protocol.

Extracellular solution:
CaCl2 2 mM, MgCl2 1 mM, HEPES 10 mM, KCl 4 mM, NaCl 145 mM, Glucose 10 mM. pH = 7.4 with NaOH, osmolarity = 305 mOsm with sucrose (before adjustment 285 – 295 mOsm)

Intracellular solution for ChR2 experiments:
CsF 140 mM, EGTA/CsOH 1 mM / 5 mM, HEPES 10 mM, NaCl 10 mM. pH = 7.3 with 3 M CsOH, osmolarity = 320 mOsm with sucrose

Intracellular solution for Rubi-GABA and iC++ experiments:
KCl 90 mM, KF 50 mM, MgCl2 1 mM, HEPES 10 mM, EGTA 11 mM, Mg-ATP 4 mM. pH = 7.35 with KOH, osmolarity = 300 mOsm (before adjustment 280 – 290 mOsm)

Extracellular solution for HCN2/bPAC experiments
NaCl 145 mM, CaCl2 2 mM, MgCl2 1 mM, HEPES 10 mM, KCl 4, BaCl2 2 mM/10mM

Intracellular solution for HCN2/bPAC experiments
KCl 70 mM, K2SO4 50 mM, HEPES 10 mM, KOH 25 mM, EGTA 5 mM, HEDTA 5 mM, Na2-ATP 5 mM, MgCl2 3.83 mM, CaCl2 3.38 mM. pH = 7.0 with KOH, Osmolarity = 320 mOsm with sucrose

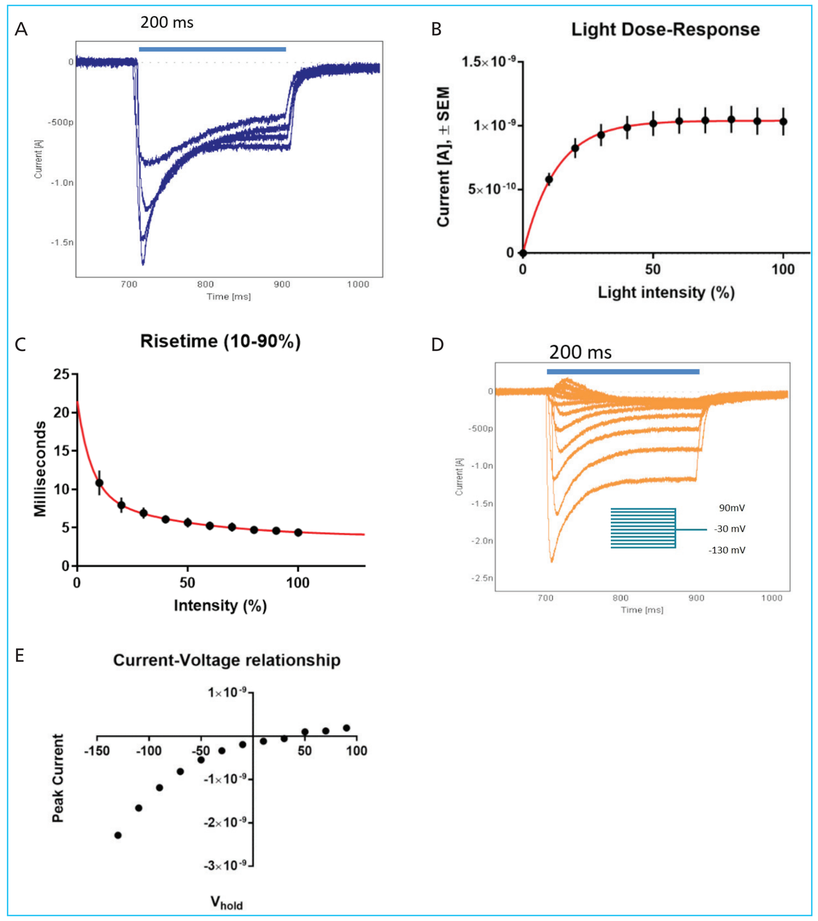

Fig. 1: **The ChR2 - mediated current could be manipulated in both a light and voltage dependent manner.**
A) Typical light-evoked current at a light output intensity of 10%, 20%, 40% and 100% and a holding potential of -90 mV. B) Intensity-response relationship for light from 0 % to 100 % output intensity. C) Fast activation kinetics: Rise time calculated as time from 10-90% of maximum current amplitude. Data is represented as mean ± SD of n=12. D) ChR2: Left: Representative light-evoked current traces E) Current-Voltage relationship. The maximum current amplitude plotted against holding potential.

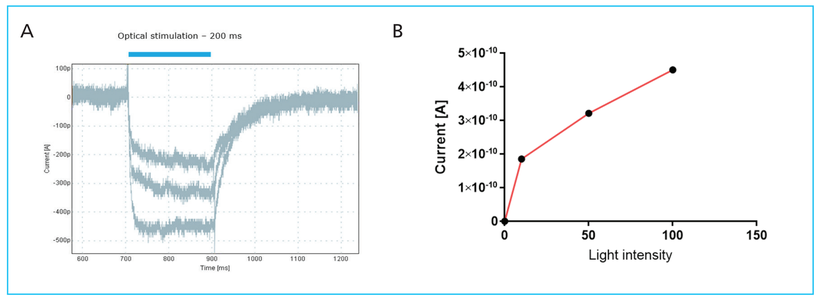

Fig. 2: **Chloride conducting channelrhodopsin (iC++)**
A) iC++ sweep plots when exposed to light output intensities of 10%, 50% and 100%. The holding potential was clamped at -100 mV. B) Dose-response relationship for the peak current of the traces seen left.

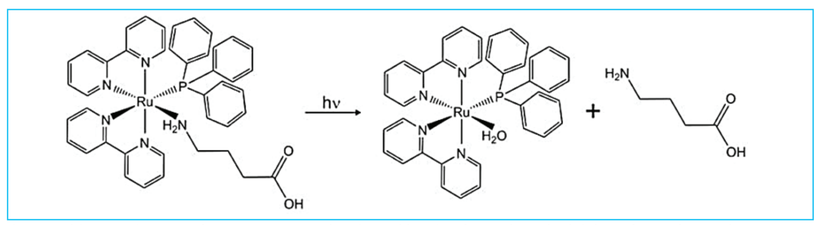

Fig. 3: Rubi-GABA is a caged GABA compound activated by visual wavelengths (Rial Verde et Al., 2008).

References

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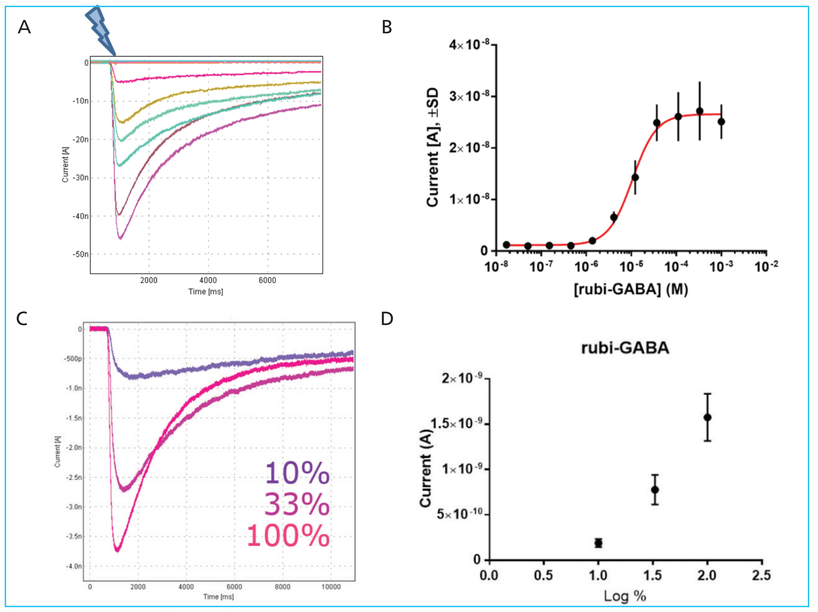

Fig. 4: **Photo-release of caged GABA**
A) Concentration-response relationship: HEK 293 cells expressing $GABA_A$ ($\alpha_5\beta_3\gamma_2$) were exposed to varying concentrations of Rubi-GABA (Tocris), by a 200 ms light pulse (475 nm) at 100% light output. B) Non-cumulative concentration-response curve for Rubi-GABA of a 3-fold dilution series. C) Optical dose-response relationship: $GABA_A$ ($\alpha_5\beta_3\gamma_2$) expressing cells were exposed to 1 mM Rubi-GABA and GABA was photo-released. Light pulse: 200 ms D) Intensity-response relationship for Rubi-GABA at intensities of 10%, 33% and 100% light output. Data is represented as mean ± SD of n=32.

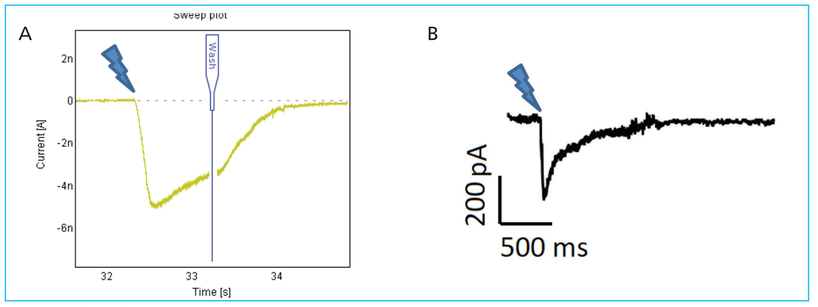

Fig. 5: **Fast ligand exposure**
With the new optical capability of Qube Opto 384 in combination with the microfluidic system of the QChip 384X, the ligand exposure time can be better controlled and drastically reduced. A) RuBi-GABA photoactivation followed by wash-out (200 µM, 100 ms light pulse). B) Uncaging during perfusion: 330 µM Rubi-GABA was washed in and during perfusion, GABA was uncaged by a 20 ms light pulse, resulting in an instantaneous wash-out of uncaged GABA.

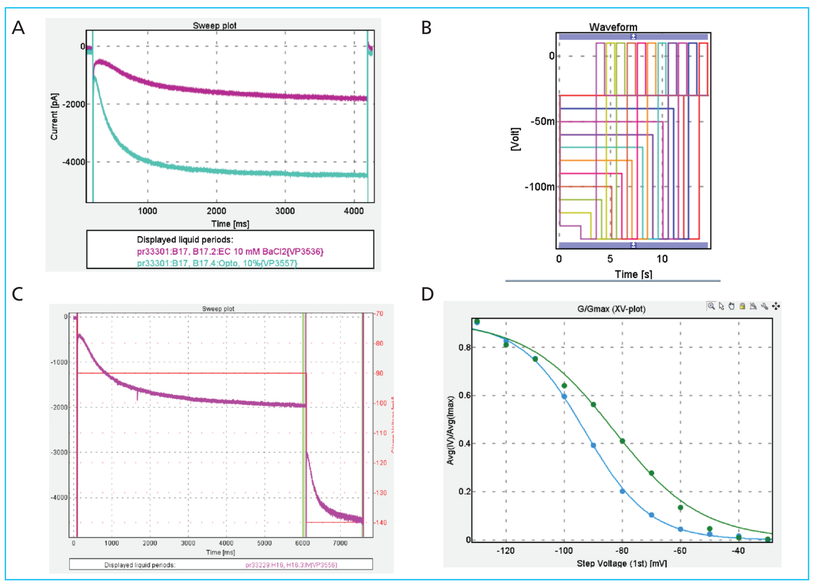

Fig. 6: **Ion channel modulation through secondary messenger**
Through activation of the photoactivatable adenylyl cyclase, bPAC with 500 ms long light pulses at λ = 475 nm with a frequency of 0.5 Hz, it was possible to increase intracellular levels of cAMP and thereby modifying the co-expressed HCN2 channel.
A) The typical current response to a single voltage step from -30 to -90 mV before (purple) and after (green) 5 minutes of optical stimulation. A monoexponential curve was fitted to the data to determine the activation kinetics (not shown). The average tau value shifted from 903 ± 32 ms to 357 ± 14 ms (n=324) following light exposure B) Step voltage protocol to assess activation kinetics with various durations for the individual test voltages.
C) Representative current trace for test voltage -90 mV (voltage step shown in red). Average current was determined in the green shaded curser interval.
D) Representative steady-state activation curve before (red) and after (blue) optical stimulation of the cells. Currents were normalized to values recorded at V = -140 mV (Imax) . A Boltzmann function was fitted to the I/Imax data to estimate $V_{½}$. In average, optical stimulation caused a shift from $V_{½}$ = -90.7 ± 5.8 mV to -81.5 ± 4.9 mV (n=324).