Using Automated Patch Clamp Technology to Assess VGCC Function and Modulation by Cannabinoids

András Horváth[2], Kyle R. Jensen[1], Thea Sandler[2], Mette Christensen[2] and Chris Bladen[3]

[1]Sophion Bioscience, Inc.
213 Burlington Road, Suite 105,
Bedford, MA 01730, USA

[2]Sophion Bioscience A/S,
Industriparken 39,
2750 Ballerup, Denmark
info@sophion.com - sophion.com

[3]Macquarie University
Wallumattagal Campus
Macquarie Park NSW 2109,
Australia

Abstract

There is mounting evidence that cannabinoids can be used to treat harmful neurological conditions like epilepsy and autism. One means by which cannabinoids are thought to exert their therapeutic benefits is through the modulation of ion channels. Voltage-gated calcium channels (VGCCs), for instance, play important roles in neurotransmitter release, synaptic transmission, and neural plasticity and thus represent a major therapeutic target for many neurological diseases. Multiple types of cannabinoids are thought to interact with and inhibit VGCCs, but there are still many questions regarding the cellular and molecular mechanisms of these interactions and the efficacy of cannabinoids (especially rare cannabinoids and synthetically created cannabinoids) in modulating ion channel function. With the goal of determining how rare cannabinoids and synthetic cannabinoids can modulate VGCC function, Sophion Bioscience has partnered with the Bladen Lab to optimize an assay which uses automated patch clamp (APC) electrophysiology to measure T-type VGCC currents in HEK293 cells. We show that Sophion's QPatch Compact semi-automated patch clamp robot can reliably record currents from up to eight cells in parallel. We demonstrate that these currents follow biophysical characteristics as described in the literature and are sensitive to drugs that interfere with VGCC function. By understanding how T-type VGCCs are modulated by rare and synthetic cannabinoids, we hope to aid in the creation and characterization of cannabinoid drugs that could potentially be used to treat conditions like epilepsy.

Summary and results

From this study, we found that CBDVA, CBGA, and CBD each powerfully inhibited T-type VGCCs. Each of these cannabinoids modulated VGCC subtypes differently, and there was strong concordance between both APC platforms (Qube and QPC) when using the same cannabinoid on the same cell line. Moreover, the results from the automated patch clamp experiments align well with the manual patch clamp data making the automated patch clamp a good tool to increase the ease of screening. Finally, these results suggest that cannabinoids could potentially be used as treatments for epilepsy via their strong inhibition of VGCC.

Table 1: Summary of IC_{50} values obtained from different cannabinoids on three different voltage gated calcium channels, $Ca_V3.1$, $Ca_V3.2$ and $Cav_V3.3$. The results were obtained using two different automated patch clamp instruments, QPatch Compact and Qube as well as manual patch clamp and calcium flux assay.

	$Ca_V3.1$ QPC	$Ca_V3.1$ Qube	$Ca_V3.1$ Manual	$Ca_V3.1$ Flex	$Ca_V3.2$ QPC	$Ca_V3.2$ Qube	$Ca_V3.2$ Manual	$Ca_V3.2$ Flex	$Ca_V3.3$ QPC	$Ca_V3.3$ Qube	$Ca_V3.3$ Manual	$Ca_V3.3$ Flex
CBD	5.46 µM	-	3.4 µM		-	4.43 µM			-	-		>10 µM
CBDVA	-	6.16 µM	7.0 µM	2.0 µM	8.84 µM	23.67 µM	10.0 µM	11.0 µM	-	-		>10 µM
CBGA	4.27 µM	4.42 µM	6.4 µM	6.2 µM	2.29 µM	2.13 µM	6.0 µM	6.4 µM	11.36 µM	-	5.7 µM	>10 µM

The QPC and Qube can reliably record T-type VGCC currents

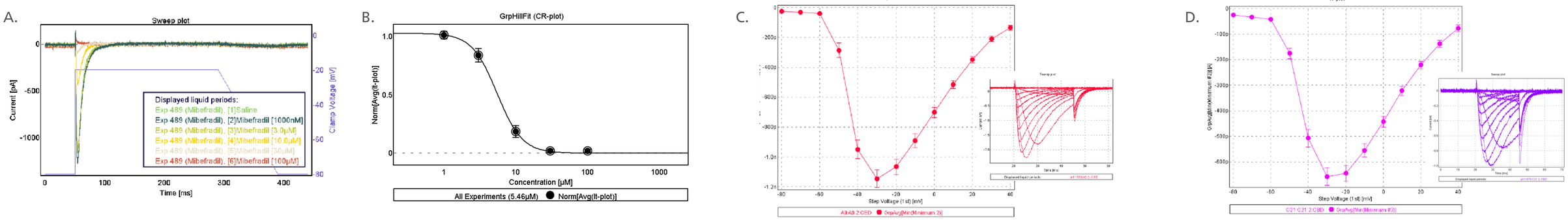

Fig. 1: Whole-cell voltage clamp recordings were made using HEK293 cells expressing either $Ca_V3.1$ or $Ca_V3.2$ channels. Using QPatch Compact a 5-point concentration-response curve was generated using the calcium blocker, mibefradil. (A) illustrates an example trace of the current obtained with increasing concentrations of the blocker. An average concentration-response curve (B) gave an IC_{50} value of 5.46µM, n=6. Apart from QPatch Compact the Qube was also utilized having a success rate of 74 % on average when filtering out cells having a whole cell capacitance of <4pF and a whole cell resistance of <100 MOhm. (C, D) shows IV curves of $Ca_V3.1$ (n=208) and $Ca_V3.2$ (n=173) respectively. The IV curves were obtained by applying 10 mV depolarizing steps.

The rare cannabinoid cannabidivarinic acid (CBDVA) modulates T-type VGCCs

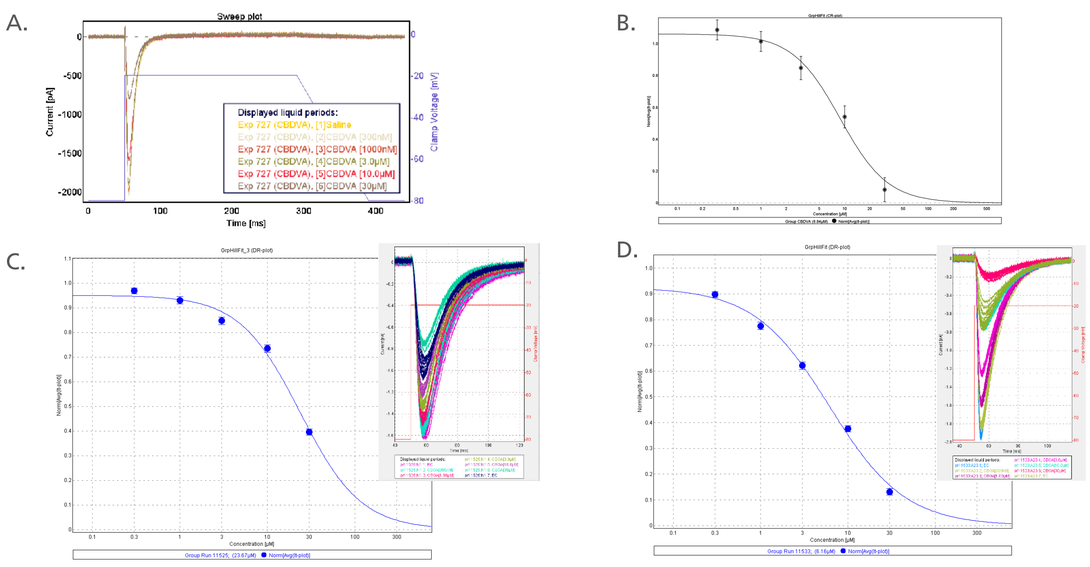

Fig. 2: We found that CBDA potently inhibited VGCC currents in the investigated channels. (A) shows an example trace of the current obtained from HEK293 cells expressing $Ca_V3.2$ with increasing concentrations of CBDVA on QPatch Compact. (B) provides an average IC_{50} value of 8.84 µM (n=10). (C) The same experiments were made on Qube resulting in an IC_{50} value of 23.67 µM (n=131). (D) tests $Ca_V3.1$ on Qube (n=198) giving an IC_{50} value of 6.16 µM.

Cannabidiol (CBD) modulates T-type VGCCs

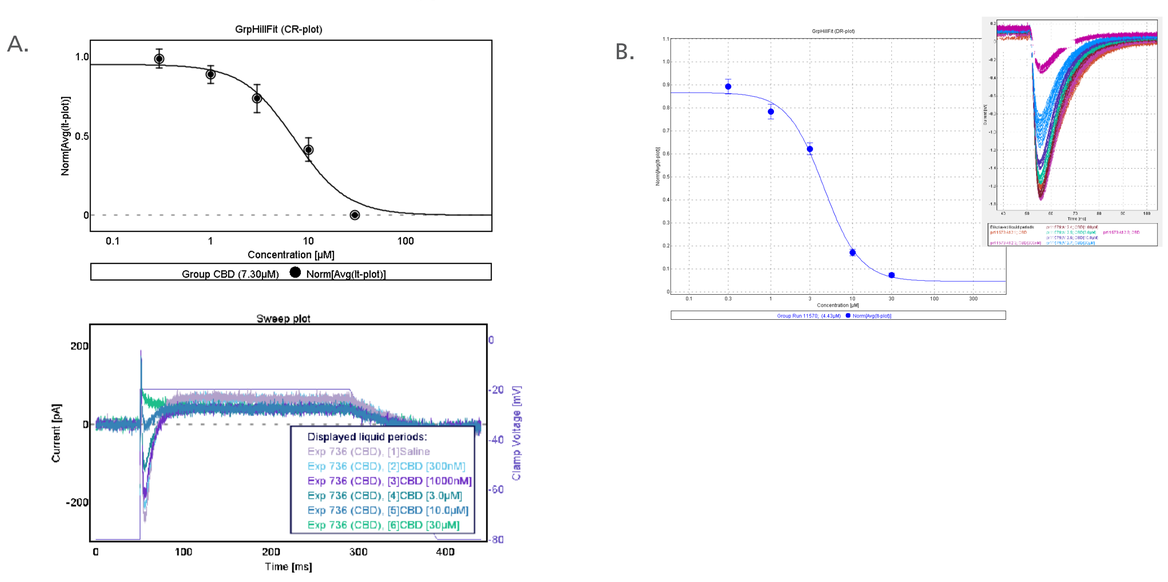

Fig. 3: (A) An example trace together with a 5-point concentration-response curve of CBD inhibition of $Ca_V3.1$ resulting in an IC_{50} value of 7.30µM (n=4) on QPatch Compact. (B) shows the same for $Ca_V3.2$ obtained on Qube, IC_{50} = 4.43µM (n=219).

Cannabigerolic Acid (CBGA) modulates $Ca_V3.3$, $Ca_V3.2$ and $Ca_V3.1$

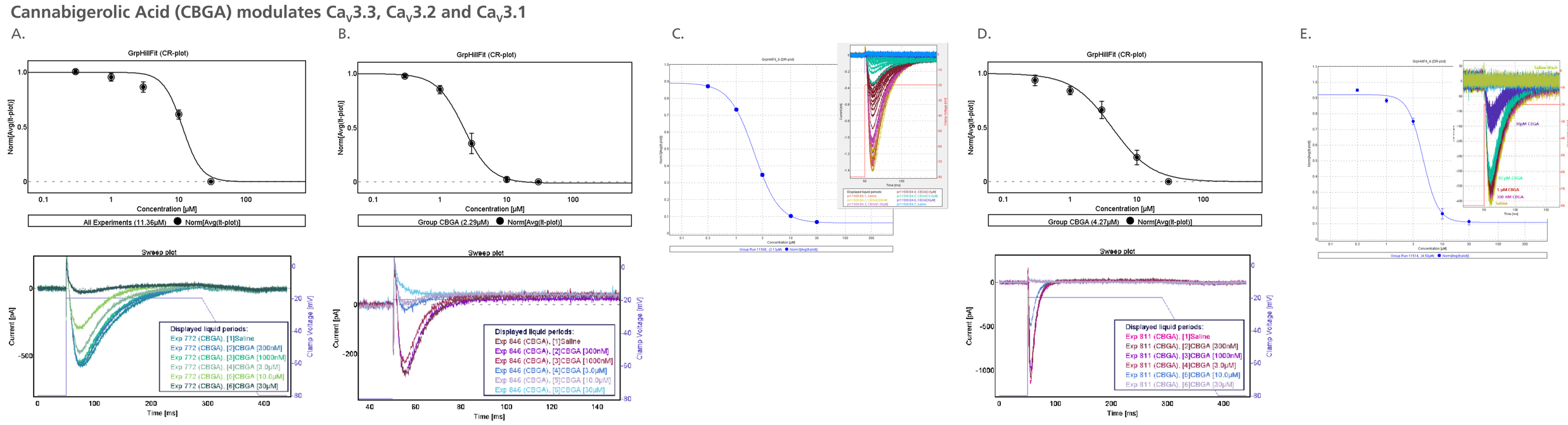

Fig. 4: Cannabigerolic acid (CBGA) was tested with all three T-type voltage-gated calcium channel subtypes to create a 5-point concentration-response curve for each of them. (A) It can be seen that CBGA potently inhibited $Ca_V3.3$ with an IC_{50} of 11.36µM, n=11. This was measured on QPatch Compact. (B,C) show results from $Ca_V3.2$ on QPatch Compact and Qube respectively. QPatch Compact, IC_{50} = 2.29µM, n = 7. Qube, IC_{50} = 2.13µM, n = 161. (D,E) show results from $Ca_V3.1$ on QPatch Compact and Qube respectively. QPatch Compact, IC_{50} = 4.27µM, n = 7 Qube, IC_{50} = 4.42µM, n = 11.

Sophion instruments used for automated patch clamp recordings

QPatch Compact (QPC)

- Gigaseal recordings in up to 8 cells asynchronously in parallel
- Water-based temperature control at each measurement site
- Giga-Ω seal in physiological Ringer's solutions – no need for seal enhancers or fluoride
- Manual liquid additions supported by light and audio guidance
- Fast and complete liquid exchange via microfluidic channels
- On the run changing of protocols
- Glass surfaces to prevent adherence of sticky compounds
- Ready-to-use individual electrode pairs – no need to ever re-chloride electrodes again

Qube 384

- Giga-Ω seal ion channel recordings in 384 cells at once
- A true walk-away screening solution to meet demanding timelines
- Accurate control of temperature, via sensor and feedback loop on the measurement site
- Patented centrifuge for spin down and preparation of cells
- Automatic cell handling
- Individual electrode pairs – no need to ever re-chloride electrodes again

Background

- VGCCs are highly implicated in epilepsy.
- VGCCs are heavily expressed throughout the brain and are involved with many functions.
- T-type VGCCs ($Ca_V3.1$, $Ca_V3.2$, $Ca_V3.3$) regulate neuronal excitability, contribute to neurotransmitter release, and are critically involved in generating intrinsic burst firing of hippocampal pyramidal neurons in TLE.
- Numerous anti-epileptic drugs modulate T-type VGCC function (succinimides, zonisamide, valproate, phenytoin).
- Cannabinoids, like CBD, inhibit VGCCs, and this may contribute to its anti-epileptic properties.

Methods

HEK293 cells expressing $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ were provided by Chris Bladen.

Standard operating procedures for cell culturing and suspension preparation were based on Sophion standards. Single-hole consumables were used in all experiments.

Solutions: Extracellular solution (in mM): 10 $CaCl_2$, 10 HEPES, 4 KCl, 145 NaCl, pH = 7.4 with NaOH, Osmolarity 315 mOsm with sucrose. Intracelluar solution (in mM): 112 CsCl, 34 CsF, 2 NaCl, 10 HEPES, 8.2 EGTA, 1 CsOH, 4 ATP, 0.6 GTP, pH = 7.2 with NaOH, 295 mOsm.

Presented @5th ECCC 2025