Current Clamp Recordings on QPatch:

Action potentials and pharmacological effects measured in HL-1 immortalized atrial cardiomyocytes

SOPHION BIOSCIENCE A/S
Baltorpvej 154
DK - 2750 Ballerup
DENMARK
info@sophion.com
www.sophion.com

Copenhagen University[1]
Blegdamsvej 3
DK - 2200 Copenhagen N
DENMARK

sophion
Part of Biolin Scientific

SØREN FRIIS | HERVØR LYKKE OLSEN | RIKKE SCHRØDER PERRIER | RASMUS BJØRN JACOBSEN | KIRSTINE CALLØ[1] | MORTEN RYTTER SUNESEN

Introduction

Cardiovascular side effect is one of the most important reasons for drugs not reaching the market and for drugs to be withdrawn from the market. Currently, safety testing in small molecule drug discovery is carried out on the hERG channel as an FDA-set standard as this channel has been found to be involved in prolongation of the heart action potentials. However, hERG is only one of the channels involved in the cardiac rhythm and as such important information about the concerted action of all ion channels involved is missing. Current clamp measurements offer this additional important information to the understanding of the often complex pharmacological effects as these measurements are done using cells that more closely resemble the situation in the heart. Up to now, current clamp has been very tedious work generating very few data points per man hour spend.

HL-1 cells are immortalized atrial cardiomyocytes that beat spontaneously in culture. They provide a unique opportunity to validate the current clamp recording mode of the QPatch. In this poster electrophysiological properties and pharmacological profiles of the HL-1 cell line recorded with the current clamp feature of the QPatch are presented. Comparable biophysical characterizations of the HL-1 cells were obtained using manual patch-clamp technique. Furthermore, we demonstrate the ability to induce action potentials using rapid switching from voltage to current clamp during recording.

Materials and methods

HL-1 cells were obtained from Professor Claycomb at the University of Louisiana and grown according to SOP.

The cells were patched in physiological solutions containing (mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose, pH=7,4 (w. NaOH) in the extracellular solution and 5.374 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 120 KCl, 4 Na_2-ATP. pH=7.2 in the intracellular solution. The internal solution also contained 10 µM β-Ecsin, a poreforming agent, in order to use the perforated whole cell configuration.

The patching was done using the QPlate – a microfluidic based patch clamp device that provides high resistance seals and has integrated glass fluid channels for compound delivery.

The patching and whole-cell formation was set up using the QPatch Assay Software – the cells were positioned using a -100 mbar negative pressure pulse and then left with a -20 mbar holding pressure for the remaining of the experiment. No pressure pulses were used for whole cell formation since this is not necessary when using β-Escin.

The voltage- and current clamp protocols were also set up in the QPatch Assay Software. The in-sweep switch from voltage clamping at voltage between -70 and -90 mV to 0 pA current clamping was the most efficient way to induce action potentials with QPatch. Manual patch clamp data was recorded on a standard patch clamp rig using HEKA amplifiers.

Current clamp recordings on cardiomyocytes is an important preclinical safety marker

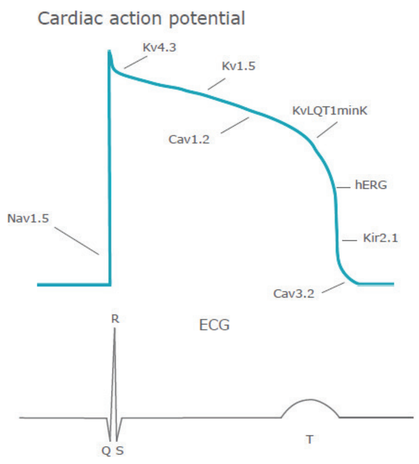


Figure 1. Ion channels are responsible for action potential propagation and cardiac contraction. Drugs affecting one or more of these ion channels can induce long QT that can lead to cardiac arrhythmia and sudden death.

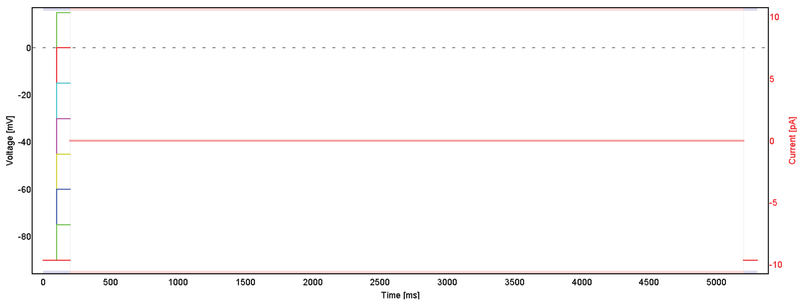


Figure 2. QPatch allows for switching between voltage clamp (blue segment) and current clamp (red segment) within the same sweep. For provoked action potentials a voltage step protocol (- 90 mV to + 15 mV followed by a switch to 0 pA) was applied to provoke the action potential.

Conclusion

This study demonstrates the ability of the QPatch to perform current clamp experiments. We show that the current clamp results on QPatch HT is consistent with manual current clamp data and literature values.

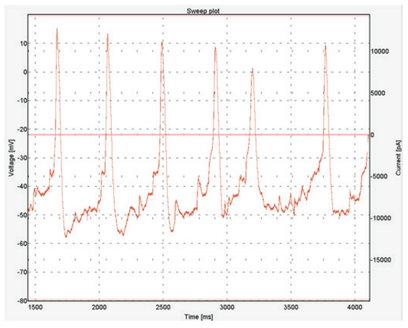
QPatch can seamlessly switch between voltage clamp and current clamp mode hereby facilitating unattended current clamp experiments. In this poster we show current clamp data from the immortalized cell line HL-1 recorded on QPatch.

We also demonstrate the comprehensive analysis methods from QPatch Assay Sofware including APD_{90}, rise time and phase plot analysis in order to make a complete evaluation of the obtained results.

With the results presented here, we clearly demonstrate that the QPatch in current clamp mode is capable of taking current clamp into higher throughput scenarios: it is now possible to reproducibly induce action potentials and study pharmacology - unattended and automated.

Spontaneous actions potentials

QPatch recordings



Manual recordings

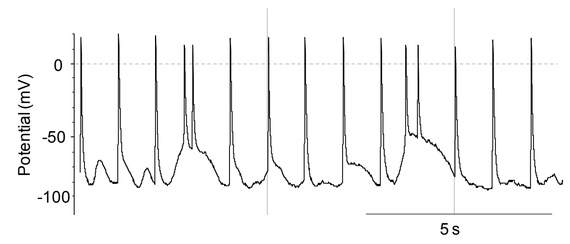


Figure 3. Recordings of spontaneous action potentials in HL-1 cells on QPatch and manual patch clamp.

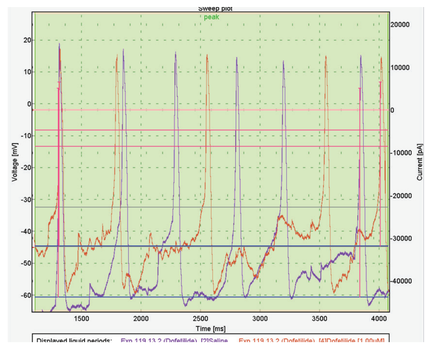
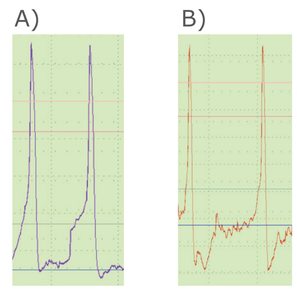


Figure 4. Dofetilide (1 µM) prolongs the peak to peak interval by increasing the duration of the action potentials.



A) Action potentials recorded at a frequency of 2.21 Hz (control).

B) Action potentials recorded at a frequency of 1.74 Hz in the presence of dofetilide.

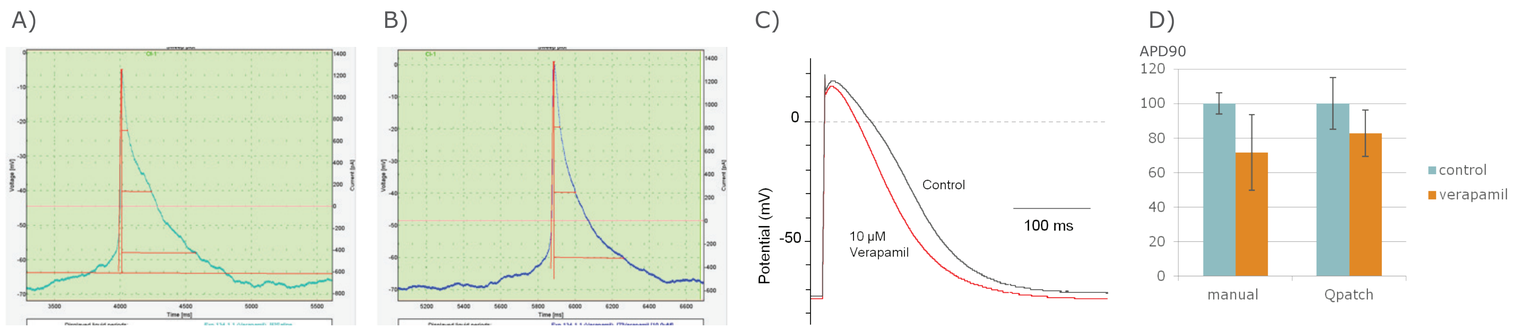
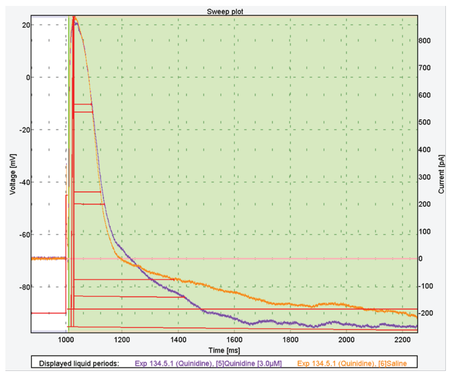


Figure 5. Shortening of APD_{90} with 10 µM Verapamil - an L-type calcium channel blocker. A) Control APD_{90} 387.81±58.25n=5. B) Verapamil APD_{90} 321.48±43.41 n=5. Similar data obtained with manual patch clamp recordings. See figure C and D.

Provoked action potentials

A)



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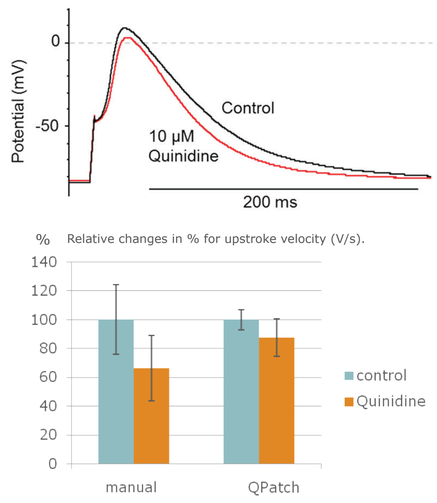


Figure 6a. Slow upstroke velocity with Quinidine (3 µM) - Quinidine blocks Nav1.5 & hERG. Upstroke velocity is slowed, but no prolongation of APD_{90}.

Figure 6b. The manual current-clamp control recordings are consistent with QPatch data - upstroke velocity is slightly reduced in presence Quinidine (10 µM).

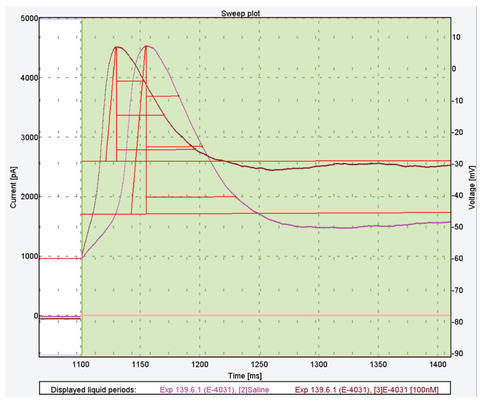
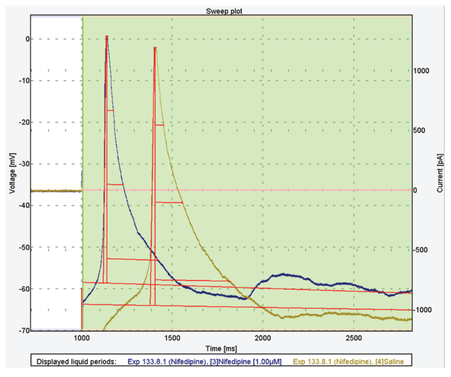


Figure 7. Early afterdepolarisations (EAD) induced by E-4031 (100 nM). Normal repolarisation is not completed.

A)



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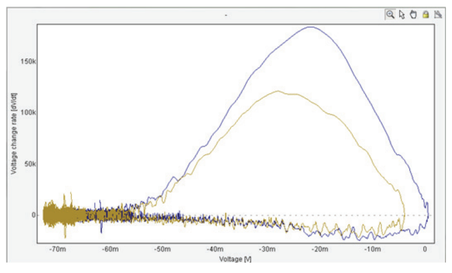

Figure 8. Nifedipine (L-type calcium blocker) increases upstroke velocity and shortens the action potential.

A): Action potential recorded in presence (yellow) and absence (blue) of nifedipine (1 µM).

B): Phase plot; showing speed of voltage change as a function of voltage saline blue, nifedipine yellow.