SOPHION BIOSCIENCE

Application Report

QPatch Compact: Patch clamping at your fingertips

QPatch Compact provides reliable electrophysiological recordings from voltage-gated, ligand-gated, and temperature-sensitive ion channels

Summary

The purpose of this application report is to showcase the versatility of QPatch Compact both for voltage clamp on voltage-gated and ligand-gated ion channels as well as current clamp and other relevant applications of the instrument. This is done by highlighting different examples of assays undertaken in our applications laboratory.

This report is intended to work as a reference collection for anyone interested in knowing more about the capabilities of QPatch Compact. The selection of these user cases is, however, not exhaustive and represents a subset of assay variations that are applicable to QPatch Compact.

With this report, we aim to show a glimpse of the plethora of patch clamp applications that are available using QPatch Compact.

Introduction

QPatch Compact is an 8-channel semi-automated patch clamp system with automated efficiency and manual control. With QPatch Compact, anyone can perform high-quality patch clamp recordings without the challenges of using a manual patch clamp. We claim that any laboratory training personnel can learn to patch clamp in less than an hour. While being easy to use, small, and maintenance-free, it allows the user to make real-time and easy adjustments to experiments while recording in both voltage- and current-clamp.

QPatch Compact uses a unique microfluidics liquid exchange system ensuring precise concentration and complete liquid exchange and is ideal for concentration-response measurements and ligand-gated ion channel recordings. Based on proven QPatch technology, it enables true giga-Ohm seal formation without using seal-enhancing agents.

A water-based temperature control unit ensures constant temperature between 18 and 40°C (64–104°F) independently of varying ambient temperature in the laboratory. The temperature control enables recordings at physiologically relevant temperatures.

In this application report, we showcase some of the applications which have been conducted on QPatch Compact both in voltage-gated and ligand-gated configuration, using single-hole or/and multi-hole consumables. We illustrate that QPatch Compact can be used to successfully record high quality data from several cell lines expressing different ion channels in stably expressed cell lines as well as a glimpse into the potential of recording on human induced pluripotent stem cell (hiPSC) derived cardiomyocytes in the current-clamp configuration.

For any questions regarding this application report, please contact us at info@sophion.com.

Results and discussion

Voltage gated ion channels

$Na_V1.5$

Voltage-gated sodium channels are responsible for the initiation and propagation of action potentials in excitable cells such as cardiomyocytes. Here, we focused on $Na_V1.5$ subtype, a critical channel maintaining the physiological electrical function in cardiomyocytes, as it is the heart's main depolarizing current[1].

We used QPatch Compact to measure the biophysical characteristics of $Na_V1.5$ and determine the pharmacological profiles of two different compounds (tetrodotoxin and tetracaine) dependent upon the conformational state of the ion channel.

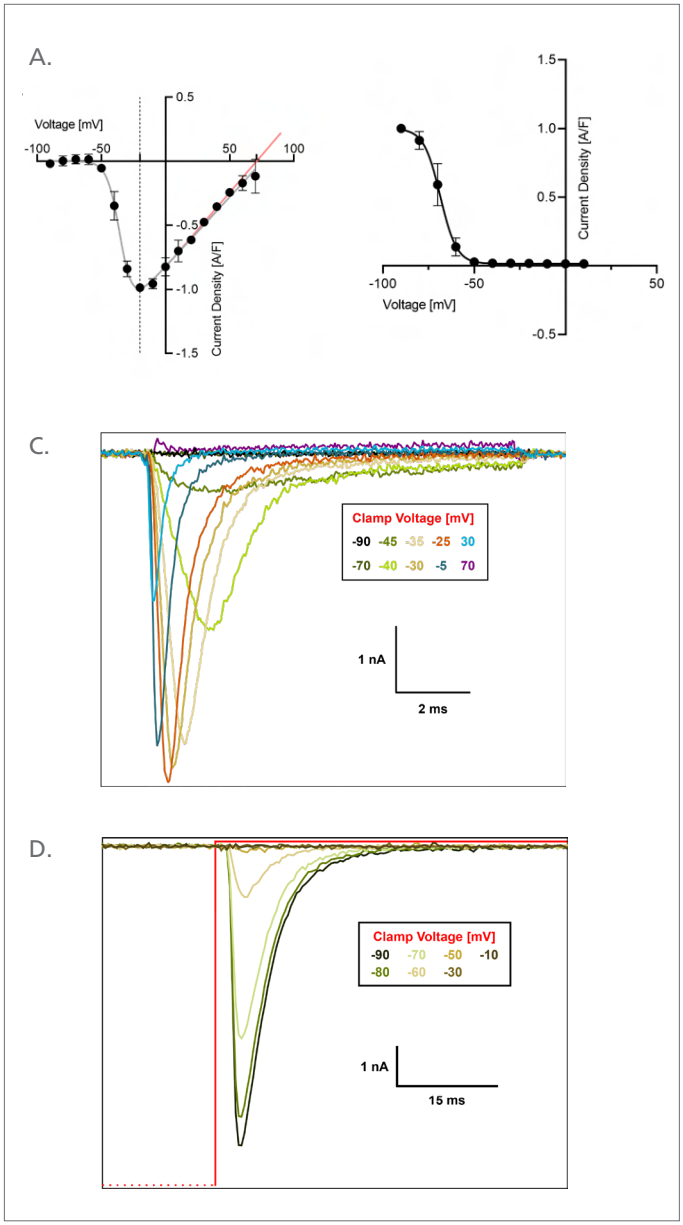

Fig. 1: **Evaluation of $Na_V1.5$ biophysical properties using QPatch Compact.** A) Activation curve representing Na_V current density as a function of voltage (n=6 cells). The dotted line indicates the voltage at the maximum current. The red line represents the linear fit between 5 and 55 mV that provided the reversal potential of the ion channel, V_{rev}=70.9 mV. **B)** Representative traces of $Na_V1.5$ current in response to specific steps of voltage (indicated on graph). **C)** Steady-state inactivation current-voltage relationship curve (n=40 cells). **D)** Representative traces of Na_V currents in response to a voltage step after inactivation ($V_{½}$=-67.4 mV). A Boltzmann function was fitted to the data to estimate $V_{½}$ values. Data are presented as mean ± SD.

Adaptive voltage feature

When experimenting on voltage-gated ion channels, it is imperative that the voltage applied to the cells is accurate. This is especially important when testing state-dependent compounds. For the most accurate compound activity determinations, ideally, the precise value for the $V_{½}$ of inactivation should be used for each cell.

QPatch Compact is equipped with the unique adaptive voltage clamp feature, allowing measurement of Vxx values for individual cells. Using this protocol, QPatch Compact can separately define the voltage applied to each individual well for both the activation and inactivation of the channels. Adaptive voltage clamp has been shown to reduce the data variability compared to traditional standard methods[2].

In the following experiments, we use this feature to evaluate the efficiency and potency of two compounds, tetracaine and tetrodotoxin (TTX), when half of $Na_V1.5$ channels are in an inactivated state.

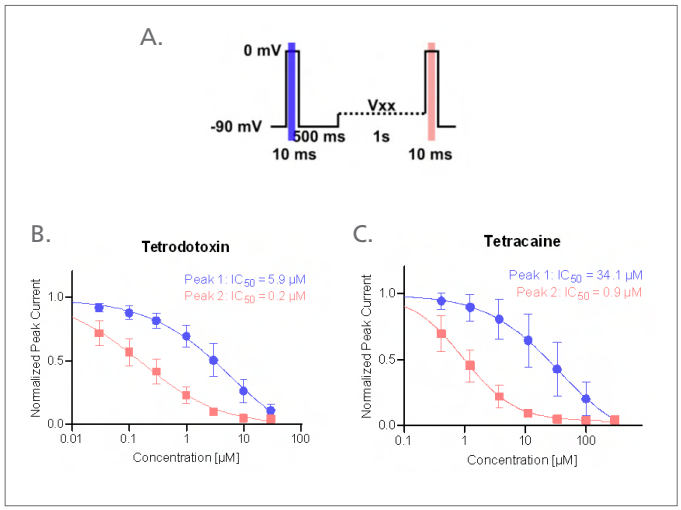

Fig. 2: **Pharmacological characterization of $Na_V1.5$ depending on its conformational state. A)** Voltage protocol used to measure Na_V current during activation (peak 1, blue) and afterwards (peak 2, red**)** applying the adaptive voltage feature (Vxx, dotted line) to inactivate half of the ion channels. **B)** Concentration-response curves representing current inhibition by TTX during activation state (peak 1, blue dots) and inactivation state (peak 2, red boxes). **C)** Concentration-response curves representing current inhibition by tetracaine during activation state (peak 1, blue dots) and inactivation state (peak 2, red boxes). IC_{50} values were determined by a Boltzmann fit using the Sophion Analyzer software. Error bars represent mean ±SD.

hERG

hERG channel, also known as I_{Kr}, is a voltage-gated potassium channel that plays a crucial role in action potential repolarization. hERG can influence action potential duration in cardiac cells and is commonly measured in a cardiac safety environment[3].

In these experiments, we demonstrate that QPatch Compact is a reliable system to evaluate the pharmacology of frequently used compounds in cardiac drug safety assessment by providing IC_{50} values in agreement with the literature and benchmark automated patch clamp systems.

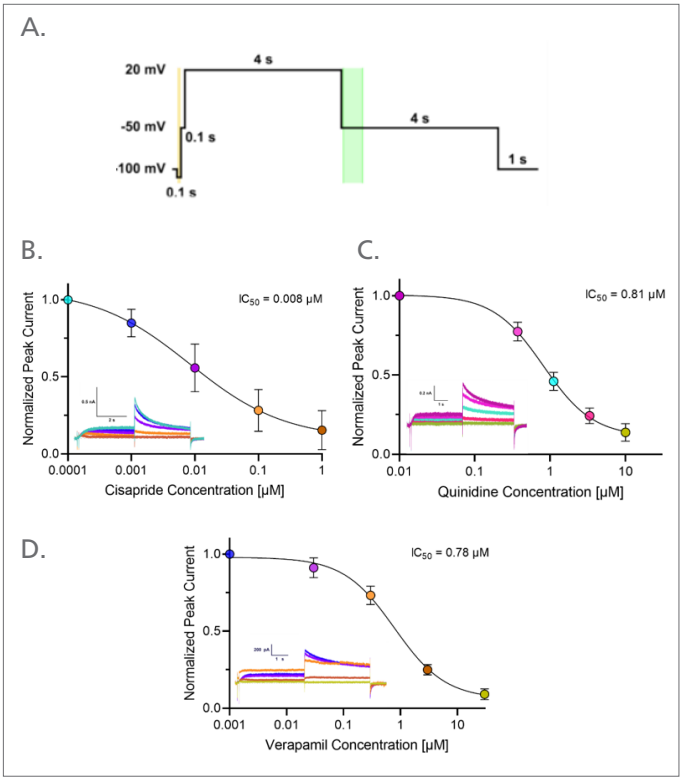

Fig. 3: **Pharmacological characterization of hERG channel using QPatch Compact. A)** Voltage protocol applied to the cells to open hERG channels. The inhibition by the compounds was measured on the tail hERG current (green cursor). Concentration-response curves showing hERG current inhibition by **B)** cisapride (n=10), **C)** quinidine (n=14) and **D)** verapamil (n=10). Experiments were recorded at room temperature. Error bars represent mean ± SD.

Table 1: IC_{50} values (in µM) of four different hERG inhibitors measured on our platforms (QPatch Compact & QPatch II) and compared to the literature.

Compound	QPatch Compact	QPatch II	Literature[4-8]
Cisapride	0.01	0.05	0.02-0.16
Quinidine	0.81	0.98	0.2-0.9
Verapamil	0.78	0.86	0.37
Erythromycin	445	>300	199-387

Ligand-gated ion channels

$GABA_A$

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the central nervous system. Dysregulations in GABA signaling have been linked to neurological and neuropsychiatric disorders, including epilepsy, schizophrenia, and major depression[9-10].

Exploring GABA physiopathology using patch clamp recordings plays a crucial role in drug development. Here, we used QPatch Compact to investigate the pharmacology of GABAα5β3γ receptor using GABA as an agonist and bicuculline as a competitive antagonist.

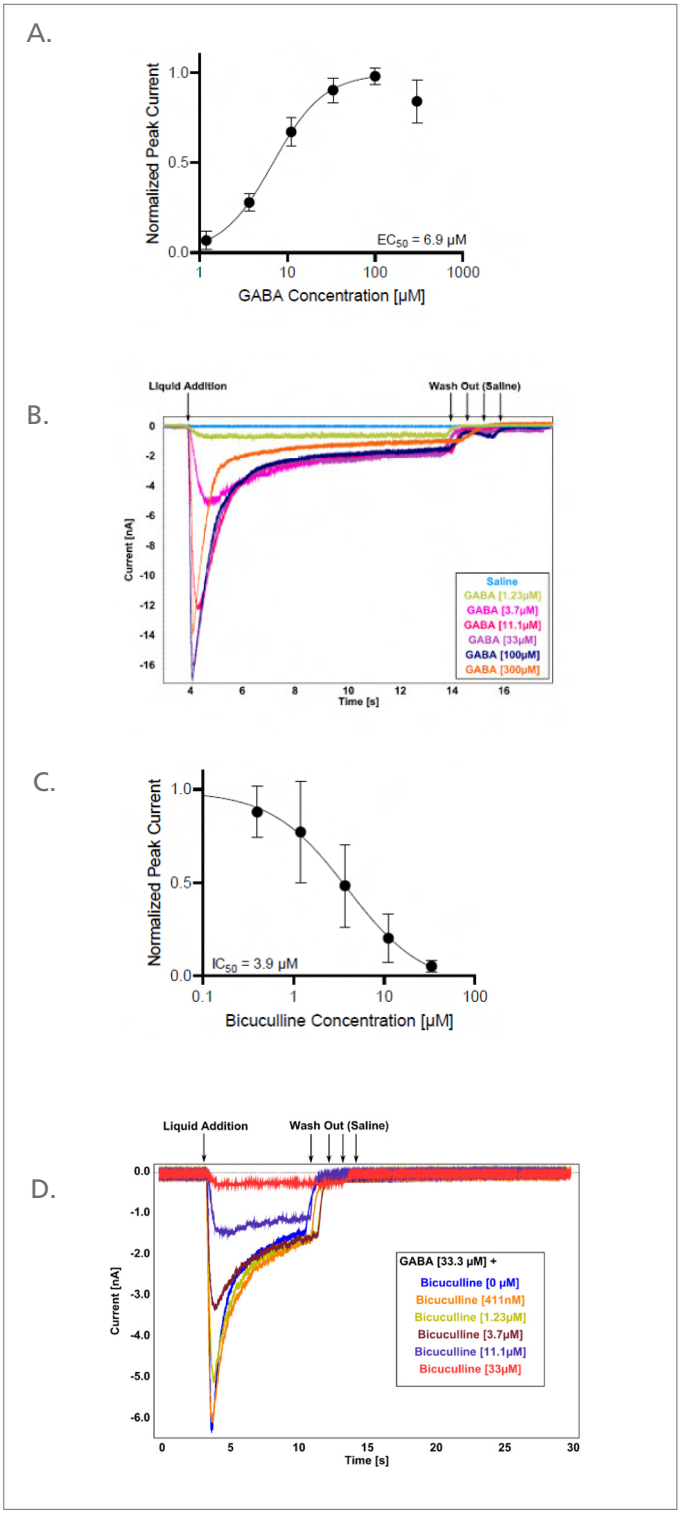

Fig. 4: **Pharmacological characterization of $GABA_A$ using QPatch Compact. A)** Cumulative concentration-response relationship of GABA binding to the GABAα5β3γ receptor (n=20). **B)** Representative current traces recorded in response to increasing concentrations of GABA. **C)** Cumulative concentration-response curve of the competitive antagonist bicuculline in presence of 33.3 µM of GABA (n=18). **D)** Representative traces of GABA currents in response to an increasing concentration of bicuculline associated with 33.3 µM GABA. Error bars represent mean ± SD.

Nicotinic acetylcholine receptor

The nAChR is a ligand-gated channel activated by endogenous (e.g. acetylcholine) and exogenous (e.g. nicotine) molecules, mainly expressed in the central and peripheral nervous system. The nAChRα1 subunit is prominently expressed in skeletal muscle cells and mediates fast transduction of motor neuron electrical signals[11].

In the following assay, we show that QPatch Compact with its microfluidic flow channels and fast liquid exchange, allows the recordings of fast-desensitizing ligand-gated channels like nAChR, even with repeated agonist exposure.

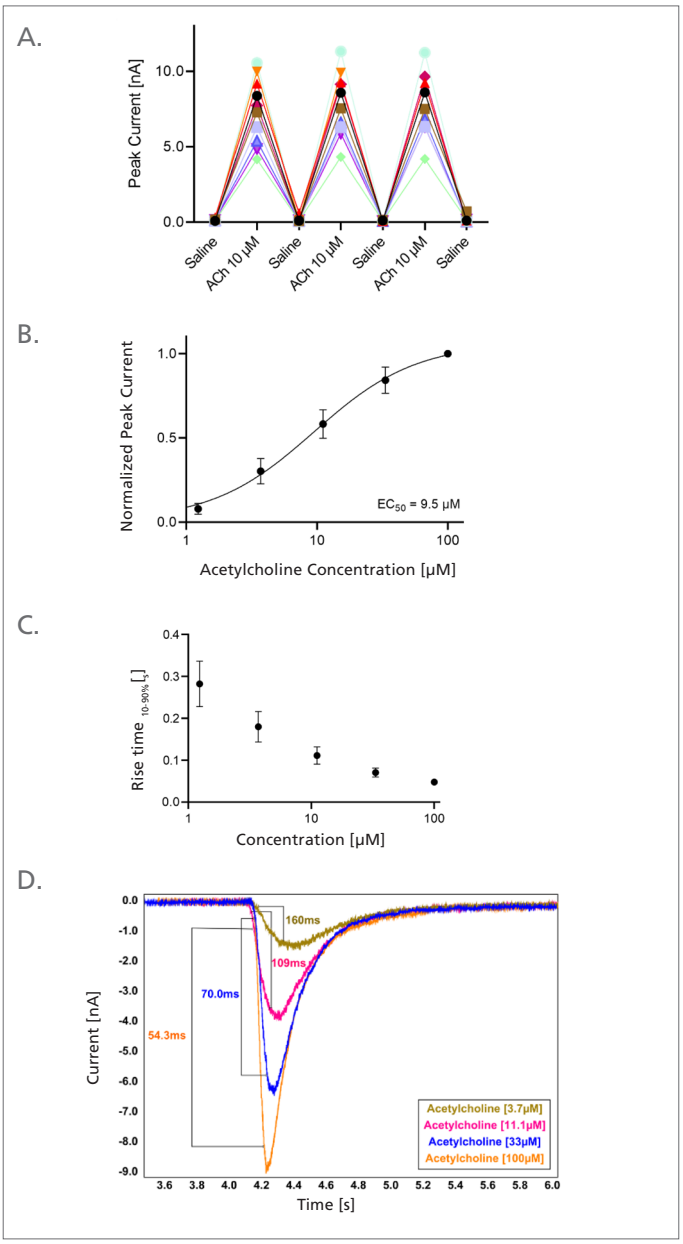

Fig. 5: **Characterization of nAchRα1 using QPatch Compact. A)** Repetitive stimulation of the nicotinic receptor with the same acetylcholine concentration (Ach, 10 µM, n=10) showing no significant differences in current size. **B)** Cumulative concentration-response relationships of acetylcholine binding the nAchRα1 channel (n=11; EC_{50}=9.49 µM). **C)** nAchRα1 opening time constant plotted against acetylcholine concentrations showing an inverse relationship between these parameters (n=11). **D)** Representative currents and rise times in response to increasing concentrations of acetylcholine. Error bars represent mean ± SD.

Temperature-sensitive ion channels

TRPM8

TRPM8 channel is known to be activated by chemical cooling agents (e.g. menthol) and innocuous cold temperatures, suggesting that it mediates the detection of cold thermal stimuli by primary afferent sensory neurons[12-13].

Here we demonstrate that QPatch Compact can be used to record TRPM8 currents after cold activation and pharmacological modulation.

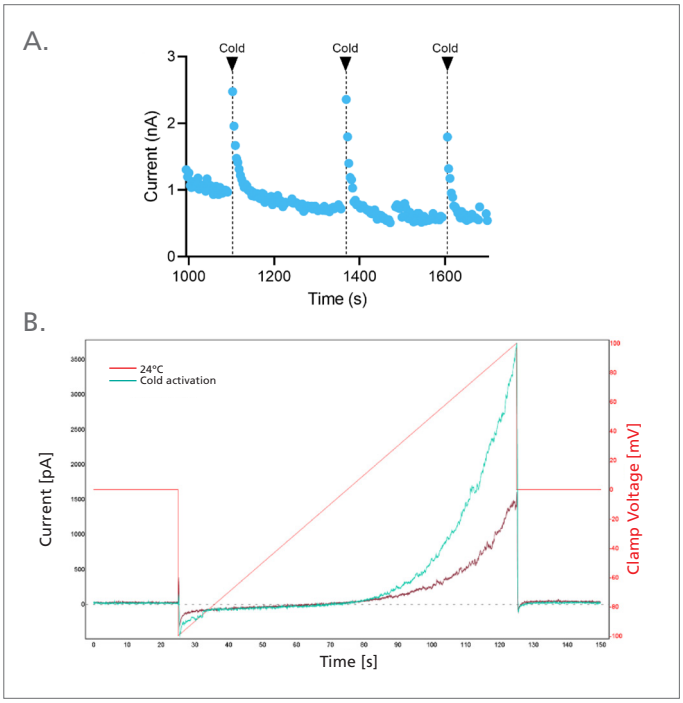

Fig. 6: **TRPM8 activation by cold temperature on QPatch Compact. A)** TRPM8 current (measured at 80 mV) plotted against time. The arrows represent the addition of cold saline liquid into the well. **B)** Representative traces of TRPM8 current at 24°C (brown trace) and 3 seconds after cold stimulation (green trace) by adding saline solution kept in ice (4°C).

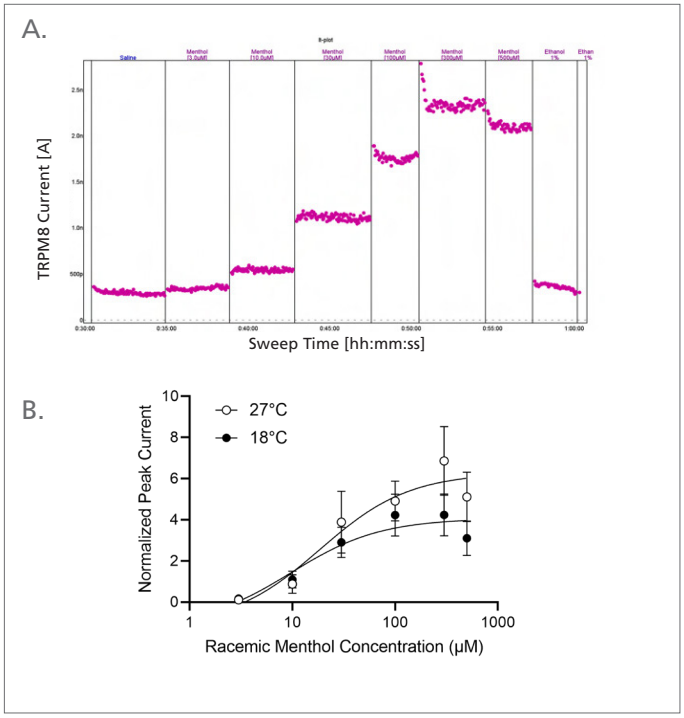

Fig. 7: **Pharmacological modulation of TRPM8 at two different temperatures A)** Representative TRPM8 current over time plot in response to increasing concentrations of racemic menthol. A depolarizing ramp from -100 to +100 mV over 100 ms was used as a protocol. It is important to notice the decrease in response with the highest agonist dose (500 µM) due to desensitization. **B)** Racemic menthol cumulative concentration-response relationship at 18°C (black dots, EC_{50}=9.74 µM, n=9) and 27°C (white dots, EC_{50}=18.63 µM, n=12). Data were normalized to the 2nd saline addition and the highest concentration was not considered for the fitting of the curve due to the desensitization. Error bars represent mean ± SD.

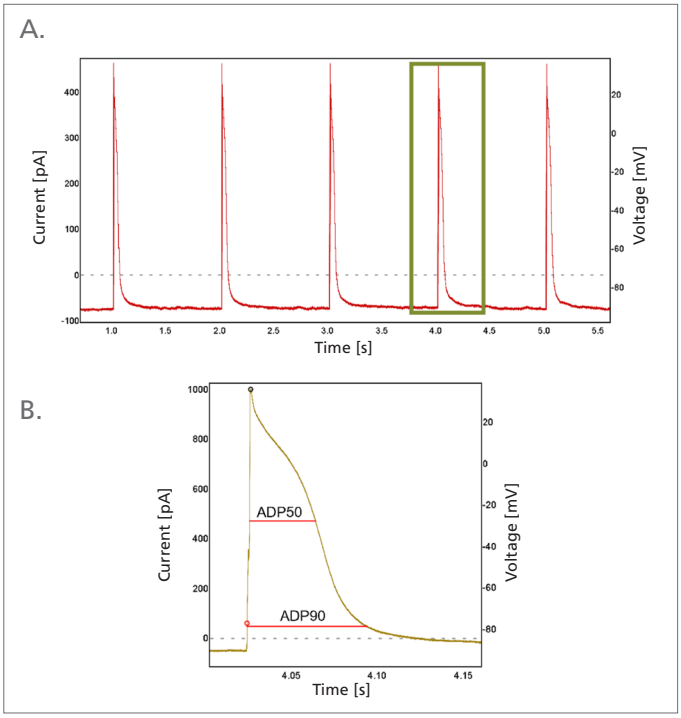

Fig. 8: **QPatch Compact measurements of paced action potentials. A)** Paced action potentials from an individual hiPSC-CM measured in current clamp. **B)** Zoom of the framed action potential from the left figure (green box) displaying the action potential duration at 50% (ADP50) and at 90% (ADP90) repolarization measured by the Sophion Analyzer software.

hiPSC-derived cardiomyocytes

Human-induced pluripotent stem cells (hiPSCs) have been shown to be a promising tool for disease modeling, drug discovery and personalized medicine, especially in the cardiac biology field[14].

Recording hiPSC-derived cardiomyocytes (hiPSC-CM) on a patch-clamp system (both manual and automated) can be challenging due to their immature phenotype, high variability of cell quality between vendors, batch-to-batch variation, and cell population heterogeneity[15].

We have previously demonstrated the usability of high-throughput automated patch clamp platforms, Qube 384 and QPatch II, for the characterization of both hiPSC-derived cardiomyocytes and the neurons[16-17].

In the following experiments, we show the potential of QPatch Compact to record action potentials in hiPSC-cardiomyocytes (hiPSC-CM) using the current clamp configuration.

Temperature control

Temperature control is integrated in QPatch Compact allowing for temperature regulation ranging from 18-40°C with high precision and accuracy of ±0.5°C.

The temperature regulation is performed using circulating water in the bed of nails (BON) close to the measurement sites to ensure precise control with minimum fluctuations. The ambient temperature is accounted for by using an algorithm. All data is recorded and stored in the database together with the electrophysiology data. Temperature data is thus readily available for analysis after an experiment run.

Read more about our temperature control design on our website here.

In fig. 9 and table 2, you can observe example data for hERG at room temperature and physiological temperature recorded on QPatch Compact using our temperature control system.

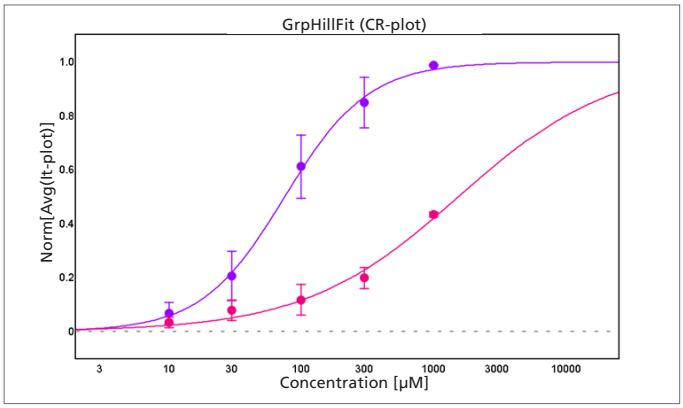

Fig. 9: **Pharmacological modulation of hERG at two different temperatures** The curves represent the erythromycin cumulative concentration-response relationship at room temperature (~20°C) (pink curve, IC_{50}=1540.1 µM) and at physiological temperature (~35°C) (purple curve, IC_{50}=75.2 µM).

Table 2: IC_{50} values (in µM) of hERG inhibitor erythromycin at two different temperatures measured on our platforms (QPatch Compact & Qube) and compared to the literature.

Erythromycin	IC_{50} [µM]	Reference*	Reference#
Ambient temperature (20°)	1540.1	>>600	1410
Physiological temperature (35°C)	75.2	55	115

* Temperature control on Qube 384 – pharmacological dependency of hERG reference compounds, #Kirsch et al., 2004. Journal of Pharmacological and Toxicological Methods 50 (2004) 93–101.

Methods

Cell lines:

- CHO-hERG (B'SYS GmbH)
- CHO-$Na_V1.5$ (B'SYS GmbH)
- TE671/RD expressing nAChR (ATCC)
- HEK293-TRPM8 (MilliporeSigma)
- HEK293-GABAα5β3γ2 (Charles River Laboratories International Inc.)
- Ncyte Cardiomyocytes (Ncardia Belgium NV)

- Data analysis was performed using the Sophion Analyzer Software and GraphPad Prism 9 (GraphPad Software Inc.).

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