SOPHION BIOSCIENCE

Application Report

hiPSC-derived cardiomyocytes: A robust and reproducible QPatch® II assay for $Na_V1.5$ measurements

Including biophysical characterization and concentration-dependent block of $Na_V1.5$

Summary

The $Na_V1.5$ channel is critical in maintaining the physiological electrical function in cardiomyocytes (CMs). Consequently, high-throughput measurements of $Na_V1.5$ currents (I_{Na}) in hiPSC-CMs are useful e.g. in the characterization of cardiac disease phenotypes and compound testing. QPatch II has been demonstrated as a capable platform for such measurements[1], and here we present a high-performance $Na_V1.5$ assay using iCell® Cardiomyocytes[2] (hiPSC-CMs) from FUJIFILM Cellular Dynamics with:

- Assay success rates ~45% with more than 85% hiPSC CMs expressing I_{Na} with peak currents $|I_{Na}|$> 200 pA (at -30 mV)
- Biophysical characterization with activation/inactivation potentials in agreement with literature values
- Cumulative compound concentration-response of the local anesthetic agent tetracaine with IC_{50} values in agreement with literature values

Results and discussion

Assay success rate and measurement quality

We obtain an assay success rate of ~ 45%, calculated as the percentage of measurement sites, out of the full measurement plate (48 sites), that pass our quality criteria (membrane resistance, R_{mem} > 200 MΩ, cell capacitance, C_{slow} > 4 pF). In 85% of hiPSC-CMs we record I_{Na} with peak current $|I_{Na}|$ > 200 pA. See Figure 1 for a partial measurement plate view, displaying I_{Na} current-voltage (IV) relationship recorded in 42% of the sites.

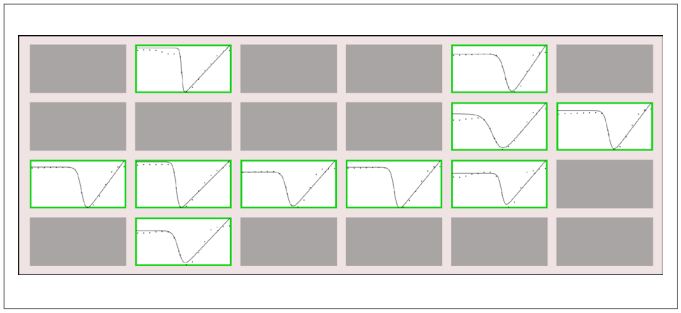

Fig. 1: Plate overview of half a QPlate consumable (24 measurement sites out of 48) displaying I_{Na} IV relationship recorded in individual hiPSC-CMs. In this experiment we obtained a 42% success rate.

R_{mem} and C_{slow} values are plotted in Figure 2A for all recorded hiPSC-CMs (~30% of 48 sites) that maintained a good seal for the duration of the concentration-response experiment. Note that hiPSC-CMs display a large distribution of sizes as observed in the C_{slow} values that varied from 4 pF to 40 pF (Fig. 2A, right). To ensure good voltage clamping we reduced the extracellular concentration of Na^+, see Figure 2B for a representative I_{Na} trace. The peak I_{Na} was recorded at -30 mV and correlated well with cell size. See Figure 2C for plots of $Na_V1.5$ peak current, I_{Na}, and peak current density.

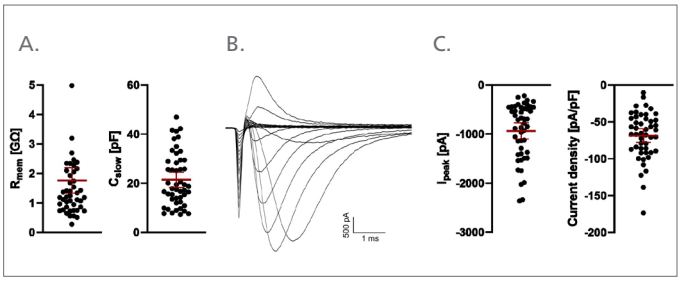

Fig. 2: Measurement quality and representative hiPSC-CM I_{Na} current. **A)** Seal resistance, R_{mem} [GΩ], (left) and cell capacitance, C_{slow} [pF], (right) (mean and 95% CI are shown in red, N_{CMs} = 40, $N_{QPlates}$ = 3). **B)** Representative $Na_V1.5$ current trace in response to a voltage step protocol (see methods). **C)** $Na_V1.5$ peak current, I_{Na} [pA], (left) and peak current density [pA/pF] (right) (mean and 95% CI are shown in red, N_{CMs} = 48, $N_{QPlates}$ = 3).

Biophysical characterization

We quantified the biophysical properties of the I_{Na} recorded from hiPSC-CMs. The experiments were conducted at a reduced extracellular sodium concentration ($[Na^+]$ = 10 mM), to ensure good voltage clamping of the cells. The I_{Na} activated around – 60 mV and peaked around – 30 mV (Fig. 3A). The half maximal activation was ~ -44 mV and the half maximal inactivation was ~ -83 mV, in agreement with reported values for $Na_V1.5$[2,3] (Fig. 3B).

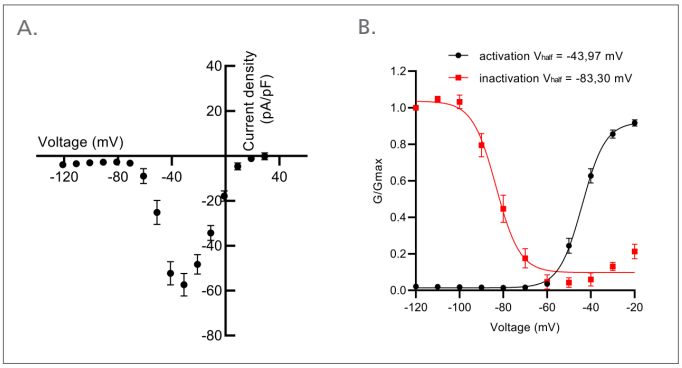

Fig. 3: Biophysical characterization of the I_{Na} current. **A)** Average I_{Na} current density versus step voltage (voltage steps of 10 mV from a holding potential of -130 mV, see Methods) (N_{CMs} = 40). **B)** Normalized conductance (G/G_{max}) versus step voltage was plotted for the activation (black, N_{CMs} = 39) and inactivation (red, N_{CMs} = 49) and fitted with the Boltzmann equation (solid lines) to yield the voltage at half maximum activation (V_{half} ~ -44 mV) and inactivation (V_{half} ~ -83 mV). All data points are mean ± sem.

Cumulative concentration-response of tetracaine

As a proof-of-concept concentration-response experiment, we recorded the I_{Na} sensitivity to increasing concentrations (0.01 µM to 100 µM) of the local anesthetic agent tetracaine, using a two-pulse voltage protocol, including an adaptive protocol for V_{half} measurement for improved control of $Na_V1.5$ state before measurement[4] (see Methods). Tetracaine blocked I_{Na} in a state-dependent manner, and in agreement with previous APC measurements on immortal cell lines expressing the $Na_V1.5$ ion channel[5], we quantify an IC_{50} value of ~2.4 µM for the second peak of the double pulse.

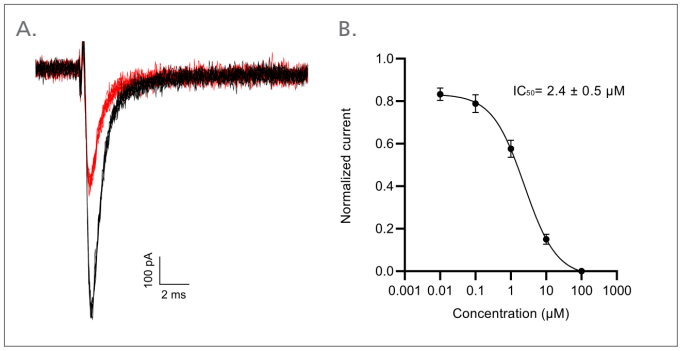

Fig. 4: Concentration-dependent block of I_{Na} in hiPSC-CMs. **A)** Representative I_{Na} current (recorded in the second pulse of the voltage protocol) before (black) and after (red) the addition of 1 µM tetracaine. **B)** Normalized current (I_{Na}) as a function of tetracaine concentration (N_{CMs} = 24). Fitting of the Hill equation (solid line) to the data yielded an IC_{50} value of ~ 2.4 µM. Data points are mean ± sem.

Results and discussion

In conclusion, we demonstrate a successful $Na_V1.5$ assay using iCell Cardiomyoctes² (hiPSC-CM) from FUJIFILM Cellular Dynamics, with assay success rates up to 45%. The cell line shows robust and reproducible I_{Na} current in more than 85% of recorded hiPSC-CMs. The biophysical and pharmacological features measured using QPatch II, are in concordance with I_{Na} previously reported, from hiPSC-CMs[3,6].

Methods

iCell Cardiomyocytes² (hiPSC-CMs) were kindly provided by FUJIFILM Cellular Dynamics. They were cultured according to vendor SOP and assayed (7 – 21) days post-thawing the frozen vials. The $Na_V1.5$ current density was stable within this time frame.

Cell dissociation

On experiment day the cells were dissociated using a Sophion SOP, specifically developed for iPSC-CMs and further optimized for iCell Cardiomyocytes². For more information contact the authors directly or through Sophion at info@sophion.com.

Solutions

Extracellular solution (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 10 NaCl, 135 $C_5H_{14}ClNO$, 10 Glucose, pH = 7.4 with NaOH, osmolarity = 305 mOsm with sucrose

Intracellular solution (in mM): 10 NaCl, 10 HEPES, 1 EGTA, 5 CsOH, 140 CsF, pH = 7.3 with HCl, osmolarity = 280-290 mOsm with sucrose

Whole-cell protocol

We used a whole-cell protocol, specifically developed for iPSC-CMs. For more information contact the authors directly or through Sophion at info@sophion.com.

Voltage protocols

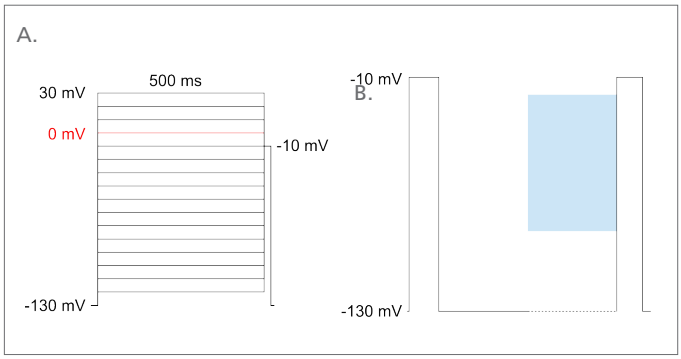

Fig. 5: Applied voltage protocols. A) Voltage step protocol for recording I_{Na} current-voltage relationship. The conductance (G) was calculated as G = I/(V-V_{rev}), with the activation current being measured during the 500 ms step pulses from – 120 mV to +30 mV, and the steady state fast inactivation current being measured during a 20 ms test pulse to -10 mV following the pre-pulse step voltages. B) Double pulse for measurement of state-dependent I_{Na} compound effects. Individually recorded V_{half} values (voltage at half maximum activity) were applied before the second pulse (illustrated by the blue square) using our adaptive protocol for measurements of V_{half}[4].

References

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FUJIFILM Cellular Dynamics

iCell Cardiomyocytes² (CM²) were kindly provided by FUJIFILM Cellular Dynamics, Inc.

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