Application Note

Ion Channels:
hERG

Cells:
CHO-K1

Tools:
SyncroPatch 384

Characterization of hERG current expressed in CHO-K1 cells from ION Biosciences on the SyncroPatch 384

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Summary

The hERG gene encodes a potassium channel responsible for the repolarization of the I_{Kr} current in cardiac cells[1]. This channel is important in the repolarization of the cardiac action potential. Abnormalities in this channel can lead to long or short QT syndrome, leading to potentially fatal cardiac arrhythmia. Given the importance of this channel in maintaining cardiac function, and disturbances of channel activity by certain compounds such as anti-arrhythmias and anti-psychotics, it has become an important target in compound safety screening.

A large range of therapeutic agents with diverse chemical structures have been reported to induce long QT syndrome by inhibiting the hERG channel. These include antihistamines (e.g. Terfenadine), gastrointestinal prokinetic agents (e.g. Cisapride), amongst others[2]. Therefore, it is important to test new therapeutics for actions on the hERG channel early on in the drug discovery process. Testing on the hERG channel using patch clamp electrophysiology has been largely successful in preventing potentially unsafe drugs from reaching the market. With the introduction of the CiPA initiative in 2013, a larger repertoire of cardiac ion channels are required to be tested for cardiac safety including hERG, $Na_V1.5$ (peak and late) and $Ca_V1.2$, amongst others. The aim of the CiPA initiative is to ensure that drugs that reach the market are safe, by identifying candidate compounds with the potential to cause potentially fatal arrhythmia, but also to reduce the number of false positives ensuring that potentially useful (and safe) drugs do reach the market.

Access to good quality, affordable cell lines is essential for high throughput automated patch clamp (APC) screening of cardiac (and other) ion channels. We have characterized a new CHO-K1 cell line stably expressing the hERG channel available from ION Biosciences using the SyncroPatch 384. We have tested different voltage protocols, investigated activation and inactivation properties and pharmacology of the hERG channel using this cell line.

Results

The CHO-K1 cells stably expressing the hERG channel were captured to the patch clamp aperture of the NPC-384 single hole (1X) chips with a success rate of 96% for cells with R_{Seal} >500 MΩ. Peak current size using a 2-step voltage protocol was 0.38 ± 0.04 nA (n = 82) and average R_{Seal} = 5.6 GΩ (n = 82). Voltage protocols were run and activation and inactivation IV plots were constructed (Fig. 1) revealing a V_{half} of activation of -87.5 mV (n = 152), in good agreement with the literature[3], and a V_{half} of inactivation of -1.87 mV (n = 138). When we used multihole chips (4 holes per well; 4X) the peak current was increased to 0.94 ± 0.04 nA (n = 173). Recordings were

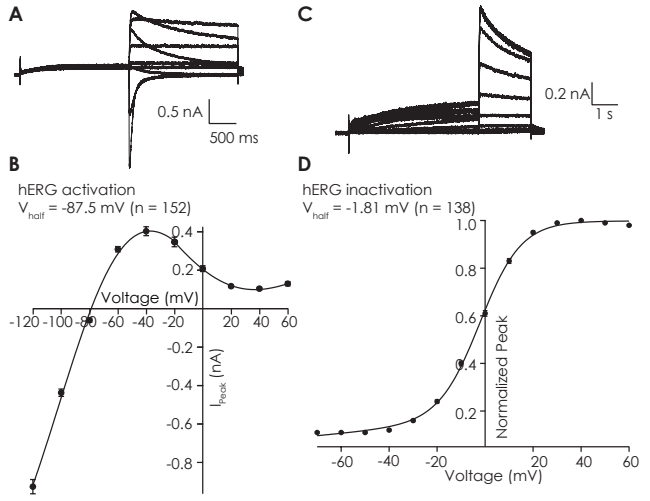

Figure 1: A *Example traces of a hERG activation IV protocol.* **B** *Activation IV curve for an average of 152 wells.* **C** *Current responses from an example cell following an inactivation voltage protocol.* **D** *Inactivation IV curve for an average of 138 wells.*

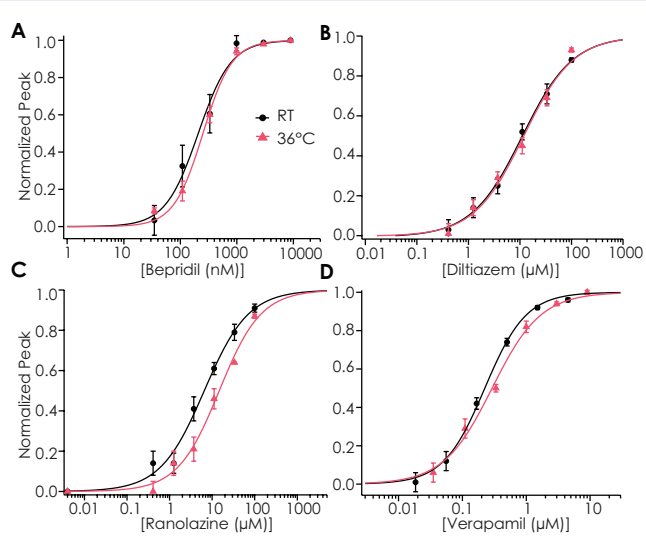

Figure 2: *Concentration response curves for* **A** *Bepridil,* **B** *Diltiazem,* **C** *Ranolazine and* **D** *Verapamil are shown at RT and 36°C overlaid.*

made at room temperature (RT; 21°C) and upon increasing the temperature to 36°C there was a 78% increase in peak current amplitude to 1.67 ± 0.05 nA (n = 173). Recordings were highly stable with a rundown rate of <2%/min over a 5 minute recording period at RT and <0.7%/min at 36°C (total recording time was 10 mins). We tested 4 different compounds at RT or 36°C and found no difference in the concentration response curves (CRC) at the different temperatures, with the exception of ranolazine which appeared to be slightly less potent at 36°C (Fig. 2, Table 1). The IC_{50} values were in excellent agreement with the literature (Table 1, refs. 4 & 5).

	Bepridil (nM)	Diltiazem (µM)	Ranolazine (µM)	Verapamil (nM)
RT	226 (23)	12.2 (24)	6.9 (23)	236 (23)
36°C	254 (21)	12.0 (21)	14.8 (20)	289 (19)
Lit.	160[4]/149[5]	13.2[4]/6.6[5]	6.5[5]	250[4]/499[5]

Table 1: IC_{50} values for 4 compounds at RT and 36°C, number of wells used for the CRC shown in parentheses. Third row shows literature values for RT[4] and 36°C[5].

In summary, the CHO-K1 cells stably expressing the hERG channel provided by ION Biosciences could be used on the SyncroPatch 384 with excellent success rates for R_{Seal} and current amplitudes large enough for screening using APC. Currents were stable over time (>10 mins) and could be used for pharmacology experiments at both RT and physiological temperature and IC_{50} values obtained agreed well with the literature[4,5]. The hERG CHO-K1 cell line is an excellent tool for cardiac safety testing using APC.

References

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5. Crumb, W.J., *et al*. 2016. 81: 251-262 doi: 10.1016/j.vascn.2016.03.009.

Methods

Cells

CHO-K1 cells stably expressing hERG (C1202) were kindly provided by ION Biosciences and were thawed and cultured according to Nanion's standard procedures.

Electrophysiology measurements

Cells were harvested according to Nanion's standard procedures. Cells were resuspended in Nanion's external recording solution (# 08 3001) and stored in the CellHotel of the SyncroPatch 384 shaking at 200 rpm at 15°C before use. Nanion's standard internal (# 08 3007) and external solutions (#08 3001 and #08 3004) were used for recordings. Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384. Activation IVs were recorded using a voltage step protocol and analyzed using DataControl 384. IV curves were fitted with a Boltzmann equation. For pharmacology experiments, either a double step or step-ramp protocol was used and a single concentration of compound was added to each well, the CRCs were constructed over multiple wells in DataControl 384. CRCs were fit with a Hill equation. For recordings at 36°C, cells were first captured and sealed to the chip and the temperature then raised to 36°C prior to control and compound additions.

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