Application Note

Channels: hERG, $Na_V1.5$, $Ca_V1.2$, Kir2.1, KvLQT1
Cells: HEK, CHO, iPS-derived cardiomyocytes
Tools: SyncroPatch 384/768PE, Patchliner, CardioExcyte 96

High Throughput Screening of Cardiac Ion Channels

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Summary

In 2013 the Cardiac Safety Research Consortium (CSRC), the Health and Environmental Sciences Institute (HESI), and the US Food and Drug Administration (FDA) proposed a new paradigm to improve assessment of the proarrythmic risk of therapeutic compounds. Until now, drug safety testing has focussed on interaction with the hERG channel and QT prolongation which can lead to potentially fatal torsades de pointes (TdP). Although this approach has been largely successful in preventing new drugs reaching the market with unexpected potential to cause TdP, it is also possible that potentially valuable therapeutics have failed due to this early screening. The new paradigm, the Comprehensive In-vitro Proarrhythmia Assay (CiPA) was introduced to provide a more complete assessment of proarrythmic risk by evaluating and implementing currently available high throughput methods[1,2]. An important part of this remains electrophysiological evaluation of not only hERG, but also other cardiac channels including $Na_V1.5$, $Ca_V1.2$, KvLQT1 and Kir2.1. Additionally, new technologies, such as impedance measurements, and cells such as stem cell-derived cardiomyocytes, may provide useful tools for high throughput safety assessment.

Here we present high quality data with reliable pharmacology on hERG expressing CHO cells, $Na_V1.5$, $Ca_V1.2$ or KvLQT1 expressed in HEK293 cells and Kir2.1 expressed in RBL cells on the SyncroPatch® 384PE or Patchliner. Additionally, electrophysiological recordings on the Patchliner and Impedance measurements on the CardioExcyte 96 of stem cell-derived cardiomyocytes are shown.

Results

CHO cells expressing hERG cells were recorded on the SyncroPatch® 384PE with a high success rate (>80%). Figure 1 shows the current-voltage relationship for hERG.

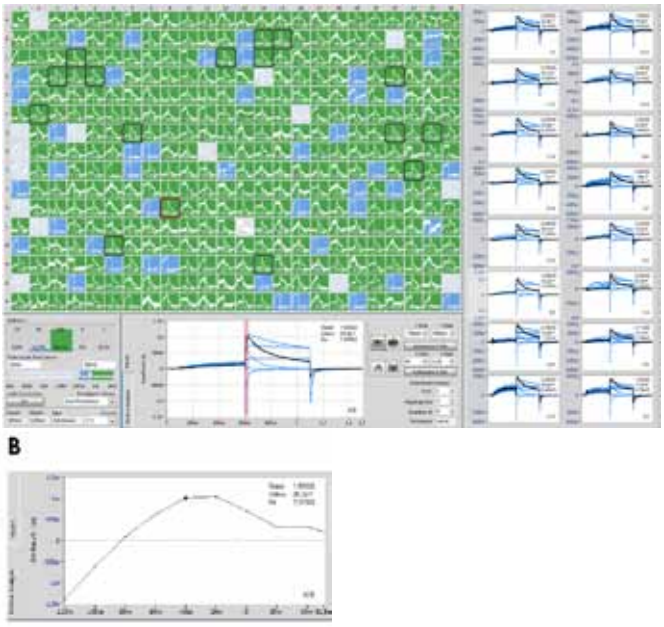

Figure 1: Typical recording from hERG expressed in CHO cells on the SyncroPatch® 384PE. The screenshot shows the data acquisition and analysis software used on the SyncroPatch® 384PE. Wells are color-coded based on seal resistance (green: Rmemb > 500 MΩ, blue: Rmemb = 100 - 500 MΩ, light blue or grey: Rmemb < 100 MΩ or disabled). Shown are hERG-mediated currents elicited using a voltage step protocol from -120 mV to 80 mV increasing in 20 mV increments (after a depolarizing step to 60 mV). The black trace highlights the maximum current in response to a test potential of -40 mV. **B** The current voltage relationship from an exemplar cell shows the typical IV curve of hERG channels.

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Pharmacology of hERG-mediated currents could be recorded on the SyncroPatch® 384PE with a high success rate for completed experiments. Figure 2 shows the concentration response curves for four known blockers of hERG with IC_{50} values as expected in the literature. A summary of the IC_{50} values is given in Table 1.

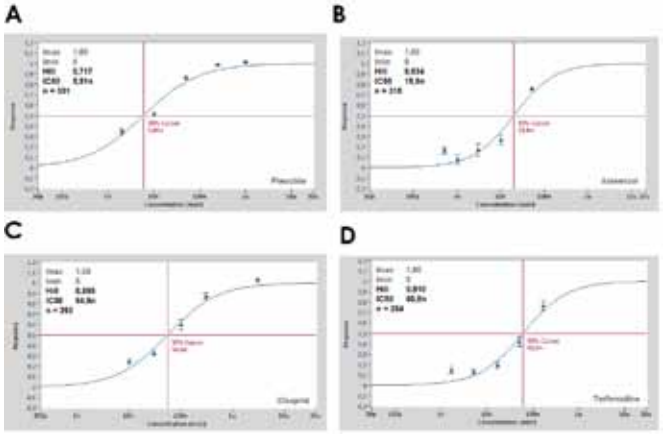

Figure 2: Average concentration response curves for 4 different hERG blockers on the SyncroPatch® 384PE. The concentration response curves were constructed across the whole plate (single concentration per cell). The SyncroPatch® 384PE analysis software (DataControl® 384) was used to calculate the average concentration response curves, normalized to maximum block and fitted with a standard Hill-equation. A summary of the IC_{50} values and success rates is shown in Table 1.

Figure 3 shows the current-voltage relationship of $Na_V1.5$ expressed in HEK293 cells recorded on the SyncroPatch® 384PE. An average activation and inactivation IV plot is shown in Panels C and D, respectively. The Vhalf of activation was -51 mV and for inactivation was -84 mV in good agreement with the literature[3]. Pharmacology of the $Na_V1.5$ channel could be recorded on the SyncroPatch® 384PE with success rates of up to 76% for completed experiments.

Compound	IC_{50} (nM)	Success rate (%)	Literature value (nM)
Pimozide	5.9 (301)	78	1 - 18[4,5]
Astemizole	19.8 (315)	82	26[5]
Cisapride	54.6 (292)	76	7- 26[4,5]
Terfenadine	60.8 (264)	69	7 - 101[4,5]

Table 1: IC_{50} values for pimozide, astemizole, cisapride and terfenadine on hERG-mediated currents recorded on the SyncroPatch® 384PE. Shown are IC_{50} values (number of cells shown in brackets), success rate for completed experiments and the expected literature IC_{50} values. All IC_{50} values recorded on the SyncroPatch® 384PE agree well with the literature values[4,5].

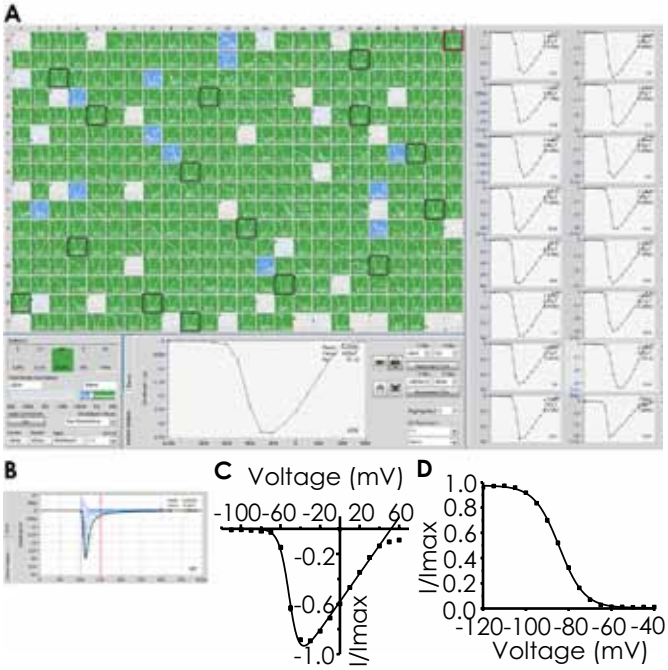

Figure 3: $Na_V1.5$ expressed in HEK293 cells recorded on the SyncroPatch® 384PE. A The screenshot shows the data acquisition and analysis software used on the SyncroPatch® 384PE. Wells are color-coded based on seal resistance (green: Rmemb > 500 MΩ, blue: Rmemb = 100 - 500 MΩ, light blue or grey: Rmemb < 100 MΩ or disabled). Shown is the online analysis where peak current is plotted against voltage to show the current-voltage relationship **B** $Na_V1.5$-mediated current responses to a voltage step protocol from -110 mV to 60 mV increasing in 10 mV increments. **C** Average current voltage plot of 337 cells. The points were fit with a Boltzmann equation and the Vhalf of activation was -51 mV. **D** Average inactivation plot for 271 cells. The points were fit with a Boltzmann equation and the Vhalf of inactivation was -84 mV.

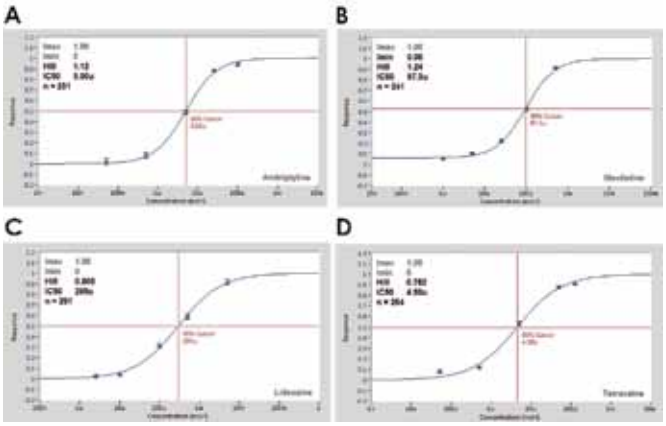

Figure 4: Average concentration response curves for 4 different $Na_V1.5$ blockers on the SyncroPatch® 384PE. The concentration response curves were constructed across the whole plate (single concentration per cell). The SyncroPatch® 384PE analysis software (DataControl® 384) was used to calculate the average concentration response curves, normalized to maximum block and fitted with a standard Hill-equation. A summary of the IC_{50} values and success rates is shown in Table 2.

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Figure 4 shows the concentration response curves for four sodium channel blockers on $Na_V1.5$-mediated currents. In these experiments holding potential was -110 mV. At more depolarized holding potential the IC_{50} for lidocaine increased by a factor of 35 (data not shown) as expected[6]. The IC_{50} values and success rates are summarised in Table 2.

Compound	IC_{50} (µM)	Success rate (%)	Literature value (µM)
Amitriptyline	5.0 (251)	65	1.6[7]
Mexiletine	97.3 (241)	63	49[8]
Lidocaine	295 (291)	76	353[6]
Tetracaine	4.5 (264)	69	9.3[9]

Table 2: IC_{50} values for amitriptyline, mexiletine, lidocaine and tetracaine on $Na_V1.5$-mediated currents recorded on the SyncroPatch® 384PE. Shown are IC_{50} values (number of cells shown in brackets), success rate for completed experiments and the expected literature IC_{50} values.

The K^+ current IK1, encoded by the Kir2.1 gene, is responsible for the final phase of the cardiac action potential and for maintaining the resting membrane potential[10]. The Kir2.1 channel is expressed endogenously in RBL cells and has also been successfully recorded on the SyncroPatch® 384PE. Figure 5 shows Kir2.1 channels activated by addition of external solution containing high K^+.

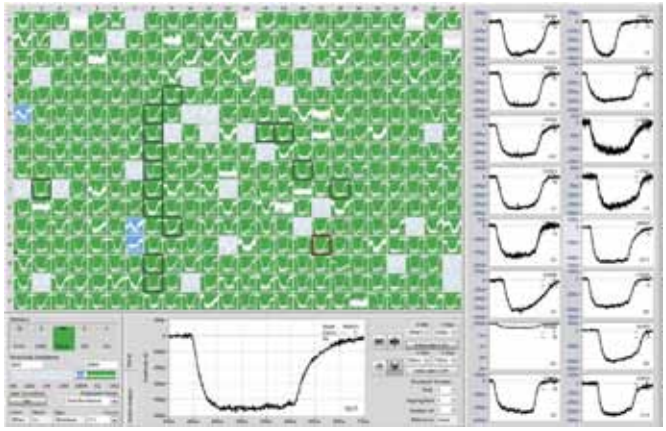

Figure 5: Kir2.1 endogenously expressed in RBL cells recorded on the SyncroPatch® 384PE. Kir2.1 was activated by addition of external solution containing high K^+ concentration. Solution exchange rate is in the order of 50 ms on the SyncroPatch® 384PE.

The 2nd phase (plateau) of the cardiac action potential is mediated by the $Na_V1.5$ and $Ca_V1.2$ channels[10]. $Ca_V1.2$ has been recorded in the voltage clamp mode on the Patchliner (Figure 6). The current was blocked by the $Ca_V1.2$ blocker, nifedipine, in a concentration-dependent manner with an IC_{50} of 12.8 ± 8.0 nM (n = 3) in good agreement with the literature[11]. Action potentials recorded from stem cell-derived cardiomyocytes on the Patchliner were also shortened by nifedipine (Figure 6C). Current clamp measurements at high throughput will soon be possible on the SyncroPatch® 384PE. Stem cell-derived cardiomyocytes offer a distinct advantage over adult ventricular myocytes given the relative ease with which large quantities can be produced and their proven success on automated patch clamp systems[12,13]. However, these cells must be properly characterized and compared with adult ventricular cardiomyocytes before their use in safety screening will become routine.

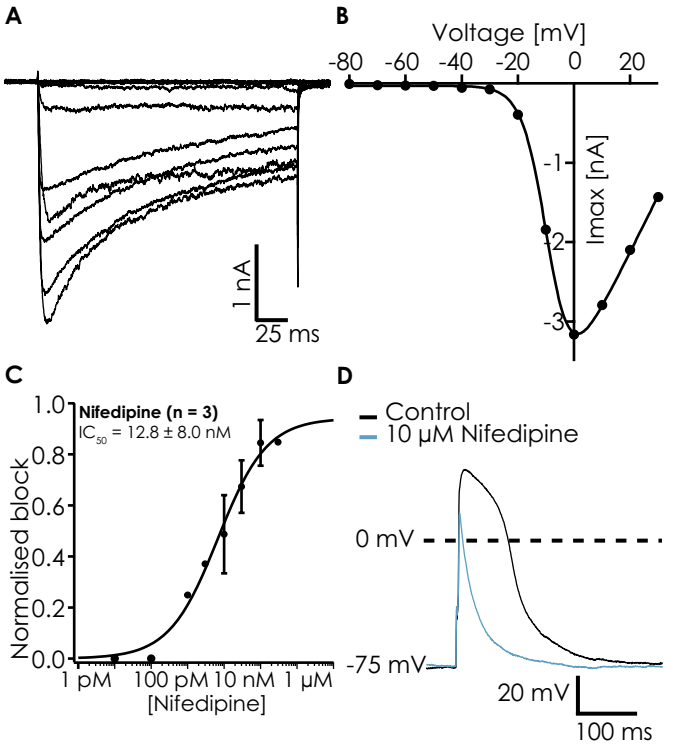

Figure 6: $Ca_V1.2$ expressed in HEK cells recorded on the Patchliner. A Current responses to a voltage step protocol (from -80 mV to +30 mV for 100 ms increasing in 10 mV increments) of an exemplar cell expressing $Ca_V1.2$. **B** corresponding IV curve. Vhalf of activation was -8.8 mV. **C** Concentration response curve for nifedipine for an average of 3 cells. IC_{50} was 12.8 ± 8.0 nM (n = 3). **D** Action potential recorded from a stem cell-derived cardiomyocyte recorded on the Patchliner. The action potential amplitude was reduced and the duration was shortened by nifedipine.

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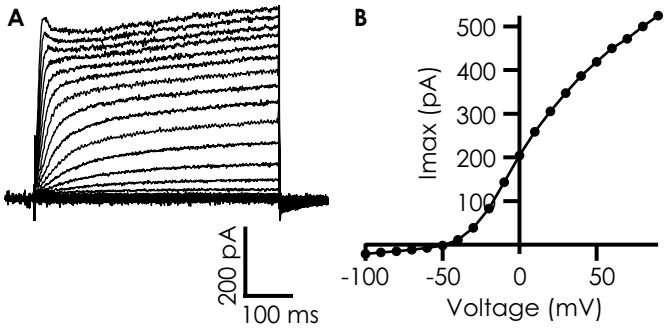

Figure 7: KvLQT1 expressed in HEK cells recorded on the Patchliner. A Current responses to a voltage step protocol of an exemplar cell expressing KvLQT1. **B** Corresponding IV plot.

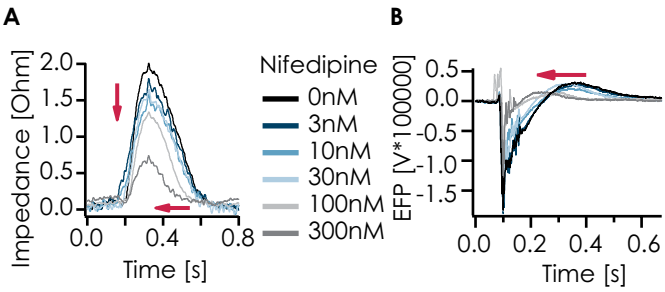

Figure 8: Dose-dependent effect of nifedipine on the impedance and the EFP. Compounds were applied as indicated to a monolayer of 20 k Cor.4U cells. The mean beats, derived from 10 s sweeps, are shown in **A** for the impedance and in **B** for the EFP. Included is the influence on the impedance amplitude and pulse-width (arrows in A), as well as on the extracellular field potential duration (FPD; arrow in B).

Repolarization of the cardiac action potential, or phase 3, is facilitated by IKs which is encoded by the KvLQT1/mink genes[10]. The KvLQT1 channel has been successfully recorded on the Patchliner (Figure 7).

Recent advances in technologies such as impedance offer the ability to measure effects of compounds on the contractility of cardiac cells. Since impedance measurements can be made on cultured cells and stem cell-derived cardiomyocytes at high throughput and at physiological temperature, this technique may prove a useful complementary technology to patch clamp electrophysiology. Figure 8 shows the effect of the $Ca_V1.2$ blocker, nifedipine, on the impedance and extracellular field potential (EFP) duration using the CardioExcyte 96.

In conclusion, Nanion Technologies offers high throughput automated patch clamp systems which are compatible with recording all ion channels important in safety screening as suggested by the CiPA initiative. This is possible in both heterologous expression systems, e.g. HEK293 and CHO cells, and potential cardiac model cells such as stem cell-derived cardiomyocytes. Additionally, the CardioExcyte 96 is an impedance system capable of testing compounds on the contractility and EFP of contractile cells such as Cor.4U cells shown here.

References

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Methods

Cells

CHO cells expressing hERG, HEK293 cells expressing $Na_V1.5$, $Ca_V1.2$ or KvLQT1, RBL or stem cell-derived cardiomyocytes

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocols.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedures for the SyncroPatch® 384PE and Patchliner.

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