Application Note

Channel: hERG
Cells: CHO
Tools: SyncroPatch® 384PE

High Throughput Pharmacology of hERG Channels on Nanion's SyncroPatch® 384PE

The electrophysiology team at Nanion Technologies GmbH, Munich.

Summary

The hERG gene encodes a potassium channel responsible for the repolarization of the IKr 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.

Here we present high quality data with reliable pharmacology on hERG expressing CHO cells at a high throughput collected on the SyncroPatch® 384PE. Current-voltage plots, and concentration response curves for the compounds pimozide, astemizole, cisapride and terfenadine are shown. The IC_{50} values for these compounds are within the expected range[3-11] and success rates of 80% for completed experiments were recorded.

Results

For the evaluation of the performance of hERG (CHO) cells, Seal Resistance, Cslow and the Series Resistance (Rseries) were determined from one experiment (Fig. 1) .

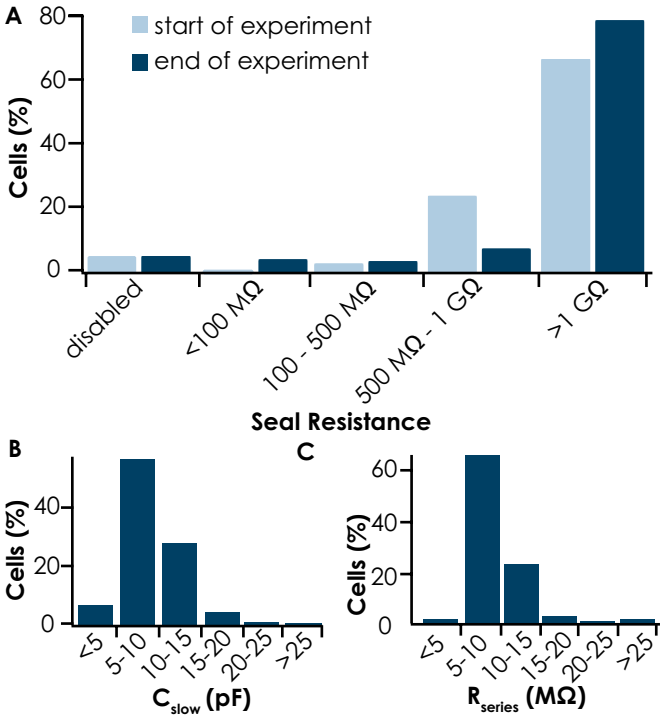

Figure 1: Statistics of hERG expressing CHO cells recorded on one NPC-384 chip on the SyncroPatch® 384PE
A Success rate (seal resistance) of individual CHO cells on the SyncroPatch® 384. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue). **B** Bar graph of cell capacitance (Cslow) and **C** Series Resistance (Rseries) values for CHO cells expressing hERG.

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Currents mediated by hERG could be reliably recorded on the SyncroPatch® 384PE with a high success rate. Figure 2 shows a screenshot of the data acquisition and analysis software of the SyncroPatch® 384PE during an experiment recording the current-voltage relationship of hERG expressed in CHO cells. In this experiment, 84% of cells had a seal resistance > 500 MΩ with a further 12% with a seal resistance > 100 MΩ.

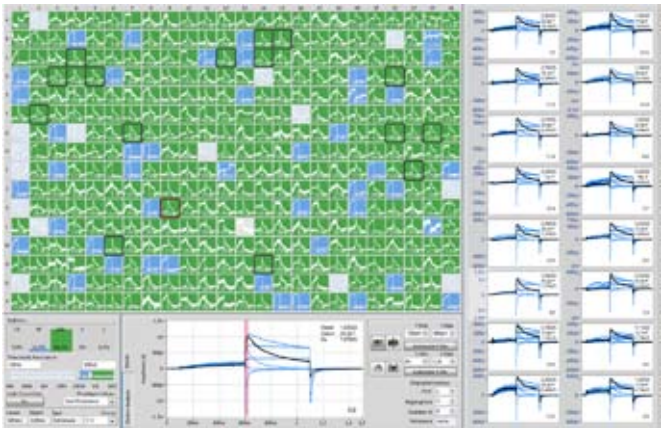

Figure 2: Typical recording from hERG expressed in CHO cells on the SyncroPatch® 384PE. The screenshot shows the data acquisition and analysis software used on the SyncroPatch® 384PE. Wells are color-coded based on seal resistance (green: Rmemb > 500 MΩ, blue: Rmemb = 100 - 500 MΩ, light blue or grey: 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.

Figure 3 shows the current-voltage relationship for an exemplar hERG-expressing CHO cell and block of the current by pimozide (250 nM). Figure 3B and 3D show the online analysis for the current-voltage relationship and the pharmacology experiment, respectively. In Panel D, the vertical lines and color-coded regions show addition and incubation of compound. The white region shows incubation in control solution (external wash solution), the grey region incubation in vehicle (0.3% DMSO) and the blue region incubation in 250 nM pimozide. Each cell was exposed to a single concentration of pimozide and the concentration response curve calculated across the whole plate. The average concentration response curves for four hERG-active compounds are shown in Figure 4. Only cells which satisfied certain quality control criteria (Rmemb, Rseries, Cm and peak current amplitude) were included in the analysis.

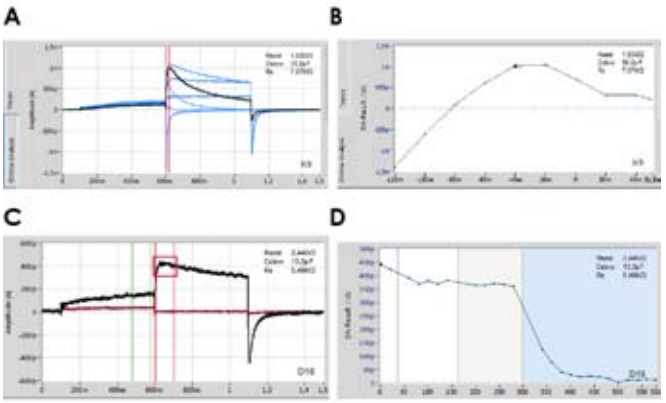

Figure 3: IV and inhibition of hERG channels expressed in CHO cells on the SyncroPatch® 384PE. A hERG-mediated current responses to a voltage step protocol (see Fig. 2). **B** The corresponding current voltage relationship from (A) shows the typical IV curve of hERG channels. **C** Current response of an exemplar cell elicited using a voltage step to -40 mV for 500 ms after a depolarizing step to 60 mV for 500 ms (holding potential -110 mV). The red trace shows inhibition by 250 nM pimozide and the cursors and red bounding box indicate the maximum peak amplitude used for plotting the online analysis timeplot. **D** Corresponding online analysis showing peak amplitude plotted against time. The online analysis is color-coded, the grey region indicates the application of control solution including vehicle (0.3% DMSO) and the blue region indicates application of 250nM pimozide.

A summary of IC_{50} values and success rates for the 4 compounds is shown in Table 1. The estimated IC_{50} values agree well with those found in the literature[3-11]. The success rates for completed experiments were between 69 and 82%.

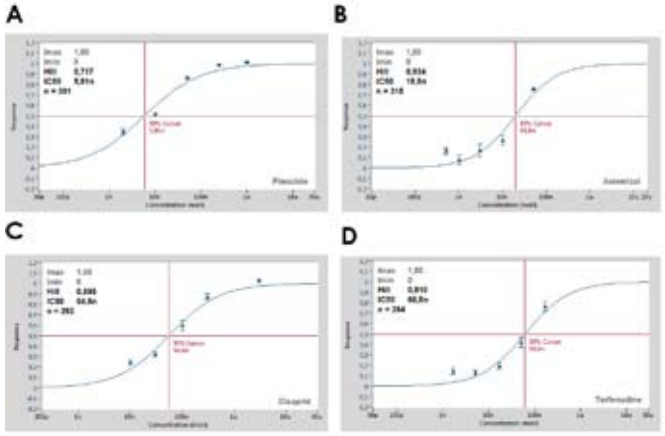

Figure 4: Average concentration response curves for 4 different hERG blockers on the SyncroPatch® 384PE. The concentration response curves were constructed across the whole plate as shown in Figure 5. The SyncroPatch® 384PE analysis software (DataControl® 384) was used to calculate the average concentration 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.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

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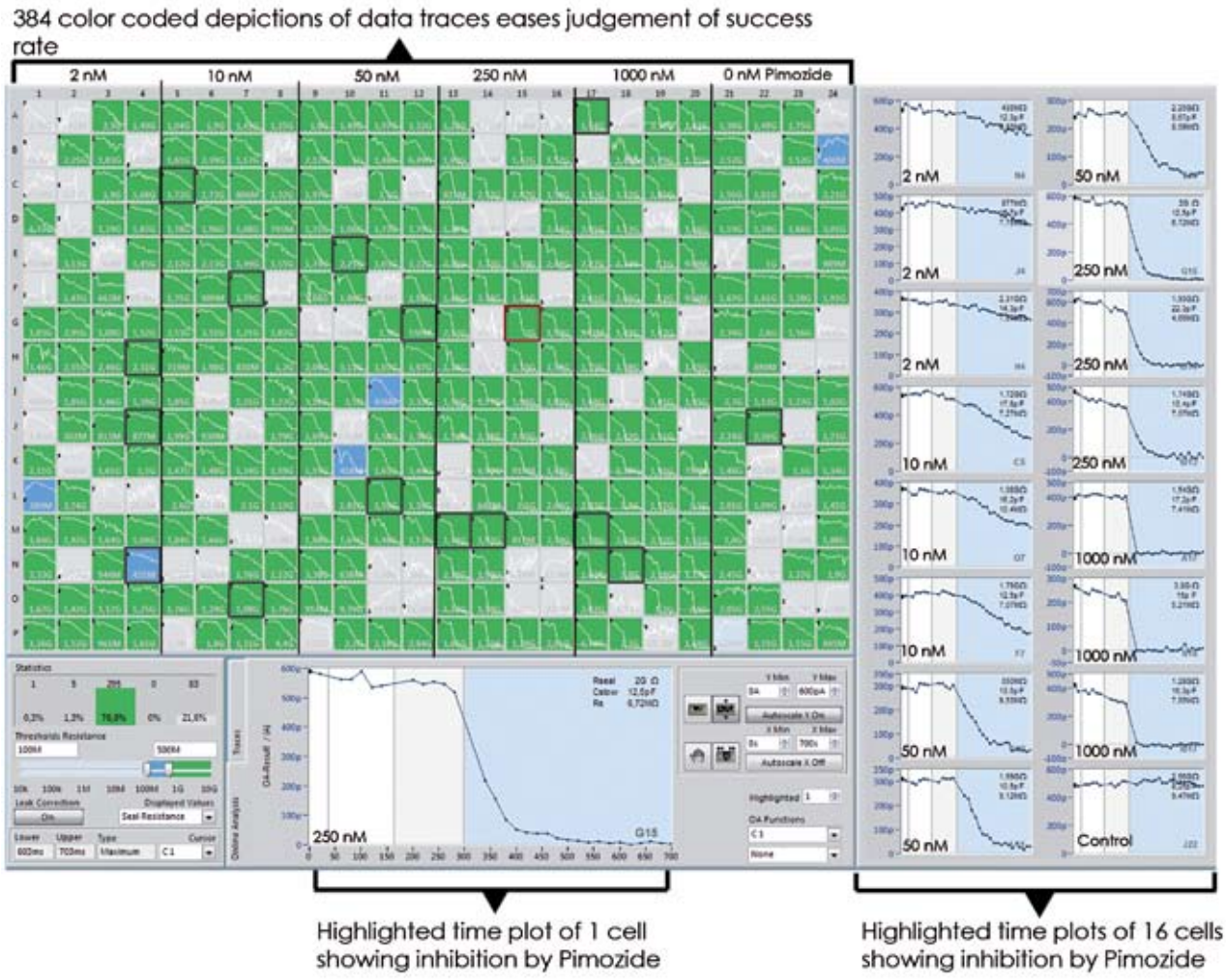

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of hERG expressing CHO cells as recorded on one NPC-384 patch clamp chip. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Depending on the seal resistance, pictures are green (Rmemb > 500 MΩ), blue (Rmemb = 100 – 500 MΩ), light blue or grey (Rmemb < 100 MΩ or cells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes of hERG channels during the voltage step to -40mV during application of control solution (white region), vehicle (0.3% DMSO; grey region) and inhibition by pimozide (blue region).

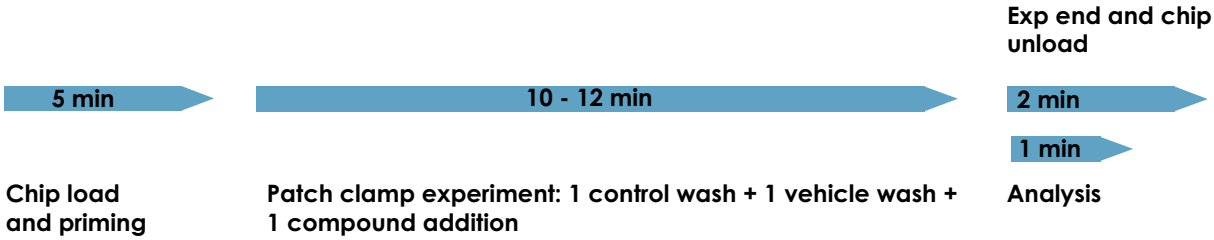

Figure 6: Timeline of an experiment on the SyncroPatch® 384PE. The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a single point concentration response curve on hERG-mediated currents took approximately 17-19 min.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

Compound	IC_{50} (nM)	Success rate (%)	Literature range (nM)
Pimozide	5.9 (301)	78	1 - 18[3,4]
Astemizole	19.8 (315)	82	0.9 - 26[5,6,7]
Cisapride	54.6 (292)	76	6.5 - 44[3,4,6,8]
Terfenadine	60.8 (264)	69	7 - 204[3,6,9,10,11]

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[3-11].

Figure 5 shows a screenshot of the SyncroPatch® 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can choose whether to visualize raw traces or online analysis. Here, online analysis is chosen and the graphs represent current amplitude versus time. An individual well can be highlighted to monitor progression of the experiment. In the Online Analysis view, the time points at which solution additions have been made are indicated by vertical lines, as well as different background colors. In this example, white shows incubation in control solution, grey is vehicle (0.3% DMSO) and blue is pimozide.

In conclusion, hERG expressed in CHO cells can be recorded on the SyncroPatch® 384PE with high success rates for completed experiments (typically >70%). The timeline of each experiment was about 17-19 minutes (start – end) and included wash with control solution, wash with vehicle and > 5 min incubation in 1 concentration of blocker. The current-voltage relationship of hERG recorded on the SyncroPatch® 384PE is in good agreement with the literature[1]. The IC_{50}'s calculated using the SyncroPatch® 384PE's analysis software, DataControl® 384, of pimozide, astemizole, cisapride and terfenadine were in good agreement with those found in the literature[3-11].

The SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording hERG currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

1. Sanguinetti, M. C., *et al.*, 1995. Cell. 81: 299-307
2. Brown, A.M. & Rampe, D. 2000. Pharm. News. 7(4): 15-20
3. Kirsch, G.E., *et al.*, 2004. J. Pharmacol. Toxicol. Methods. 50: 93-101
4. Kang, J., *et al.*, 2000. Eur. J. Pharmacol. 392: 137-40
5. Zhou, Z., *et al.*, 1999. J. Cardiovasc. Electrophysiol. 10(6): 836-43
6. Wang, J., *et al.*, 2003. Am. J. Physiol. Heart Circ. Physiol. 284: H256-H267
7. Chiu, P.J.S., *et al.*, 2004. J. Pharmacol. Sci. 95: 311-319
8. Walker, B.D., *et al.*, 1999. Br. J. Pharmacol. 128: 444-50
9. Davie, C., *et al.*, 2004. J. Cardiovasc. Electrophysiol. 2004. 15(11):1302-9
10. Lacerda, A.E., *et al.*, 2001. Eur. Heart J. Suppl. 3: K23-K30
11. Crumb, W.J. Jr., 2000. J. Pharmacol. Exp. Ther. 292(1): 261-4

Methods

Cells

CHO cells expressing hERG

Cell culture

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

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch® 384PE. A voltage step protocol from -110 mV (holding potential) to +60 mV followed by a step to -40 mV was applied to the cells every 10 s for pharmacology experiments. Peak amplitude at -40 mV was used for analysis.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

nan][on