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Comparative study of hERG channels under physiological temperature (CiPA) conditions using high-throughput automated patch-clamp QPatch and Qube

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Introduction

Cardiac ion channel activity is crucial for generating cardiac action potentials with proper timing and duration. Drug-induced impairment of these ion channels can cause abnormal cardiac activity, including QT interval prolongation, ventricular arrhythmia, and, in the most severe cases, sudden death. These adverse effects are among the leading reasons for drug withdrawal from the market or the denial of regulatory approval for new therapeutic candidates. The ICH E14/S7B Q&A released in August 2022 provided recommended conditions for best practices for *in vitro* assay of I_{Kr}/hERG to maintain reproducibility and consistency in evaluations. These recommendations include testing under physiological temperature conditions, as well as considering factors such as voltage protocols. In this study, we assessed the inhibitory effects of a compound on hERG channels under physiological temperature conditions (35° C) through whole-cell patch clamp experiments using the automated patch clamp system QPatch and Qube 384.

Discussion

Using the temperature control feature of the QPatch and Qube system, we investigated the effects of temperature changes on hERG currents. When the temperature was set at physiological temperature conditions (35° C), the consistent change in current kinetics has been observed compared to the one at room temperature conditions at 25° C in both instruments (Figure 1 and Table 1). This change has been already reported in the previous study using QPC (Table 1). The influence on the kinetics of hERG channels suggests that under physiological temperature conditions, hERG channels are more easily activated, thereby facilitating the binding of compounds with state-dependent and use-dependent properties to hERG channels. Indeed, for many of the compounds tested in this study, an enhancement in the inhibitory effect on hERG channel currents at physiological temperature (35-37° C) compared to 25° C was observed, albeit to varying degrees (Table 2). Furthermore, we developed a high-pressure whole-cell protocol for recording CHO hERG under CiPA conditions including high temperature (35-37° C) and fluoride-free internal solution on the QPatch (Figure 2). Recording conditions can be stable for upwards of 40 mins allowing measurement of five-point cumulative concentration response curves, allowing rapid measurement of IC_{50} values across several compounds simultaneously (Figure 3, Table 3).

Conclusion

In this study, the temperature control feature of the QPatch and Qube enabled detection of temperature-dependent changes in hERG channel currents and variations in the inhibitory effects of compounds. Combined with previous research, we demonstrated that consistent results on the temperature-dependent biophysical and kinetic behavior of hERG channels and drug toxicity effects can be obtained using all three instruments: QPC, QPatch, and Qube. Using QPatch, we are further able to record under CiPA conditions including 35° C and fluoride-free internal solution, allowing cumulative concentration response experiments for upwards of 40 mins. Testing under physiological temperature on automated patch clamp systems allows faster and accurate measurement of the inhibitory effects of compounds on ion channels, increasing the predictive value of in vitro electrophysiological data when evaluating cardiac safety risks of preclinical compounds.

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Results

Temperature effect on kinetics of hERG current

Under the physiological temperature condition (35° C), the average tail current value was larger compared to that at 25° C (Table 1, Fig. 1B and 1C). Additionally, the rise of the tail current and the transition to the steady state were faster, indicating that the dynamics of the hERG channel were accelerated at 35° C. All of these results were consistent with the results on QPC in the previous study (Table 1).

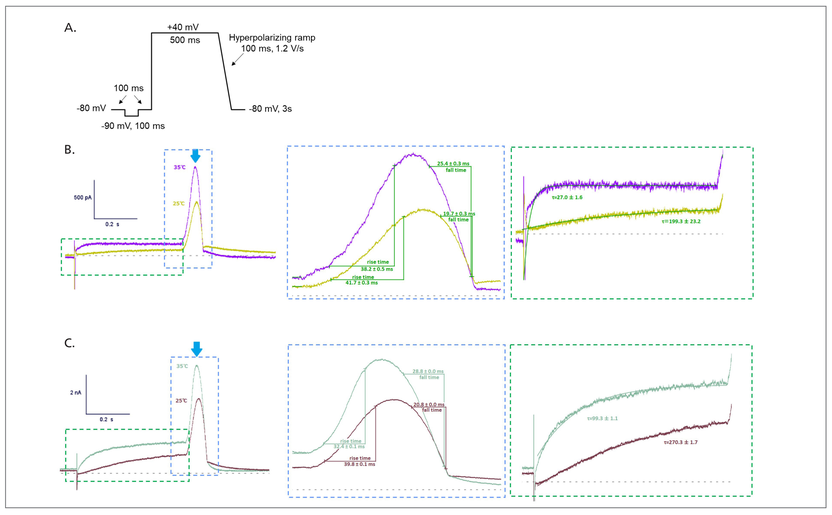

Fig. 1: Voltage protocol applied to hERG channel-expressing cells and the temperature dependence of the elicited hERG currents. **(A)** Stimulation voltage protocol. **(B)** Sweep of hERG currents measured on QPatch demonstrating temperature-dependent changes. The maximum value of tail current size (arrow head) was measured as a tail current amplitude. Tail current (blue dotted area) and steady state current (green dotted area) are shown in enlarged images. Average rise and fall times from 10% to 90% are indicated in tail current image. The average time constant at each temperature is indicated **(C)** Sweep of hERG currents measured on Qube.

Table 1: Tail current amplitude, rise time, fall time, steady-state current amplitude, and time constants of steady-state current at 25° C or physiological temperature (35° C or 37° C) conditions (mean ± S.E.). The data for QPC were obtained in the previous study.

Group Name	Tail current amplitude [pA]	Tail rise time [ms]	Tail fall time [ms]	SS current amplitude [pA]	Steady-state τ [ms]	n
QPC 25°C	661.7 ± 110.0	38.1 ± 0.9	19.3 ± 0.8	120.1 ± 16.6	150.1 ± 24.1	17
QPC 37°C	1875.4 ± 311.8	31.8 ± 1.7	28.0 ± 1.3	399.9 ± 61.3	12.7 ± 2.1	17
QPatch 25°C	846.0 ± 35.8	41.7 ± 0.3	19.7 ± 0.3	123.4 ± 7.0	199.3 ± 23.2	168
QPatch 35°C	1059.0 ± 43.8	38.2 ± 0.5	25.4 ± 0.3	228.3 ± 16.8	27.0 ± 1.6	135
Qube 25°C	2761.0 ± 21.4	39.8 ± 0.1	20.8 ± 0.0	713.7 ± 7.1	270.3 ± 1.7	1290
Qube 35°C	5909.2 ± 45.1	32.4 ± 0.1	28.8 ± 0.0	2002.1 ± 18.8	99.3 ± 1.1	925

We would like to express our sincere gratitude to B'SYS GmbH for generously providing us with CHO cells that stably express the hERG (K_v11.1) channel.

Comparison of hERG inhibitors at 25° C and 35° C

We examined the inhibitory effects of hERG channel currents at both 25° C and 35° C with QPatch and Qube for a total of seven compounds, including erythromycin, known for its temperature-dependent inhibition effect (Table 2, Figure 2). The most significant difference between the two temperatures was observed with erythromycin, yielding results consistent with previous experimental findings and literature values[1,2]. For dofetilide, moxifloxacin and ondansetron, IC_{50} values consistent with those shown in the ICH training material[4] and other literature[5,6,7]. The IC_{50} values for other compounds were also within a range consistent with literature values, without contradictions[1,2,3].

Table 2: Overview of IC_{50} values of selected compounds under conditions at 25° C or physiological temperature (35-37° C) tested on QPC, QPatch or Qube and literature values of IC_{50} for each compound. IC_{50} values in the blue cells were obtained from cumulative concentration response experiments.

	IC_{50} [μM]						ref. IC_{50} value range [μM]
Temperature	25° C	25° C	25° C	36-37° C	35° C	35° C	
System	QPC	QPatch	Qube	QPC	QPatch	Qube	
Dofetilide	0.029 (n=21)	0.038 (n=10)	0.015 (n=177)	0.0069 (n=16)	0.017 (n=11)	0.0044 (n=84)	0.013 (37° C)[5] , 0.01[4]
Moxifloxacin	108 (n=14)	116 (n=9)	154 (n=163)	32 (n=14)	38 (n=6)	39 (n=114)	65 (RT)[6], 29 (35° C)[6], 62[4]
Ondansetron	1.6 (n=15)	1.5 (n=8)	1.7 (n=159)	1.2 (n=21)	0.69 (n=7)	0.76 (n=125)	0.81[7], 1.4[4]
E-4031	0.031 (n=7)	0.040 (n=7)	0.015 (n=177)	0.0093 (n=5)	0.012 (n=12)	0.0048 (n=106)	0.14 (22° C)[1], 0.012 (35° C)[1]
Erythromycin	1336 (n=4)	891 (n=9)	286.3 (n=165)	75 (n=4)	147 (n=6)	80 (n=110)	1410 (22° C)[1], 115 (35° C)[1], 72.2 (34° C)[2]
Quinidine	0.91 (n=4)	1.0 (n=12)	1.6 (n=163)	0.62 (n=8)	0.63 (n=7)	0.90 (n=140)	0.72 (RT)[3]
Sotalol	240 (n=4)	282 (n=4)	155 (n=152)	77 (n=5)	128 (n=8)	67 (n=138)	810 (22° C)[1], 269 (35° C)[1], 74-169[2]

Cumulative concentration-response under CiPA protocol

For GLP or CiPA compatible safety screening, it is required to measure drug interactions under physiological temperature (35-37° C) without fluoride. Here we developed a whole-cell protocol for QPatch using physiological solutions (Sophion EC000 and IC000) at 35° C, allowing fluoride-free measurements compatible with FDA requirements for hERG cardiac safety screening (Figure 2). Experiment duration ranged from 41.1 to 44.7 mins allowing measurement across five cumulative concentrations of four hERG blockers: cisapride, dofetilide, quinidine, and verapamil (Figure 3, Table 3).

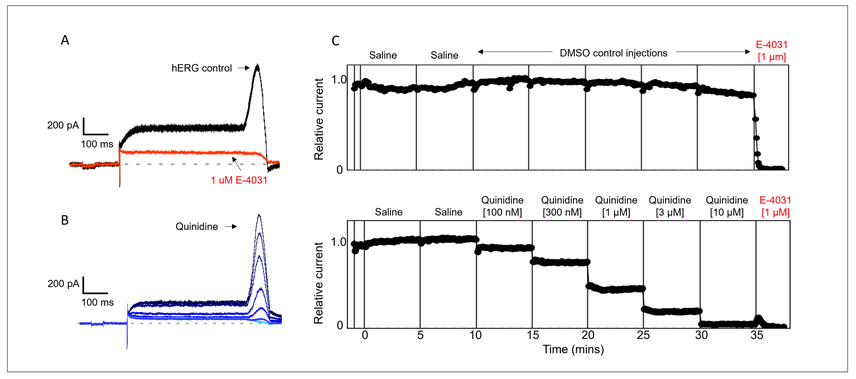

Fig. 2: Representative hERG experiment with KCl internal solution at 35° C on the QPatch. **(A)** hERG current traces under control conditions and block by reference compound 1 μM E-4031. **(B)** Cumulative inhibition by quinidine (0.1 – 10 μM). **(C)** Current-time plots for the experiments in A and B showing the relative stability of the current over 40 mins of recording at 35°C, followed by acute block with 1 μM E-4031.

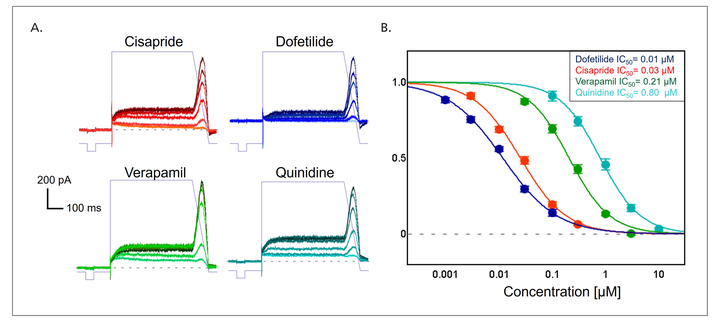

Fig. 3: Concentration-response experiments with hERG inhibitors at 35° C. **(A)** Representative hERG current traces showing concentration-dependent inhibition through 40 mins of recording at 35° C. **(B)** Concentration-response plot for all tested drugs: cisapride (red), dofetilide (blue), quinidine (teal), and verapamil (green). Reference compound IC_{50} values were calculated using a normalized Group Hill Fit and were consistent with published results (Table 3).

Table 3: Summary of IC_{50} measurements under CiPA protocol. Reference values from literature were similar to those collected on the automated system, supporting the utility of QPatch for collecting accurate IC_{50} data under CiPA conditions. n values include cells that passed quality filters of membrane resistance > 100 MΩ and C_{slow} > 4 pF. Experiment duration is shown as mean ± S.E.

Compound	Measured IC_{50} [μM]	Reference IC_{50} [μM]	Experiment duration (mins)	n
DMSO control	-	-	43.9 ± 0.3	32
Cisapride	0.022	0.010[8]	41.1 ± 0.2	10
Dofetilide	0.011	0.013[5]	44.7 ± 0.1	28
Quinidine	0.86	0.80[9]	44.7 ± 0.3	20
Verapamil	0.24	0.25[10]	44.8 ± 0.2	32

Materials and methods

Cell culture and preparation: Cells were cultured according to the Sophion SOP. CHO cells heterogeneously expressing hERG (KV11.1) channel were kindly provided by B'SYS GmbH. The cells were harvested using Detachin™ (Genlantis) and transferred to serum-free medium (EX-CELL® ACF CHO Medium, Sigma-Aldrich) supplemented with 25 mM HEPES, 40 μg/mL trypsin inhibitor, and penicillin/streptomycin. The cells were washed and resuspended in Sophion EC000 extracellular buffer.

Patch clamp experiment: All patch clamp experiments were carried out using the QPatch or Qube system (Sophion Bioscience A/S, Denmark).

Solutions: Extracellular solution (in mM): 145 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose, pH7.4. Intracellular solution (in mM): 120 KF, 20 KCl, 10 HEPES, 10 EGTA, pH 7.2. KCl intracellular solution (in mM): 120 KCl, 5.374 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 4 Na_2-ATP, pH 7.2.

Compounds: All compounds except erythromycin and moxifloxacin were dissolved in 100% DMSO and diluted 1000 times in extracellular solution to make compound solution at each test concentration. Erythromycin and moxifloxacin were dissolved in extracellular solution in the day of use at highest test concentration and then diluted to make lower test concentrations.

Whole-cell protocol: The whole-cell configuration was established using a modified whole cell protocol for CHO cells.

Voltage protocols: The voltage stimulation protocol proposed by the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative was used in this study. The protocol consisted of a +40 mV depolarizing pulse of 500 ms followed by a 100 ms hyperpolarizing ramp down from +40 mV to -80 mV, repeated at 5 second intervals. Cells were held at -80 mV between sweeps.

Test procedure and temperature control: During the experiment, the temperature at the recording site was clamped using water-circulation temperature control system. The temperature at recording site was held at 25° C or 35° C during the whole cell process and subsequent experiment. After establishment of a whole-cell configuration, vehicle control solution (0.1% DMSO extracellular solution) was added, and then the voltage protocol was executed to measure the tail current as a baseline. Subsequently, compound solutions were added cumulatively at 3 to 5 concentrations on QPatch or single concentration solution at multiple times on Qube, and the tail currents at each concentration were recorded. At the end of experiment, 1 μM E-4031 solution was applied to block the hERG current and used as a reference.