Sophion Bioscience K.K.
11-28, Asahi-cho 1-chome, Honjo-shi
Saitama 367-0026, Japan
info@sophion.com

Scan & learn more

Whole-cell patch clamp recording for hERG channel under physiological temperature using high throughput automated patch clamp system QPatch and Qube 384

Kazuya Tsurudome, Hironori Ohshiro, Taku Izumi

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.

Conclusion

In this study, the temperature control feature of the QPatch and Qube enabled us to detect 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: QPatch Compact (QPC), QPatch, and Qube. Using QPatch and Qube, it is possible to generate data of compound effect on hERG channels in higher throughput compared to QPC. Testing under physiological temperature conditions using the temperature control feature of those automated patch clamp systems allows faster and accurate measurement of the inhibitory effects of compounds on ion channels, contributing to the provision of reliable data for *in silico* modeling as advocated by CiPA.

Discussion

Using the temperature control feature of the QPatch and Qube system, we investigated the effects of temperature changes on hERG channel 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 (Fig.1 and Table 1). This change has already been 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 (Fig. 2, Table 2)

The inhibitory effect of compounds on hERG channel were measured and compared between room temperature (25°C) and physiological temperature (35°C). The IC_{50} values of dofetilide, moxifloxacin, and ondansetron obtained in this study were consistent with the reported values in the ICH training material[4]. For E-4031 and dofetilide, it is possible that the changes in IC_{50} values were due to the compound's effect manifesting more quickly at higher temperatures, as it takes longer for these compounds to exert their inhibitory effects at room temperature. As expected, these slow-effective compounds were more likely potent on single-dose repetitive application assay on Qube. Even at 25°C, the IC_{50} values for these compounds were smaller on Qube compared to the one on QPatch (Table 2).

Results

The effect of temperature on hERG current

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).

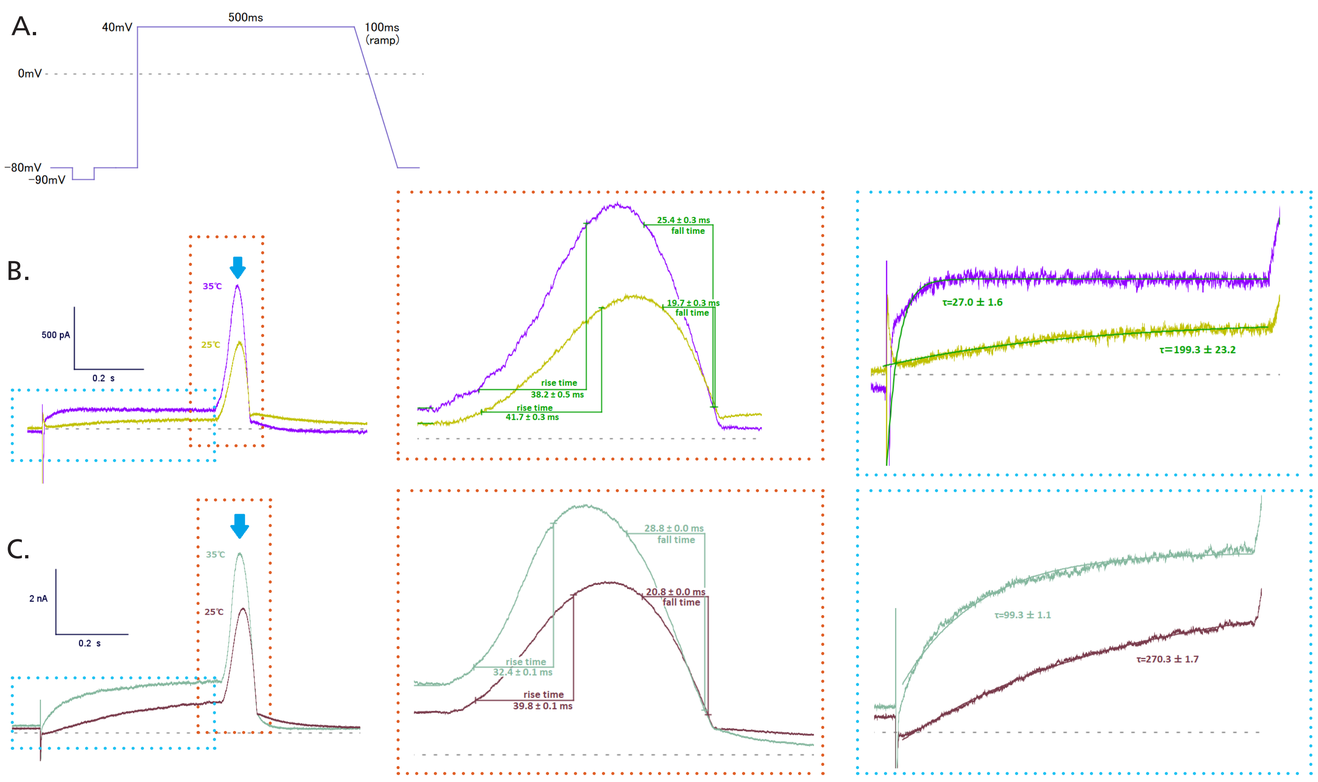

Fig. 1: Voltage protocol applied to hERG channel-expressing cells and the temperature dependence of the elicited hERG currents. **A)** Stimulation voltage protocol. **B)** Sweep of hERG currents measured on QPatch demonstrating temperature-dependent changes. The maximum tail current size (arrowhead) value was measured as a tail current amplitude. Tail current (orange dotted area) and steady-state current (blue 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

Comparison of potency of compounds on hERG current at 25°C and 35°C

IC_{50} values of selected compounds with cumulative application at 25°C or 35°C on QPatch & Qube

In this study, 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, Fig. 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 literatures[5,6,7]. The IC_{50} values for other compounds were also within a range consistent with literature values, without contradictions[1,2,3].

IC_{50} comparison between single dose application and cumulative application

Each cell was exposed compound solution at single concentration repeatedly to reach steady state compound effect for each concentration in Qube experiment. In the previous study, it was shown that dofetilide and E-4031 took a long time to reach a steady state of compound effect. It was suggested that repetitive application of same concentration would help to reach a steady state of compound effect. As expected, dofetilide and E-4031 showed higher potency even at 25°C compared to those observed in QPatch experiments (Table 2, Fig. 2).

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 multiple concentrations application experiments. The data for QPC were obtained in the previous study. (n=3 or 20 for each concentration for single dose application experiments on QPC or Qube, respectively).

	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]

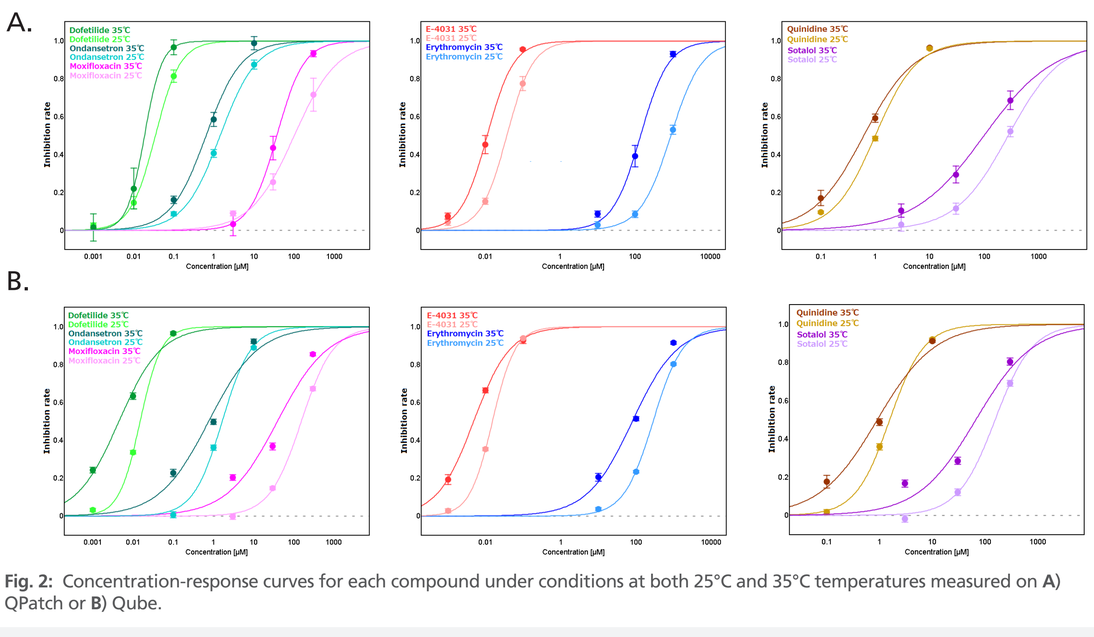

Fig. 2: Concentration-response curves for each compound under conditions at both 25°C and 35°C temperatures measured on **A)** QPatch or **B)** Qube.

Materials and methods

Cell culture and preparation: The cells were cultured according to the Sophion SOP. CHO cells heterogeneously expressing hERG (K_V11.1) channel were kindly provided by B'SYS GmbH. The cells were harvested using trypsin. Cells were 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 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 for QPatch and Qube (in mM): 145 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose, pH7.4. Intracellular solution for QPatch (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. Intracellular solution for Qube (in mM): 120 KF, 20 KCl, 10 HEPES, 10 EGTA, 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: During the compound potency measurement experiment, the cells were held at holding potential of -80 mV. The voltage stimulation protocol proposed by the CiPA (Comprehensive *in vitro* Proarrhythmia Assay) initiative was used in this study. The voltage protocol consisted of a 40 mV depolarizing pulse of 500 ms followed by a 100 ms ramp down from 40 mV to -80 mV, repeated at 5 sec intervals.

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 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 three 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.

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.

References:

1. Kirsch, G. E. et al. Variability in measurement of hERG potassium channel inhibition: effects of temperature
1. Kirsch, G. E. et al. Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. *J. Pharmacol. Toxicol. Methods* **50**, 93–101 (2004).
2. Redfern, W. S. et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: Evidence for a provisional safety margin in drug development. *Cardiovasc. Res.* **58**, 32–45 (2003).
3. Kramer, J. et al. MICE Models: Superior to the HERG Model in Predicting Torsade de Pointes. *Sci. Rep.* **3**, (2013).
4. ICH E14/S7B Q&A Training Material Examples Supplemental File (https://database.ich.org/sites/default/files/ICH_E14-S7B_TrainingMaterial_2022_0407.pdf). *Ema/chmp/ich/415588/2020 Comm. Hum. Med. Prod. File* **ICH e14/s7**, (2022).
5. Orvos, P. et al. Evaluation of Possible Proarrhythmic Potency: Comparison of the Effect of Dofetilide, Cisapride, Sotalol, Terfenadine, and Verapamil on hERG and Native i_{Kr} Currents and on Cardiac Action Potential. *Toxicol. Sci.* **168**, 365–380 (2019).
6. Alexandrou, A. J. et al. Mechanism of hERG K + channel blockade by the fluoroquinolone antibiotic moxifloxacin. *Br. J. Pharmacol.* **147**, 905–916 (2006).
7. Wempe, M. F. New Insights into Ion Channels: Predicting hERG-Drug Interactions. *Int. J. Mol. Sci.* **23**, (2022).

Presented @JSPS 2025