SOPHION BIOSCIENCE

Application Report

hERG compound screening at 22°C and 35°C on QPatch II

Temperature control and ion channel kinetics: the importance of controlling the temperature in electrophysiology

Summary

- hERG channels are voltage-gated K^+ channels that play an important role in action potential repolarization;
- hERG channel is important in cardiac drug safety assessment and is commonly screened at room temperature;
- Since temperature influences ion channel conductance and gating kinetics, it is crucial to assess drug screening at physiological temperature (35-37°C);
- Several drugs and compounds acting on hERG channel have been shown to be temperature-dependent.

In this study, by using our QPatch II system and the temperature control feature, we report a significant difference in hERG kinetics at 22°C and 35°C. By testing four hERG blockers, we were able to measure channel activity and investigate compound effects at the two temperatures.

IC_{50} values obtained from the compounds tested on the QPatch II are similar to those obtained on the Qube 384 and agree with literature.

Introduction

The interplay of various ion channels influences cell behaviour and establishes a dynamic equilibrium, which is crucial for determining the firing properties in excitable cells, such as neurons or cardiomyocytes.

Among the several factors that may influence this dynamic equilibrium, temperature specifically affects ion channel conductance and gating kinetics, which in turn may change action potential properties [1, 2]. It is also well established that the effect of drugs and compounds on different ion channels can be temperature-dependent [2].

hERG channels are voltage-gated K^+ channels that play a crucial role in action potential repolarization. They are expressed in different tissues, mainly in cardiac cells [3]. hERG channels present a slow activation, which is coupled with a rapid inactivation and recovery from inactivation. Due to its properties, hERG current (I_{Kr}) influences action potential duration since its flux is active during the plateau phase. When repolarization starts, the channel recovers rapidly from inactivation, ensuring I_{Kr} presence in the action potential repolarization phase.

As reported in the literature, both the activation and inactivation kinetics of hERG K^+ channels are temperature dependent [4, 5, 6]. In addition, temperature influences the state of the channel because of the energetic changes caused by the formation of chemical bonds necessary to initiate the conformational process [7, 8].

Routinely, drug screening assays on hERG channels are performed at room temperature (22-25°C), and physiological temperature parameters are extrapolated after screening [7, 8]. This is because physiological temperature recordings (35-37°C) may be challenging since the membrane becomes more fluid, reducing the ability to form high resistance and stable electrical seals. However, as hERG channel plays a vital role in drug safety assessment, it is crucial to investigate its properties at physiological temperature.

Here, we demonstrate that by using our APC system, the QPatch II, we can perform drug screening experiments for long compound exposures times (up to 5 minutes for each concentration) at physiological temperature using physiological recording solutions.

Results and discussion

Performing hERG experiments at 22°C and 35°C

Although conducting hERG experiments at room temperature might be expected to give more long-lasting experiments, compared to physiological temperature, success rate values were comparable between the two temperatures (up to 60%) when applying the following filters (Figure 1):

- R_{mem} >100 MΩ;
- 4 pF < C_{slow} > 35 pF;
- Baseline hERG current > 200 pA

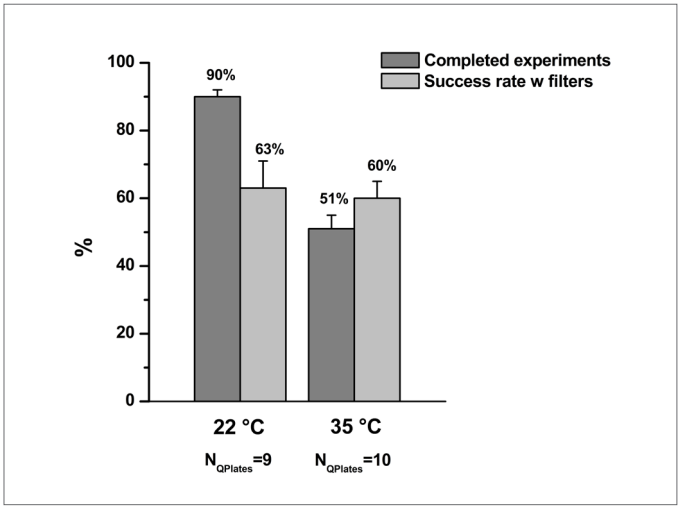

Fig. 1: Success rate comparison between 22°C (left) and 35°C (right). Values are given as average ± standard error of the mean (SEM). Completed experiments are calculated as experiments completed/experiments started; success rate w filters include incomplete experiments and is calculated with the filters mentioned above.

hERG current kinetics at 22°C and 35°C

Current parameters differed between the two temperatures (Table 1 and Figure 2), as reported in literature [4, 5, 6]. Statistical comparison of temperature-dependent effect was performed using an unpaired t-test. As shown in Table 1, the activation and the tail current density were significantly higher (P<0.0001) at 35°C compared to 22°C; the current tau decay was faster at 35°C and significantly different (P<0.0001) compared to 22°C (Table 1).

C_{slow} values (pF) were slightly lower at 35°C compared to 22°C. This difference might be explained by changes in lipid composition at high temperatures (less dense lipids and more fluid membranes), which could influence cell capacitance [9]. Since our external solution is hyperosmotic and high temperatures also affect osmolarity [10], the consequent faster water molecules movement across the membrane could also explain the difference observed in cell capacitance.

Table 1: hERG channel properties at 22°C and 35°C. Values are presented as average ± SEM.

Temperature	22° C	35° C
No. of experiments	143	140
C_{slow} (pF)	15.6 ± 0.4	11.0 ± 0.4
Activation current (pA)	63.5 ± 8.2	654.7 ± 40.9
Activation current density (pA/pF)	4.3 ± 0.5	64.0 ± 3.5
Tail current (pA)	443.9 ± 16.6	601.1 ± 28.3
Tail current density (pA/pF)	29.6 ± 1.0	58.9 ± 2.8
Current Tau decay (ms)	1480.7 ± 56.9	1027.7 ± 245.6

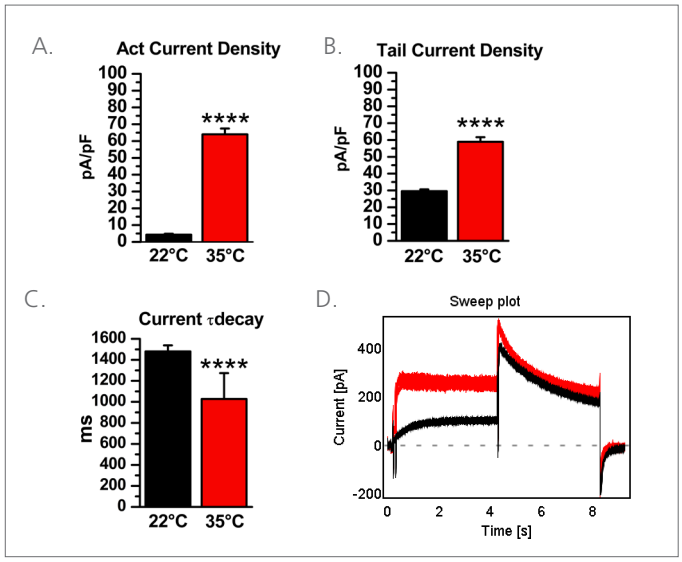

Fig. 2: hERG current comparison at 22°C (black) and 35°C (red). (A) Activation current density; (B) tail current density (C) current tau decay; (D) representative current traces. Unpaired t-test p<0.0001****. Values are presented as average ± SEM.

hERG pharmacology at 22°C and 35°C

Four-point cumulative concentration-response experiments were performed with four hERG blockers (cisapride, quinidine, verapamil, and erythromycin). All compounds tested successfully at 22°C and 35°C, and among these compounds, only erythromycin blocked hERG in a temperature-dependent manner (Figure 4). Grouped Hill-fits were created to determine IC_{50} values, shown in Table 2.

Cisapride

As reported in literature [6, 11, 12, 13, 14], IC_{50} values of cisapride showed an equally potent concentration-dependent inhibition at 22°C (IC_{50}: 0.048 µM) and 35°C (IC_{50}: 0.053 µM). Both IC_{50} values are in good agreement with previously reported data on our other APC system, the Qube [15].

Quinidine

Quinidine IC_{50} values (22°C: 0.975 µM, 35°C: 1.047 µM) were in agreement with literature [6, 12, 14] at both temperatures, with no temperature-dependent effects.

Verapamil

Verapamil IC_{50} values at 22°C (IC_{50}: 0.864 µM) were in agreement with literature [6, 12, 13, 14], but the reported values at 35°C (IC_{50}: 1.697 µM) were slightly higher than literature. No temperature-dependent effects were observed.

Erythromycin

Erythromycin exhibited increased potency at 35°C (IC_{50}: 170.9 µM at 35°C compared to IC_{50}: >>300 µM at 22°C). This has also been previously reported in literature, supported by the notion that transmembrane movement of erythromycin is temperature-sensitive [6, 12, 13]. IC_{50} values at both temperatures were also similar to values obtained on Qube [15].

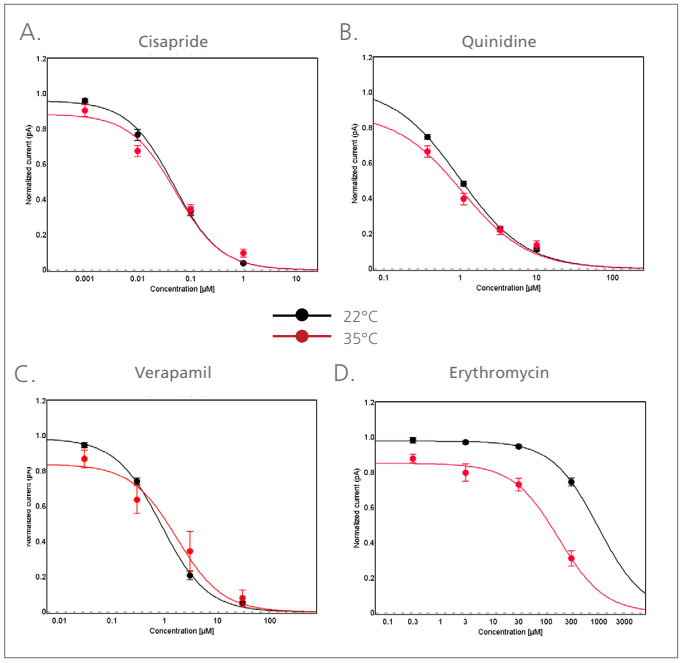

Fig. 3: Hill-fits showing compound effect at 22°C (black) and 35°C (red). (a) cisapride; (b) quinidine; (c) verapamil; (d) erythromycin. Peak tail currents were normalized to the 2nd compound addition, in this case, saline control. Hill coefficient was fixed to 1. Values are presented as average ± SEM.

Table 2: IC_{50} values of four hERG blockers at 22°C and 35°C. References 12 and 14 include data at 22-24°C; References 6, 11 and 13 include data at 22°C and 35°C. Reference 15 includes data from Qube 384.

Compound name	IC_{50} values (µM) on QPatch II		Fold change (IC_{50} 22°C/ IC_{50} 35°C)	IC_{50} values (µM) on Qube 384		Literature IC_{50} values (µM)		Literature
	22°C	35°C		18°C	34°C	20-22°C	35-37°C	
Cisapride	0.048	0.053	0.9	0.123	0.014	0.02 - 0.16	0.02 - 0.2	6, 11, 12, 13, 15
Quinidine	0.975	1.047	0.9	NN	NN	0.2-0.9	0.8	6, 12, 14
Verapamil	0.864	1.697	0.5	NN	NN	0.25-0.45	0.37	6, 12, 13, 14
Erythromycin	>>300	170.994	NN	>>600	55	1410	199-387	6, 12, 13, 15

Methods

- Cells: CHO-hERG cells kindly provided by B'SYS
- Harvesting: cells were harvested following Sophion standard procedures (SOP14097-24)
- The whole-cell configuration was established by a QPatch II standard whole-cell protocol
- A baseline current was recorded, followed by the addition of four increasing concentrations
- Voltage protocol: conditioning pre-pulse at +20 mV for 4 sec, followed by a test single-pulse at -50 mV for 4 sec. Holding potential: -90 mV. See Figure 4 below.
- Compounds: the following hERG blockers were tested at four concentrations:
- Quinidine 10 µM (3-fold dilution); verapamil 30 µM (10-fold dilution); cisapride 1 µM (10-fold dilution); erythromycin 300 µM (10-fold dilution).

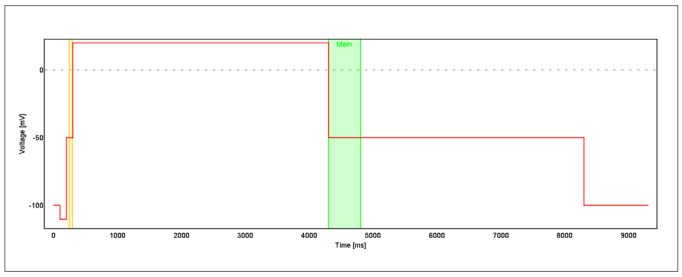

Fig. 4: Voltage protocol used for compound testing.

References

1. Chowdhury, S., Jarecki, B. W., & Chanda, B. (2014). A molecular framework for temperature-dependent gating of ion channels. Cell, 158(5), 1148-1158.
2. Windley, M. J., Lee, W., Vandenberg, J. I., & Hill, A. P. (2018). The temperature dependence of kinetics associated with drug block of hERG channels is compound-specific and an important factor for proarrhythmic risk prediction. Molecular Pharmacology, 94(1), 760-769.
3. Vandenberg, J. I., Perry, M. D., Perrin, M. J., Mann, S. A., Ke, Y., & Hill, A. P. (2012). hERG K+ channels: structure, function, and clinical significance. Physiological reviews.
4. Vandenberg, J. I., Varghese, A., Lu, Y., Bursill, J. A., Mahaut-Smith, M. P., & Huang, C. L. H.(2006). Temperature dependence of human ether-a-go-go-related gene K+ currents. AmericanJournal of Physiology-Cell Physiology, 291(1), C165-C175.
5. Zhou, Z., Gong, Q., Ye, B., Fan, Z., Makielski, J. C., Robertson, G. A., & January, C. T. (1998).Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. Biophysical journal, 74(1), 230-241.
6. Kirsch, G. E., Trepakova, E. S., Brimecombe, J. C., Sidach, S. S., Erickson, H. D., Kochan, M.C., ... & Brown, A. M. (2004). Variability in the measurement of hERG potassium channelinhibition: effects of temperature and stimulus pattern. Journal of pharmacological and toxicological methods, 50(2), 93-101.
7. Lei, C. L., Clerx, M., Gavaghan, D. J., Polonchuk, L., Mirams, G. R., & Wang, K. (2019). Rapid characterization of hERG channel kinetics I: using an automated high-throughput system. Biophysical Journal, 117(12), 2438-2454.
8. Lei, C. L., Clerx, M., Beattie, K. A., Melgari, D., Hancox, J. C., Gavaghan, D. J., ... & Mirams, G. R. (2019). Rapid characterization of hERG channel kinetics II: temperature dependence. Biophysical Journal, 117(12), 2455-2470.
9. Simons, K., & Vaz, W. L. (2004). Model systems, lipid rafts, and cell membranes. Annu. Rev. Biophys. Biomol. Struct., 33, 269-295.
10. Shin, S., & Kim, A. S. (2018). Temperature Effect on Forward Osmosis. Osmotically Driven Membrane Processes—Approach, Development and Current Status; InTech: London, UK, 87-110.
11. Walker BD, Singleton CB, Bursill JA, et al. Inhibition of the human ether-a-go-go-related gene (HERG) potassium channel by cisapride: affinity for open and inactivated states. Br JPharmacol. 1999;128(2):444-450. doi:10.1038/sj.bjp.0702774.
12. Windley, Monique J.; Abi-Gerges, Najah; Fermini, Bernard; Hancox, Jules C.; Vandenberg,Jamie I.; Hill, Adam P. (2017). Measuring kinetics and potency of hERG block for CiPA.Journal of Pharmacological and Toxicological Methods, (), S1056871916301940–.doi:10.1016/j.vascn.2017.02.017
13. Lacerda, A. E., Kramer, J., Shen, K. Z., Thomas, D., & Brown, A. M. (2001). Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs. European heart journal supplements, 3(suppl_K), K23-K30.
14. Kramer, J.et al. MICE Models: Superior to the HERG Model in Predicting Torsade de Pointes.Sci. Rep.3, 2100; DOI:10.1038/srep02100 (2013)
15. Jacobsen R., Temperature control on Qube 384 – pharmacological dependency of hERG reference compounds (2017).

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