SOPHION BIOSCIENCE

Application Report

# Temperature control on Qube 384 - pharmacological dependency of hERG reference compounds

Studies of temperature dependencies can be done efficiently and reliably on Qube 384. In this study we directly measure change in potency with change in temperature on four different compounds, emphasizing the importance of temperature when studying drug candidates.


## Summary

To better understand how compounds function in warm-blooded animals such as humans, it is key to be able to test compound potency at physiological temperature. Our automated patch clamp systems provide real time measurement of temperature, and thus the effect of temperature change. Temperature regulation is done directly at the measurement site, and not by thermal control of the whole system. Four different compounds were tested in a dose-response format on Qube 384 at differing temperatures. All four compounds showed increased effect at elevated temperature compared to room temperature. Qube is suitable for screening compounds at temperatures from 8° C to 40° C (or 8° C above dew point).


## Introduction and scope

The human Ether-à-go-go-Related Gene (hERG or KCNH2) codes for a potassium channel (the protein $K_V11.1$) which is important for the physiology of the heart. Because hERG block can lead to long QT syndrome, cardiac arrest and sudden death it is targeted for safety screening of new compounds, it is also a formal requirement by regulatory authorities.

This study shows that the effect of some hERG reference compounds is temperature sensitive.

## Result and discussion

The shift in $IC_{50}$ and Hill slope (18° C vs. 34° C) can be used, and should be noted from this report.

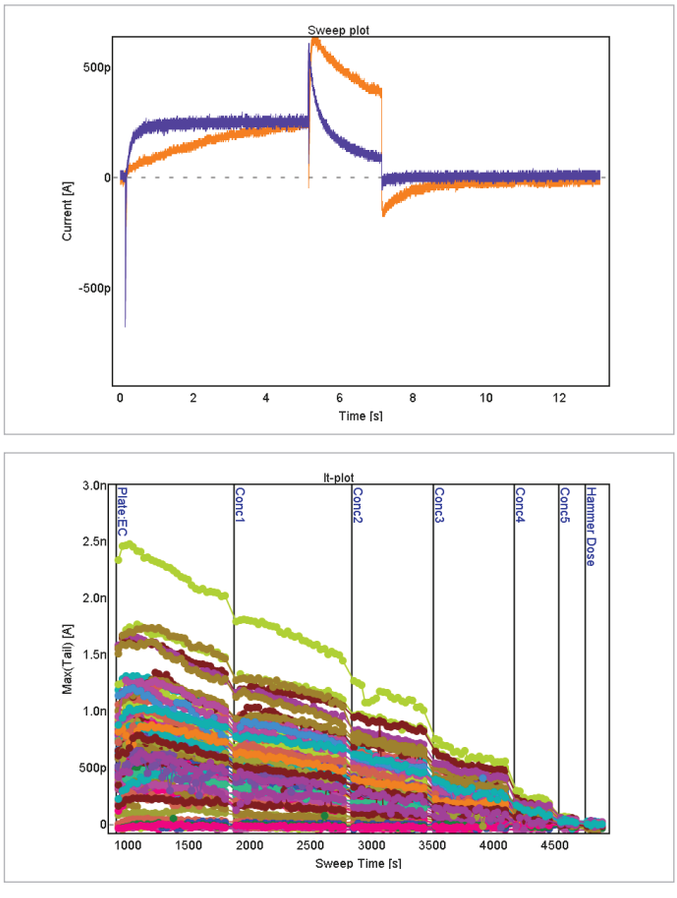



**Fig. 1:** hERG expressing CHO cells were exposed to the voltage protocol shown in Figure 4. Top; representative traces recorded at two different temperatures (Orange = 18° C and Blue = 34° C). The peak tail current at t=5 s is used for the time course (IT-plot, bottom panel). Bottom; example of time courses of peak current in the presence of increasing concentration of cisapride (1 nM to 1 µM).

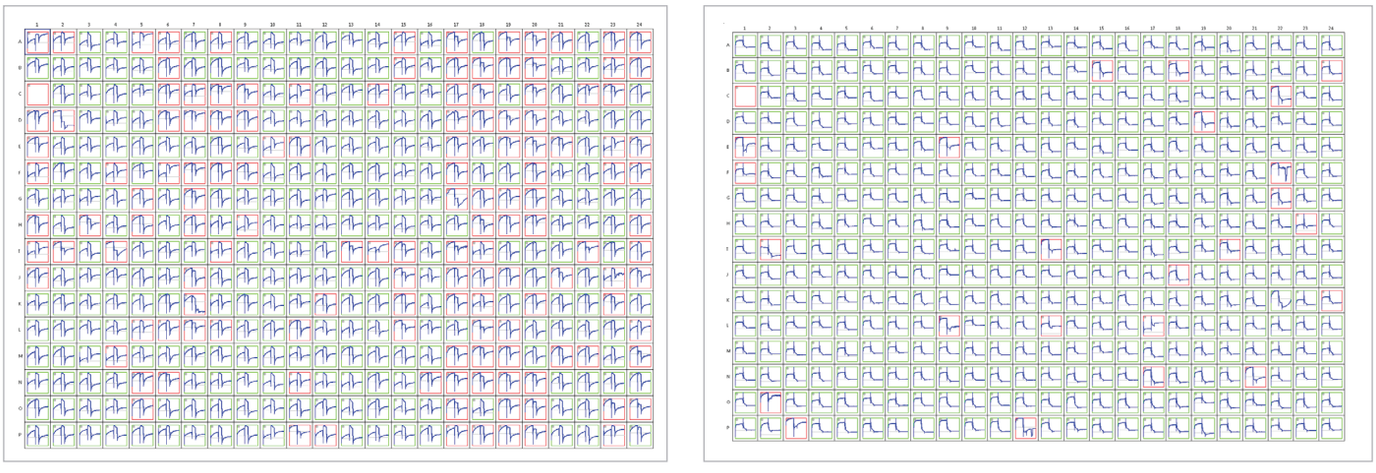
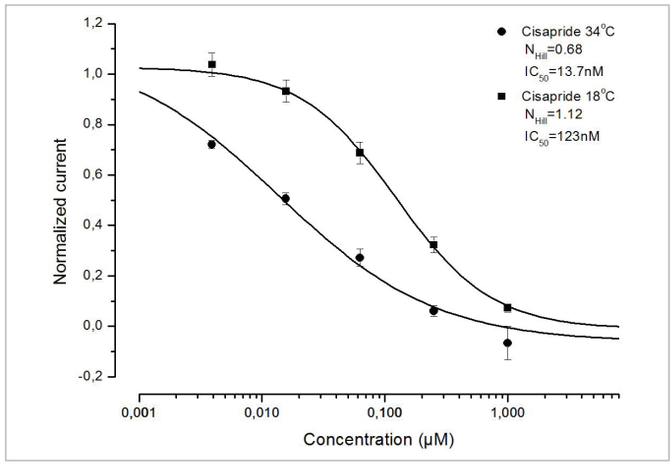
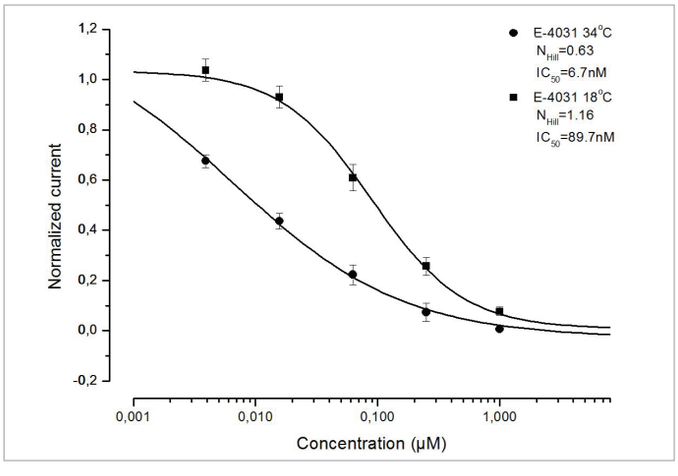


**Fig. 2:** Plate views with 384 simultaneous hERG recordings at 18° C (left) respectively 34° C (right).

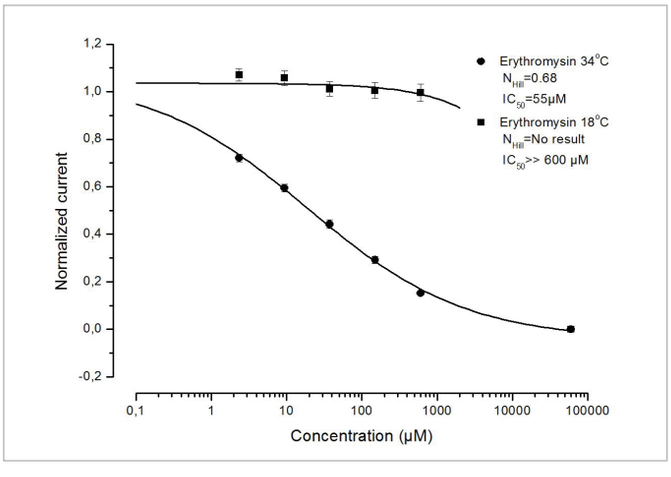
Cisapride 18° C vs 34° C




E-4031 18° C vs 34° C




Erythromycin 18° C vs 34° C




Sotalol18° C vs 34° C

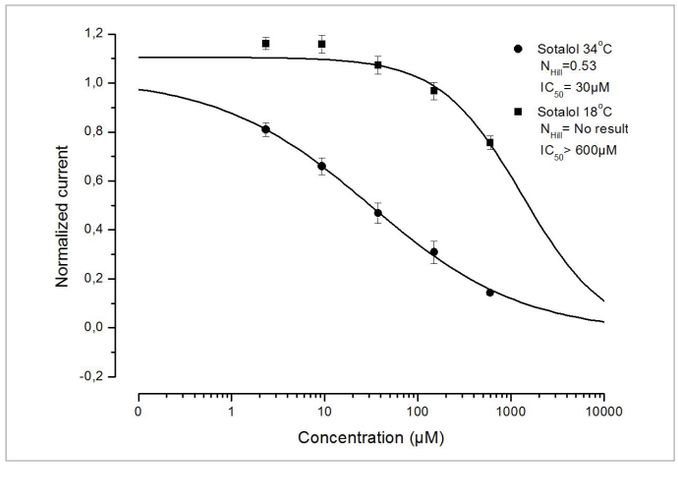


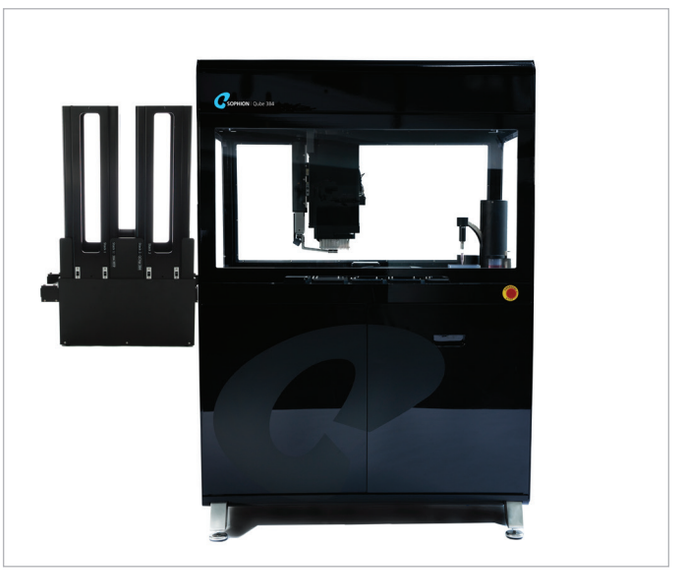

**Fig. 3:** Normalized compound effects as a function of concentration and temperature in single hole mode (see legend for specific temperatures).

**Table 1:** Potency of four reference compounds at two different temperatures in single hole mode. #) 22° C, *) 35° C.

| | $IC_{50}$ [µM] | Fold change ($IC_{50}$18°C/$IC_{50}$34°C) | Hill Slope | Literature values [µM] $IC_{50}$ |
|---|---|---|---|---|
| Cisapride; 18° C | 0.123 | 8.8 | 1.2 | 0.02[b#] |
| Cisapride; 34°C | 0.014 | | 0.7 | 0.027[a*] |
| E-4031; 18° C | 0.09 | 12.9 | 1.2 | 0.14[a#] |
| E-4031; 34° C | 0.007 | | 0.6 | 0.012[a*] |
| Erythromycin; 18° C | >>600 | NN | NN | 1410[a#] |
| Erythromycin; 34° C | 55 | | 0.7 | 115[a*] |
| Solatol; 18° C | >600 | NN | NN | 810[a#] |
| Solatol; 34° C | 30 | | 0.5 | 269[a*] |

**Table 2:** Potency of four reference compounds at two different temperatures in multi-hole mode. (Hill fit not shown).

| | $IC_{50}$ [µM] | Fold change ($IC_{50}$18°C/$IC_{50}$34°C) | Hill Slope |
|---|---|---|---|
| Cisapride; 18° C | 0.190 | 6.8 | 1.5 |
| Cisapride; 34° C | 0.028 | | 0.8 |
| E-4031; 18° C | 0.15 | 12.5 | 1.3 |
| E-4031; 34° C | 0.012 | | 0.9 |
| Erythromycin; 18° C | >>600 | NN | NN |
| Erythromycin; 34° C | 56 | | 0.5 |
| Solatol; 18° C | >600 | NN | NN |
| Solatol; 34° C | 50 | | 0.6 |



## Discussion of data

### Sensitivity to temperature on length of voltage protocol

When working at lower temperature one should be aware of the activation phase of the voltage protocol. In Figure 1 it can be seen in the orange raw trace, that the ion channels are not fully activated before depolarizing the ion channel. In this study, the voltage protocol contains a 4.9 second activation phase before depolarization, which normally would be considered as a long protocol, but one could argue that the activation phase should be even longer. It has previously been reported that many reference compounds do not show any temperature dependencies (Kirsch et. al. 2004, Walker et. al 1999). In the study done by Kirsch et al. (2004) the measurements were done at room temperature with a short activation period of hERG, which could give rise to an underestimation of $IC_{50}$.

### Effect of the four test compounds

Only two of the compounds tested in this study have previously been considered to change potency with change in temperature (Kirsch et. al. 2004). However, all four compounds showed more than a 3-fold change, which is viewed as a significant difference between the two temperatures. Other publications have been reporting temperature decencies on e.g. E-4031 (Yonezawa et.al.).

### Change in Hill slope

There is a small change in Hill slope which properly comes from run-down or run-up of the current. Maybe five different doses are not enough to estimate the Hill slope properly, and it should be investigated if the change in Hill slope are present when using 6-7 different concentrations.

## Methods

The whole cell configuration was established by the Qube standard whole cell protocol for CHO cells. A baseline current was recorded for 600 seconds, followed by addition of four accumulative increasing concentrations (Figure 1).

- Cells
  - CHO-hERG from B'SYS
- Electrophysiology
  - Automated patch clamp measurements were carried out on Qube 384 system from Sophion Bioscience.
  - The whole cells were generated with a Qube 384 standard whole cell protocol.
  - The experiment protocol had a baseline period of 20 protocol sweeps interspaced by 30 s. After this, compound was added cumulatively and a varying number of sweeps (fewer with higher concentrations) were executed.

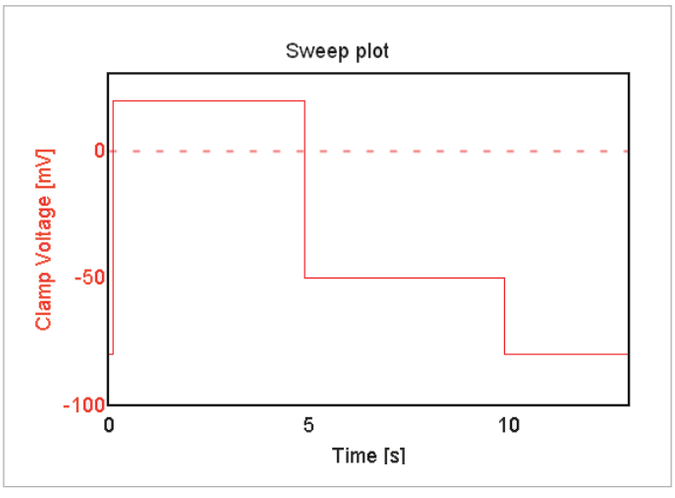



**Fig. 4:** Voltage protocols for compound testing.

## Materials and Methods

**Cells:** CHO-hERG cells kindly provided by B'SYS.


**References:**

a. Kirsch et. al., 2004. Variability in the measurement of hERG potassium channel inhibition: Effects of temperature and stimulus pattern. Journal of Pharmacological and Toxicological Methods 50 (2004) 93–101

b. Kramer et. al., MICE Models: Superior to the HERG Model in Predicting Torsade de Pointes. Scientific Reports. 2013; 3: 2100.

c. Yonezawa et. al., Influence of the temperature of extracellular solutions on the evaluation of drugs that suppress hERG potassium currents.

d. Walker et. al 1999, Inhibition of the human ether-a-go-go-related gene (HERG) potassium channel by cisapride: affinity for open and inactivated states. British Journal of Pharmacology (1999) 128, 444-450



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