Temperature effect on hERG channel pharmacology measured using the Qube automated patch clamp system

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Introduction

The human ether-à-go-go related gene (hERG) function is important for cardiac repolarization and inhibition of the channel can prolong the cardiac action potential, which gives increased risk for ventricular arrhythmias including torsade des points (TdP). Therefore, ***in vitro*** evaluations of the compound effects is performed on the hERG channel routinely in drug development projects to detect potential arrhythmic side-effects.
Usually these compound measurements are carried out at ambient temperatures. Previously it has been shown that the potency for a number of compounds has been underestimated when compared to near physiological temperature tests. Therefore, a temperature controlled measuring environment is beneficial when testing compounds for the aims as mentioned here.
Until recently, the only possibility to test compound potency under voltage control conditions has been the manual patch clamp technique. Now automated patch clamp instruments with temperature control have become available making it possible to perform up to 384 parallel recordings at controlled temperatures ranging from 8°C and above.
Here we used an automated patch clamp system, Qube, to study the effect of temperature on concentration response relationships on a panel of compounds known to block the hERG channel. Qube has a temperature controlled test environment and in these studies, we show that temperature merits being taken into consideration when evaluating for hERG pharmacology.

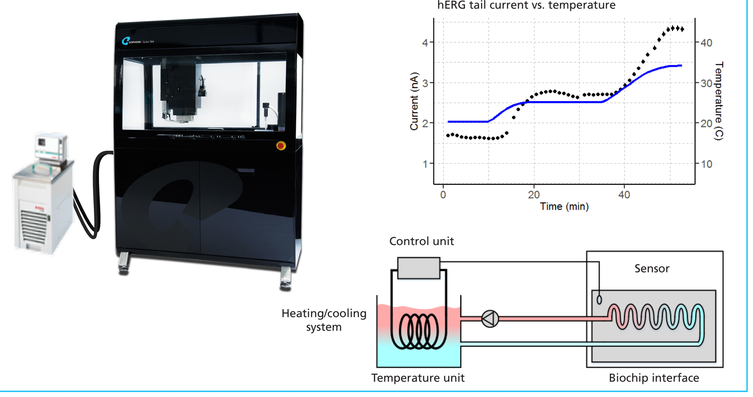


Fig. 1: The Qube automated patch clamp system can be equipped with a temperature control system making it possible to regulate the recording temperature between 8 and 40 degrees Celsius. Lower panel shows schematically the temperature control feedback loop. Upper right panel shows hERG tail current magnitude (black) as a function of temperature (blue). Increasing the temperature from 20 to 34 degrees increased the average tail current approximately 2.5 times.

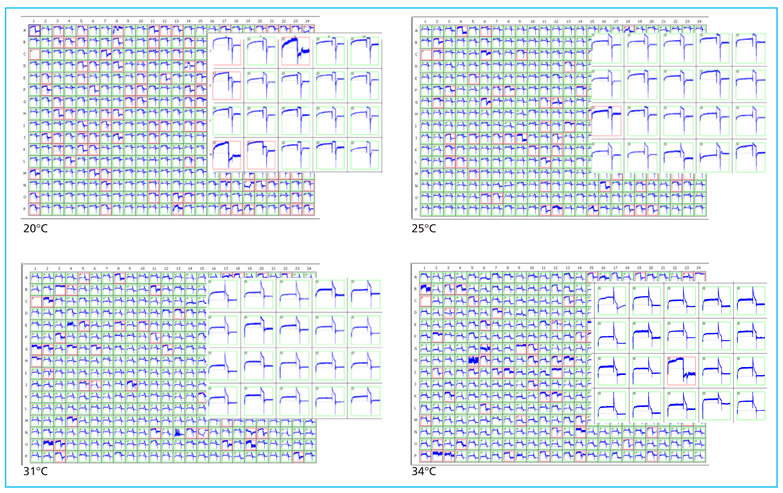


Fig. 2: Plate views of recordings from hERG expressing cells tested at different temperatures using single-hole QChips. The inserts show an excerpt on a larger scale from each experimental temperature.

Conclusions

Our biophysical and pharmacological experiments shows the importance by controlling and standardizing the temperature at the measurement site. Here we used a Qube 384 instrument equipped with a temperature control unit to regulate and standardize the temperature at the measurement site and the biophysical experiments showed an increased rate of activation, a leftward shift of steady-state inactivation and a rightward shift in steady-state inactivation with increasing temperature.

As shown previously the pharmacological response to changes in temperature depend on the compound, e.g. verapamil and quinidine potencies don't change with temperature while erythromycin, sotalol, E-4031 and cisapride showed a more or less pronounced leftward shifts when the temperature was increased from 18 to 34 degrees Celsius. These data shows the importance of temperature control at the measurement site and that the Qube 384 instrument can be used routinely for compound testing with controlled temperatures.

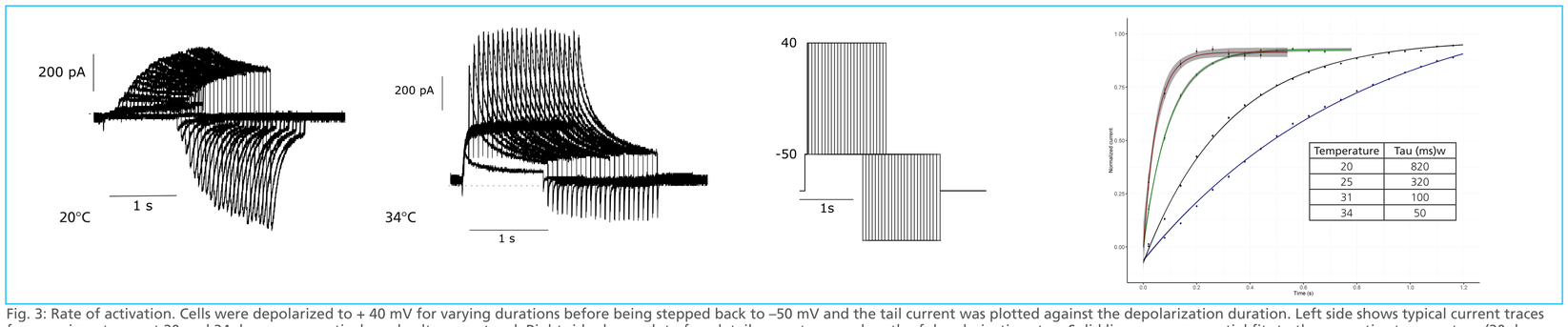


Fig. 3: Rate of activation. Cells were depolarized to + 40 mV for varying durations before being stepped back to –50 mV and the tail current was plotted against the depolarization duration. Left side shows typical current traces for experiments run at 20 and 34 degrees respectively and voltage protocol. Right side shows plot of peak tail currents versus length of depolarization step. Solid lines are exponential fits to the respective temperature (20 degrees: blue, 25: grey, 31: green and 34: red).

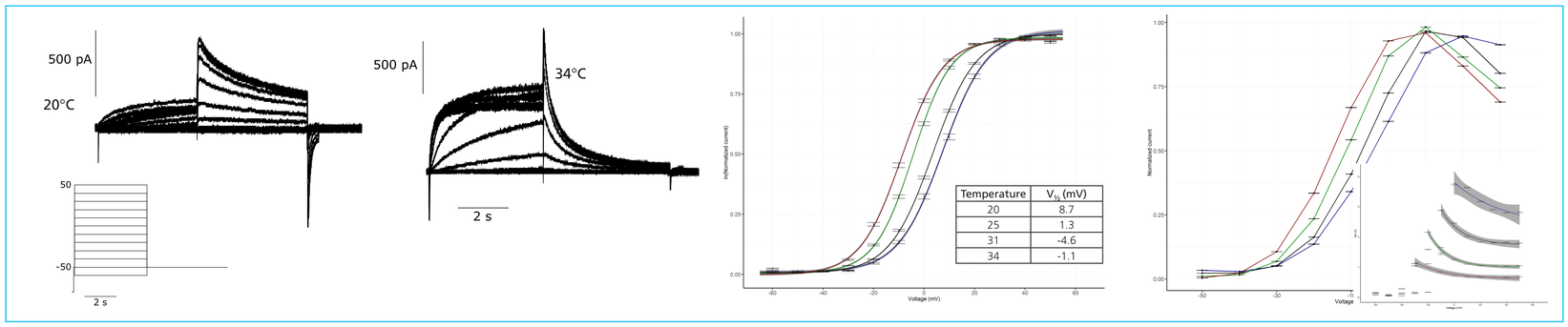


Fig. 4: Steady-state activation measured using a two-step protocol. Cells were depolarized from a holding voltage of -80 mV to varying potentials in steps of 10 mV, repolarized to -50 mV and tail currents recorded. Left side shows typical currents traces recorded at 20 and 34 degrees. Left plot shows normalized tail current plotted against step voltage. Solid lines are fits to the Boltzmann equation at the respective temperature (20 degrees: blue, 25: grey, 31: green and 34: red). Rightmost plot shows the current before repolarizing to -50 mV which shows the expected "n-shaped" curve. The insert shows tau values for tail current and the table half maximal activation at the respective temperature.

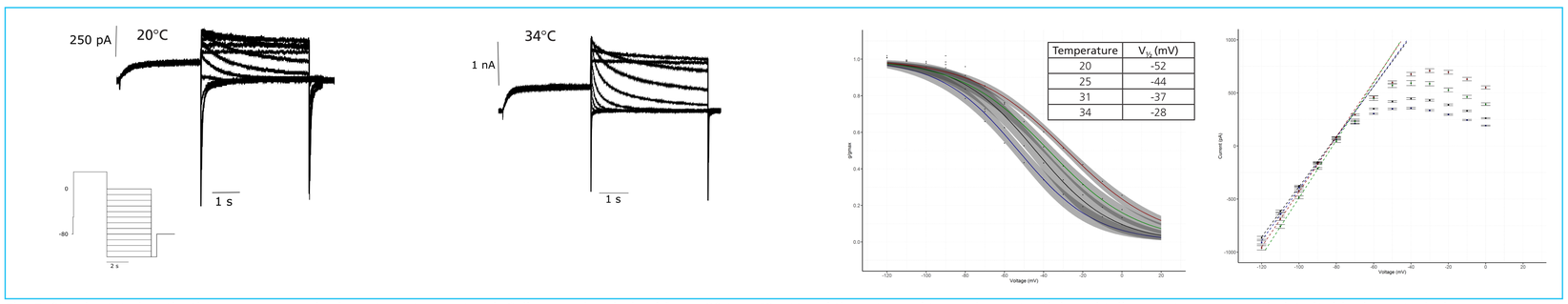


Fig. 5: Steady-state inactivation was measured using a two-step protocol. Cells were depolarized from -80 to + 40 mV and then stepped to varying potentials and tail currents were recorded. Left side shows typical current traces at 20 and 34 degrees and applied voltage protocol. Rightmost plot shows the tail current plotted against the stepped voltage, the left plot shows fits to the Boltzmann equation and the table lists the calculated $V_{½}$.

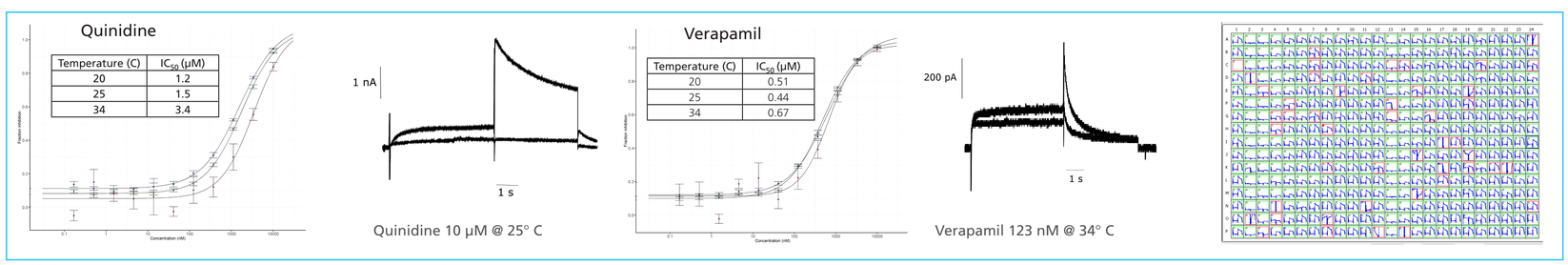
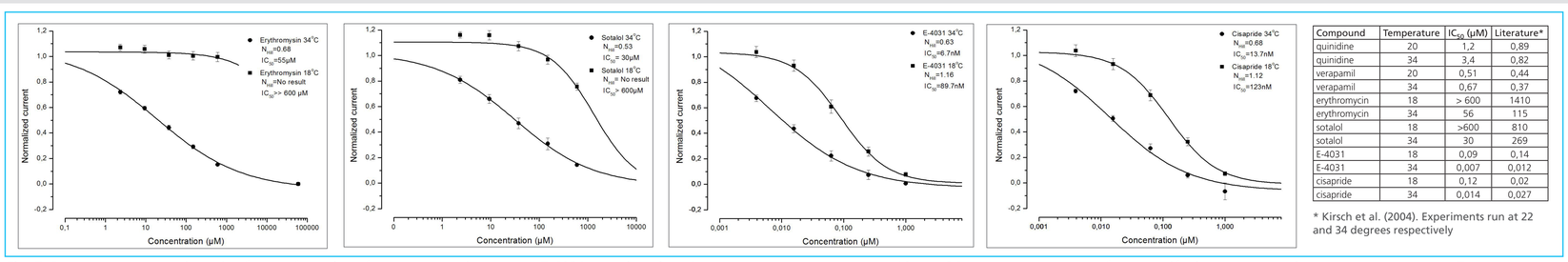


Fig. 6: Dose-response relationships for quinidine and verapamil. Left panels show the inhibition of tail current by the respective drug and right panels show typical current traces under control conditions and in the presence of respective drug at 25 and 34 degrees. Rightmost panel shows plate view of an experiment carried out at 25 degrees (negative controls in columns 1 and 24 and decreasing concentrations from left to right; quinidine columns 2 to 12 and verapamil columns 13-23).



Compound	Temperature	IC_{50} (µM)	Literature*
quinidine	20	1,2	0,89
quinidine	34	3,4	0,82
verapamil	20	0,51	0,44
verapamil	34	0,67	0,37
erythromycin	18	> 600	1410
erythromycin	34	56	115
sotalol	18	>600	810
sotalol	34	30	269
E-4031	18	0,09	0,14
E-4031	34	0,007	0,012
cisapride	18	0,12	0,02
cisapride	34	0,014	0,027

* Kirsch et al. (2004). Experiments run at 22 and 34 degrees respectively

Fig. 7: Dose-response relationships from left to right for erythromycin, sotalol, E-4031 and cisapride. All compounds were tested at 18 and 34 degrees and show leftward shifts in response to increased temperature. Table shows IC_{50} values obtained during experiment and literature values (Kirsch et al, 2004).

Methods

Cells expressing the hERG ion channel (cell line "hERG-DUO") were kindly provided by B'SYS (Witterswil, Switzerland) and cultured according to instructions. On the day of experiment the cells were harvested and maintained in serum-free medium () supplemented with HEPES (25 mM), trypsin inhibitor and penicillin/streptomycin. The intracellular solution consisted of (mM) 120 KF, 20 KCl, 10 HEPES, 10 ETGA (pH 7.2 with KOH) and the extracellular (mM) 145 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.4 with NaOH).

References

Kirsch et. al. Variability in the measurement of hERG potassium channel inhibition: Effects of temperature and stimulus pattern. J Pharmacol Toxicol Meth, 50:93–101, 2004
Vandenberg et al. hERG K+ channels: structure, function and clinical significance. Physiol Rev. 92:1393-1478, 2012.