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

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Introduction

The human ether-a-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 real 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.

Conclusions

Our biophysical and pharmacological experiments show the importance of 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 temperature.

Methods

Cells expressing the hERG on channel (cell line "hERG-DUO") were kindly provided by B'YS (Wittenwil, Switzerland) and cultured according to instructions. On the day of the experiment the cells were harvested and maintained in serum-free medium (I supplemented with HEPES (25 mM), trypsin inhibitor and penicillin/streptomycin. The intracellular solution consisted of (mM) 120 KF, 20 KCl, 10 HEPES (pH 7.2 with KOH) and the extracellular (mM) 145 NaCl, 4 KCl, 2 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 10 HEPES (pH 7.4 with NaOH).

References


Vandenberg et al. hERG K\textsubscript{+} channels: structure, function and clinical significance. Physiol Rev. 92:1393-1478, 2012.