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Estimating hERG drug binding using temperature-controlled high throughput automated patch clamp

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Introduction

The human ether-à-go-go related gene (hERG) carries one of the major repolarizing currents in the heart myocytes and block of this channel by certain drugs may lead to prolongation of the cardiac action potential and the potentially lethal arrhythmia torsade de pointes (TdP). Therefore current guidelines are that potency against hERG should be assessed in preclinical drug development. However, not all compounds inhibiting hERG are proarrhythmogenic and more integrated effects of the drug must be taken into consideration.

The comprehensive in vitro proarrhythmic assay (CiPA) initiative have introduced a new paradigm for assessing proarrhythmic risk based on a thorough understanding of the underlying cellular mechanisms leading to a more predictive estimation of the risk carried by the specific compound.

One pillar of the CiPA paradigm is to measure the major ionic currents involved in cardiac depolarization and repolarization using the high throughput automated patch-clamp (APC) systems. Until recently this has meant that the measurements were carried out at ambient temperature a but recently 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 10 to 40°C. Here we estimate potencies and binding kinetics to the hERG channel which are important parameters for the outcome of the risk assessment for a panel of clinical drugs using a Qube APC system equipped with temperature control.

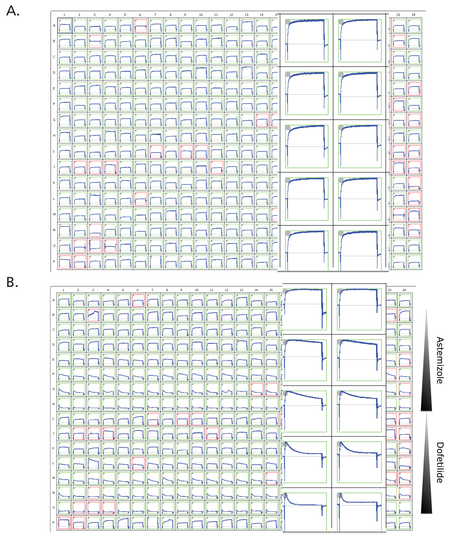

Fig. 1: Plate-view of hERG recordings at 32°C in the absence (A) and presence (B) of increasing concentrations of compound (rows A and P are controls). Currents were elicited by a modified form of the protocol used by Milnes et al (2010) with five 45 seconds long depolarization from -90 to +10 mV under control conditions followed by five 15 seconds sweeps at -90 mV in the presence of compound and lastly five 45 seconds depolarizations in the presence of compound. The longer step was chosen so that it would be minimum 2 times longer than the longest time constant (Windley et al. 2017). The plate-view in panel B shows first sweep after addition of the respective compound. Quality control indicated by green (accepted) and red (rejected).

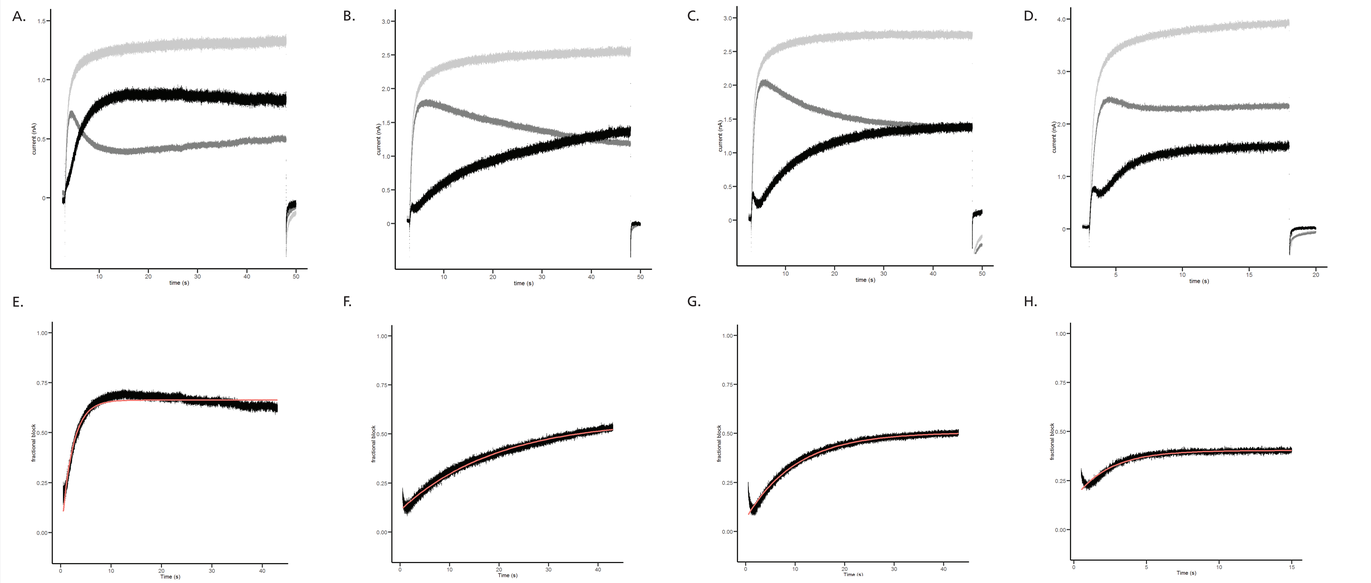

Fig. 2: Examples of original hERG traces recorded at 32°C in response to 10 or 45 seconds voltage steps from -90 to +10 mV in the absence (light grey trace) and presence of compound (dark grey) for cisapride (A), dofetilide (B), terfenadine (C) and verapamil (D) respectively. The black trace is the difference between control and compound. E to H show the normalised subtracted trace and the fitted exponential function to the data used to calculate the time-constant for the developing block. Concentrations chosen to approximately represent the IC_{50} for the respective compound. Concentrations and approximations of the time-constant in table 2.

Summary

- Here we have used of a Qube384 instrument equipped with temperature control to record hERG currents at 32°C and:
  - measured compound potency using both a step and a step-ramp instrument
  - measure binding kinetics using a step protocol.
- These results shown are in line with previously reported data.
- These types of measurements are important to the CiPA initiative for use in the developed *in silico* model (Dutta et al. 2017)

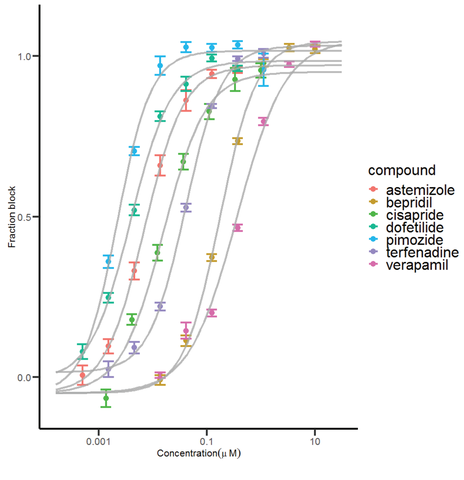

Fig. 3: Dose response plot for a set of archetypical hERG channel blockers. Fraction inhibition at 32°C for each concentration was calculated from the current at end of the fifth depolarization in the presence of compound normalized to the current in the corresponding depolarization in the absence of compound. A logistic function was fitted to the data (represented as mean ± SEM) and the calculated IC_{50} values are tabulated in table 1 ("Milnes").

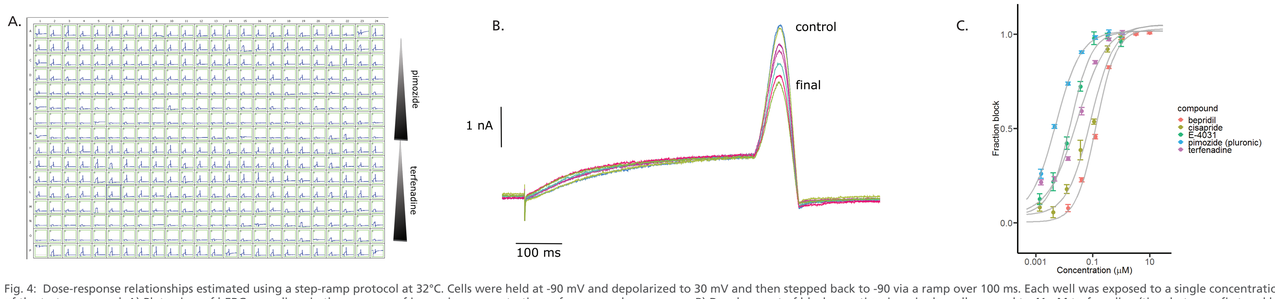

Fig. 4: Dose-response relationships estimated using a step-ramp protocol at 32°C. Cells were held at -90 mV and depolarized to 30 mV and then stepped back to -90 via a ramp over 100 ms. Each well was exposed to a single concentration of the test compound. A) Plate-view of hERG recordings in the presence of increasing concentrations of compound across rows. B) Development of block over time in a single well exposed to 41 nM terfenadine (time between first and last sweep approximately 30 minutes). C) dose-response relationships for a set of known hERG blockers. Values are tabulated in table 1 ("Step-ramp").

Table 1: IC_{50} values obtained using the Milnes and a step-ramp protocol for a set of archetypical hERG blockers.

	IC_{50} (μM)	
Compound	"Milnes"	"Step-ramp"
Astemizole	0.007	
Bepridil	0.17	0.13
Cisapride	0.016	0.08
Dofetilide	0.004	
Pimozide	0.002	0.01
Terfenadine	0.04	0.03
Verapamil	0.36	
E-4031		0.02

Table 2: Time-constants and concentrations for compounds shown in Fig. 2.

	Time constants (s)	
	Conc. (nM)	tau (s)
Verapamil	370	2.2 ± 0.1
Terfenadine	41	10.6 ± 0.6
Dofetilide	14	17.8 ± 2.1
Cisapride	37	2.3 ± 0.2

Materials and methods

CHO-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 (EX-CELL ACG CHO-medium, Sigma-Aldrich, Copenhagen, DK) 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 CaCl2, 1 MgCl2, 10 HEPES (pH 7.4 with NaOH). Astemizole, dofetilide and terfenadine were dissolved using Pluronic F-68 (Sigma-Aldrich). All compounds from Sigma-Aldrich.

All experiments were carried out at 32 degrees using multi-hole QChips (i.e. 10 patch holes per well) and a Qube 384 with temperature control.

References

Dutta et al. Optimization of an *in silico* Cardiac Cell Model for Proarrhythmia Risk Assessment. Front Physiol. doi.org/10.3389/fphys.2017.00616.

Milnes *et al.* Investigating dynamic protocol-dependence of hERG potassium channel inhibition at 37 degrees C: Cisapride versus dofetilide. J Pharmacol Toxicol Methods. 178-91, 2010.

Windley JW *et al*. Measuring kinetics and potency of hERG block for CiPA. J Pharmacol Toxicol Meth. 99-107, 2017.