Development and validation of a Qube automated patch clamp hERG assay at physiological temperatures

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Introduction

The development of Automated Patch Clamp (APC) technology over the last 20 years has transformed the research and development process for identifying novel drugs for ion channel targets[1]. Furthermore, it has been widely implemented in cardiac safety pharmacology screening by pharma and contract research organisations[1,2].

The adoption of APC as a screening tool has gathered pace over the last 10 years, which may in part be attributed to the introduction of the Comprehensive in Vitro Proarrhythmia Assay initiative (CiPA and JiCSA in Japan). Several published studies have confirmed that the high quality potency data derived from APC screening can be inserted into *in silico* models of human ventricular action potentials to accurately predict proarrhythmic risk[1,2,3,4].

Most commercially available APC cardiac safety assays have historically been performed at room temperature. However, it is widely acknowledged that temperature can affect the hERG potency of certain agents (e.g. erythromycin, sotalol)[4,5], which underlies the basis of why the FDA recommend performing GLP hERG studies at physiological temperatures[6].

Therefore, an APC assay performed at physiological temperatures will benefit from the combination of greater throughput with enhanced sensitivity for compounds that exhibit temperature dependent inhibition.

Aim

The successful development of a robust hERG assay at physiological temperatures using the 384 well Qube APC system

Methods

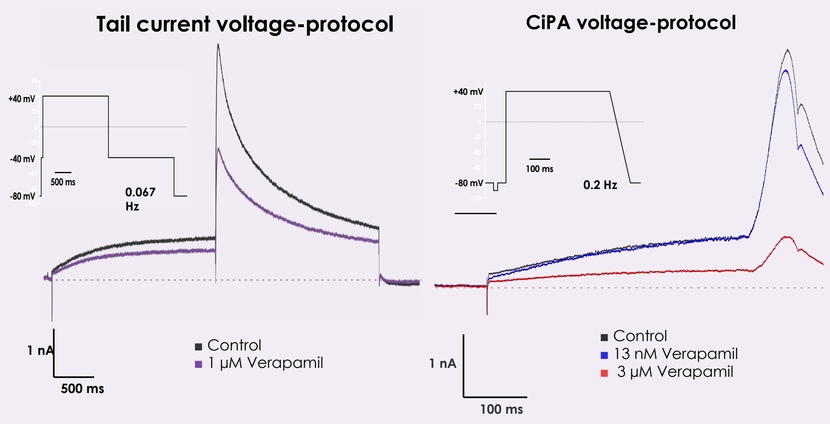
A CHO cell line stably expressing the human *ether-a-go-go related* gene potassium channel (hERG, K_V11.1) was cultured according to the vendor's instructions (B'SYS GmbH) and harvested using optimised protocols.

Experiments were performed using the Qube 384 well platform (Sophion, Denmark) in the conventional whole-cell configuration using either Single (SH) or Multi- Hole (MH, population patch) format chips. Recordings were performed at either Room Temperature (RT, 20 – 25 °C) or physiological temperature (37 °C). Qube 384 experimental parameters were set using ViewPoint V2.6 and data quality control and export controlled using Assay Software V6.6.

Solutions

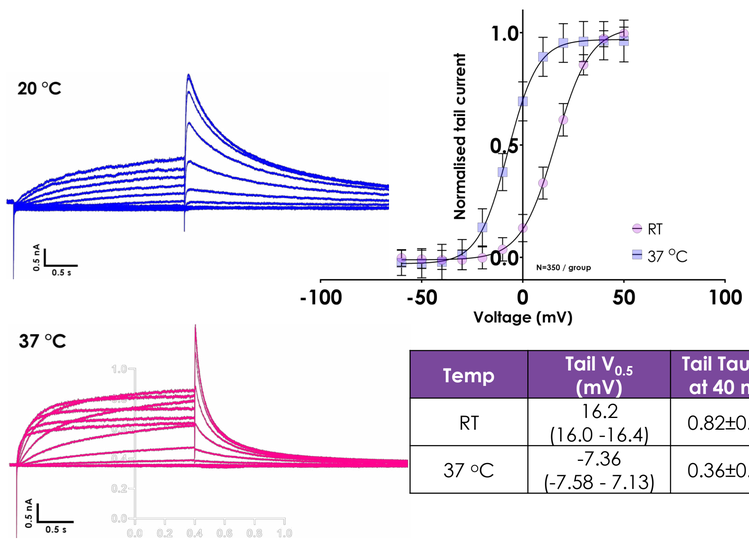
Component	Intracellular Solution (mM)	Extracellular Solution (mM)
NaCl	-	140
KCl	20 or 70	2
KF	70 or 120	-
HEPES	10	10
$MgCl_2$	-	1
$CaCl_2$	-	2
Glucose	-	5
EGTA	5	-
MgATP	5	-
pH	7.2 (KOH)	7.4 (NaOH)

Example current traces for the selected voltage-protocols



1. Effect of temperature on biophysics

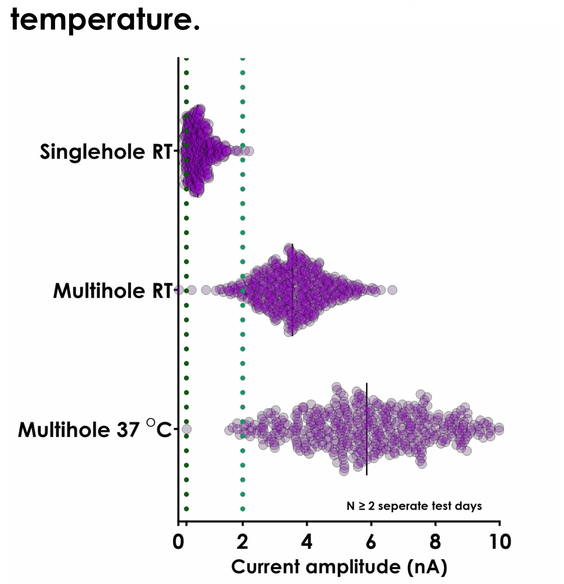
Elevated temperatures led to expected change in biophysics and kinetics for hERG.



Temp	Tail $V_{0.5}$ (mV)	Tail Tau (s) at 40 mV
RT	16.2 (16.0 -16.4)	0.82±0.11
37 °C	-7.36 (-7.58 - 7.13)	0.36±0.04

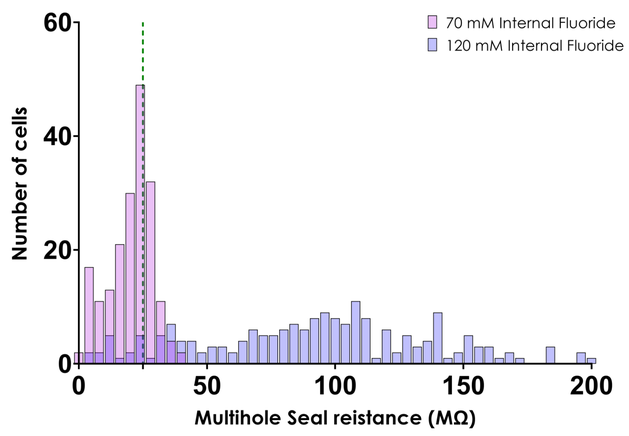
2. Current expression

Use of multihole chips provides sufficient current (>2 nA) for screening at physiological temperature.



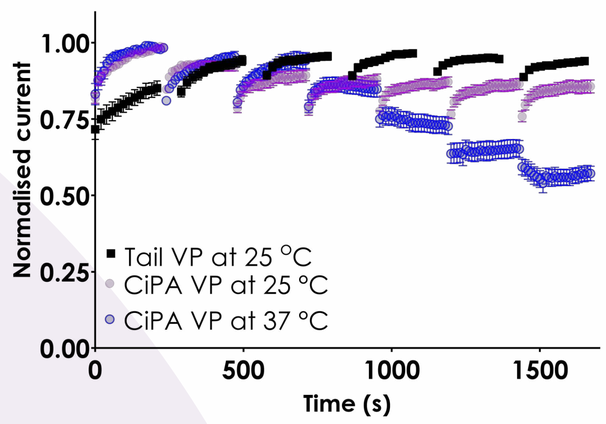
3. Multihole seal quality

A higher intracellular fluoride concentration was required to maintain whole cell input resistance >20 MΩ across the 30 minute experiment.



4. Current stability

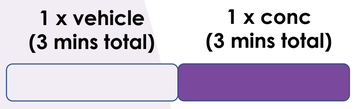
After an initial stabilisation period, rundown was 0.5%/ minute for both the Tail and CiPA VP at RT, however, at 37 °C, the rate of rundown was 2% / minute.



5. Pharmacology assessment

5a. Testing paradigm

A single concentration per cell paradigm was used due to the rundown at 37 °C. Potency was determined from a 10 point CRC with N > 3 separate cells per concentration.



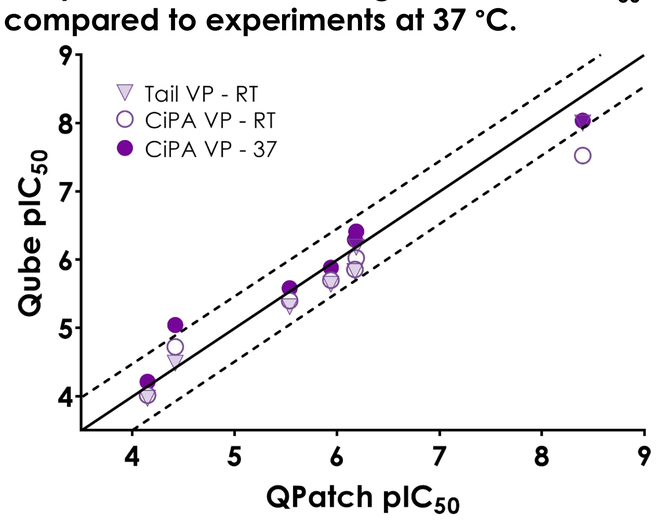
5b. Potency for a series of reference antagonists

Choice of voltage protocol and/or the temperature of recordings did not impact the potency of a series of reference antagonists.

	pIC_{50} values for each experimental condition				
Platform:	Qpatch	Qube			
Temperature:	RT	RT	RT	RT	37
Format:	SH	SH	MH	MH	MH
Voltage Protocol:	Tail	Tail	Tail	CiPA	CiPA
Dialtazem	4.42	4.59	4.48	4.72	5.04
Ibutilide	8.40	8.00	8.00	7.52	8.04
Nifedepine	4.15	3.97	3.97	4.01	4.21
Ondansetron	5.54	5.43	5.31	5.40	5.58
Quinidine	5.94	5.74	5.64	5.70	5.88
Risperidone	6.19	6.35	6.18	6.02	6.41
Verapamil	6.17	5.92	5.82	5.85	6.29

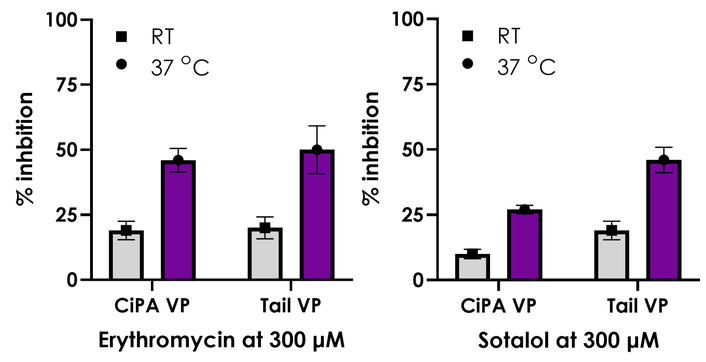
5c. Potency results

Good cross platform consistency (QPatch vs. Qube) for potency determined at room temperature with <0.5 log variation in IC_{50} when compared to experiments at 37 °C.



5d. Confirmation of temperature sensitive hERG blockers erythromycin and sotalol

Due to solubility issues, 300 µM was the highest test concentration evaluated. There was a larger hERG inhibition when tested at 37 °C and is in agreement with published manual patch clamp potency data[5].



Conclusions

- **A hERG assay on Qube at 37 °C was successfully developed**
- **Patchability, current expression and stability was optimized to be sufficient for potency assessment of compounds at 37 °C**
- **Potency for literature compounds assessed showed good agreement between two voltage protocols and across different APC platforms**
- **Known temperature dependent hERG blockers were confirmed using the assay**

References

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