The $Na_V1.5$ Late Current in WT and $Na_V1.5$-ΔKPQ Mutant Channels: An Automated Patch Clamp LQT3 Electrophysiological Assay Comparison

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Introduction

The cardiac late Na^+ current (late I_{Na}) generates persistent inward currents throughout the plateau phase of the ventricular action potential and is an important determinant of repolarisation rate, EADs and arrythmia risk[1]. As inhibition of late INa can offset drug effects on hERG and other repolarising K^+ conductances it is one of the key cardiac channels in the Comprehensive *in vitro* Pro-arrythmia Assay (CiPA) panel being developed by the FDA to improve human clinical arrythmia risk assessment[2,3]. The standard CiPA late I_{Na} assay uses the anemone toxin ATX-II to pharmacologically inhibit inactivation and produce persistent openings of wildtype (WT) $Na_V1.5$ channels, but this method is variable and non-physiological. In contrast, several mutations in the SCN5A gene cause a form of hereditary long QT syndrome (LQT3) by promoting late openings[4]. The ΔKPQ mutation deletes residues Lys 1505, Pro 1506 and Gln 1507 and results in a sustained, non-inactivating current during long depolarizations which causes prolongation of the action potential and can result in fatal ventricular arrhythmias such as Torsade de Pointes (TdP)[5,6].

Here we utilised a stable $Na_V1.5$ LQT3 mutant cell line to optimise and validate a high throughput automated patch clamp late I_{Na} assay on the SyncroPatch 384i platform. High quality gigaseal recordings were obtained with a high success rate, enabling the efficient and accurate determination of relevant biophysical and pharmacological properties of this CiPA-compliant late $Na_V1.5$ assay. The combination of the SyncroPatch 384i automated patch clamp system and $Na_V1.5$ ΔKPQ cell line created a reliable high throughput cardiac safety screening assay without the need for openers like ATX-II toxin.

Materials and methods

CHO cells expressing WT human $Na_V1.5$ gene and HEK cells expressing $Na_V1.5$ ΔKPQ mutant channel proteins were cultured and harvested according to standard protocols. Dissociated cell suspensions were kept at 10°C in the onboard cell hotel and dispensed onto NPC patch chips immediately prior to conventional whole-cell patch clamp recordings using standard sealing and patching protocols on the SyncroPatch384i platform, with minor adjustments to obtain a high proportion of gigaohm seals and stable late INa current amplitude and kinetics. Intracellular solution was CsF-based and external contained 140 NaCl. Peak and late INa currents were measured using a CiPA step-ramp voltage protocol for pharmacology screening, whilst voltage step protocols were used to determine activation and inactivation biophysical parameters, with Rs compensation enabled. Single-hole and multi-hole NPC plates were employed to assess and optimise late INa current amplitudes. Test compounds for cumulative IC_{50} screening were made up in 384 well plates at and dispensed by Biomek standard tips into each well (final 0.1% DMSO). Data was acquired Nanions' Patch Control Software with leak subtraction turned on and analysed and plotted using Nanions' DataControl software.

1. SyncroPatch384i APC ΔKPQ $Na_V1.5$ assay

Very little optimisation of standard $Na_V1.5$ cell line preparation and SyncroPatch384i APC assay conditions was required to achieve acceptable success rates for peak INa recordings, as measured by sealing and patchability QC parameters and current expression levels (Fig. 1). In contrast, resolving inward late currents in the ΔKPQ cell line was more challenging but a ~50% QC success rate was achieved using 4x multi-hole chips (Table 1).

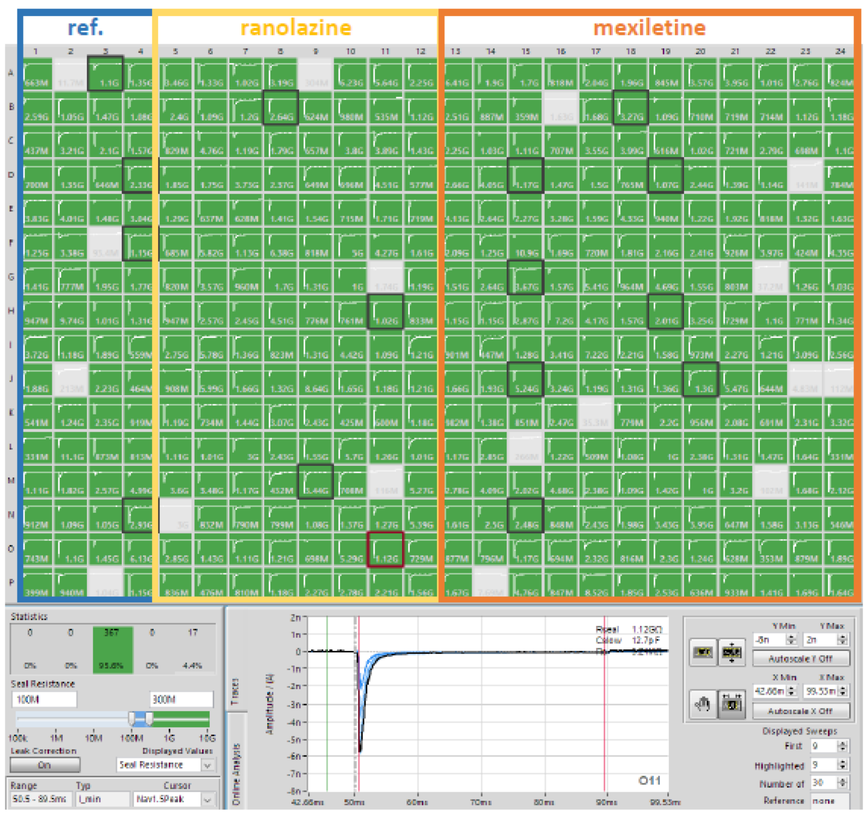

Figure 1: Graphical interface plate view of ΔKPQ $Na_V1.5$ peak current recording from 384 well single hole chip. Wells exceeding minimum QC parameters (>300pA, >300 MΩ) are shown in green, and raw current traces from highlighted wells in 0.1% DMSO, Ranolazine and Mexiletine are shown below in trace view.

Current type	Chip type	QC parameters		Success rate % wells
		pA	MΩ	
Peak ΔKPQ	1x high	-300	300	95.6
Late ΔKPQ	1x high	-200	1000	4.7
Late ΔKPQ	4x medium	-200	250	45.3

Table 1: Patchability, current expression and success rates for different Nav1.5 ΔKPQ assay conditions. Comparison of assay performance obtained using single-hole vs 4x multi-hole NPC plates. Patchability QC parameters include whole-cell seal resistance, minimum current amplitude, and experimental success rate (completion of pharmacology screening or biophysical assessment over 20 min recording).

2. ΔKPQ $Na_V1.5$ current characterisation

A major challenge to recording late openings of $Na_V1.5$ channels is their small amplitude, which is negligible in WT channels (Fig. 2) but resolvable in ΔKPQ mutant channels using single-hole APC chips and of sufficient size for reliable biophysical and pharmacological assessment using multi-hole chips.

$Na_V1.5$ current expression

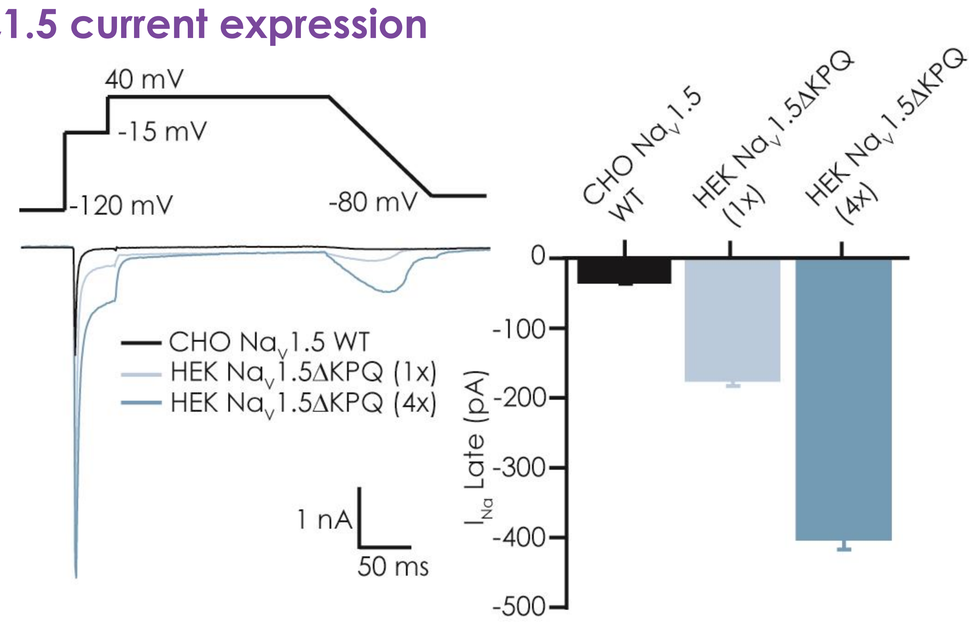

Figure 2: Comparison of peak (*left*) and late (*right*) $Na_V1.5$ current components in WT and ΔKPQ cell lines recorded using single- and multi-hole chips on SP384i using a CiPA step-ramp voltage protocol.

LQT3 ΔKPQ $Na_V1.5$ currents exhibited steady state voltage-dependent activation ($V_{1/2}$ -35 mV, left shifted compared to WT of -15 mV) and inactivation properties ($Vh_{1/2}$ -75 mV) as expected using standard protocols[5,6].

$Na_V1.5$ biophysics

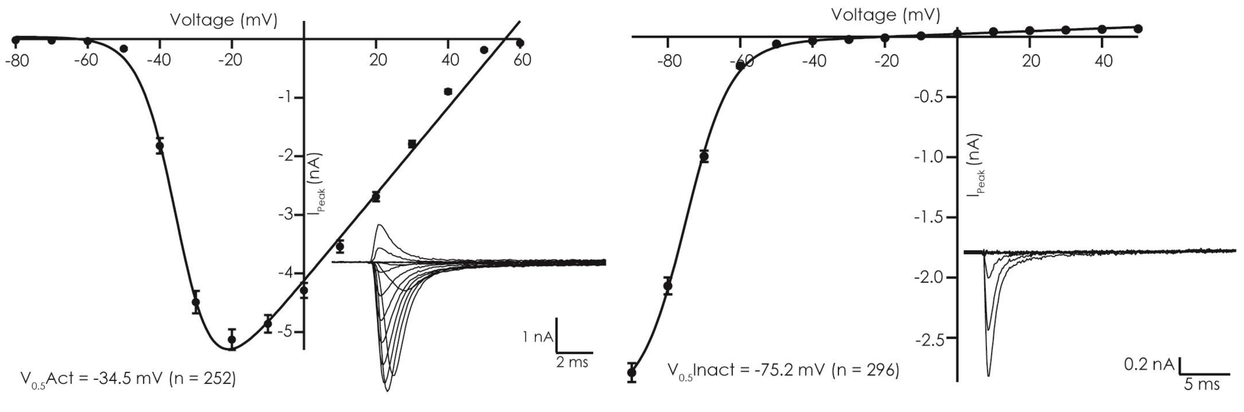

Figure 3: Voltage-dependent biophysical properties of ΔKPQ peak $Na_V1.5$ currents. Activation (left) and steady-state inactivation (right |) were determined using voltage steps delivered from a Vh of -100 mV.

3. Reference pharmacology

As there is little HTS APC data on ΔKPQ LQT3 late I_{Na} pharmacology it was important to test reference compounds and compare their potency to non-physiological ATX-II toxin-activated channels, using the CiPA step-ramp protocol. We found that Ranolazine and Mexiletine inhibited mutant late I_{Na} with an IC_{50} of 17.5 µM (n = 74 wells) and 6.4 µM (n = 73 wells), respectively (Fig. 4), in good agreement with published values[7]. Peak WT and ΔKPQ currents were less sensitive than late I_{Na} components (Table 2).

Mexiletine

Ranolazine

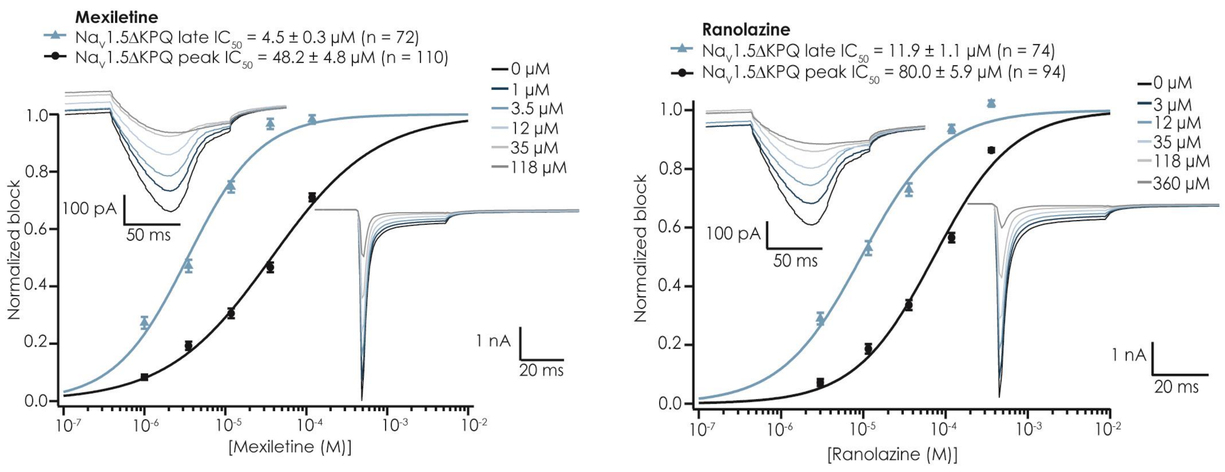

Figure 4: Pharmacological validation of ΔKPQ $Na_V1.5$ currents. The inhibition of peak (black circles) and late current (blue triangles) is plotted against applied concentration of Mexiletine (*left*) and Ranolazine (*right*), and mean IC_{50} values are shown above each figure. Inset into each figure are late currents (*upper left*) evoked at the end of a step-ramp protocol, as well as peak and non-inactivating currents evoked using a step protocol, from 4x multi-hole chip recordings; note different amplitude axis range.

Compound	CHO WT $Na_V1.5$		HEK ΔKPQ $Na_V1.5$ (1x)		HEK ΔKPQ $Na_V1.5$ (4x)		Literature*	
	Peak	Late	Peak	Late	Peak	Late	Peak	Late
Ranolazine	143	-	46.2	12.3	80.0	11.9	79.5	16.7
Mexiletine	83	-	17.1	4.7	48.2	4.5	21.9	12.2

Table 2: Comparison of reference compound inhibition (IC_{50}, µM) of peak *vs* late $Na_V1.5$ currents. Mexiletine was a more potent inhibitor of $Na_V1.5$ currents than Ranolazine, and both compounds exhibited a 4-6 fold preference for the late INa current component of ΔKPQ channels. *Potency and selectivity for late openings were similar to that in published literature using ATX-II on WT $Na_V1.5$ currents.

Conclusions

- Our collaboration was successful in using Metrion's WT and ΔKPQ $Na_V1.5$ cell lines and Nanion's SyncroPatch 384i APC platform to design, optimise and validate a CiPA-ready HTS cardiac safety screening assay suitable for prediction of human clinical pro-arrhythmia risk.
- Gigaseal quality recordings were key to resolving the small late current openings of LQT3 mutant $Na_V1.5$ channels without resorting to use of ATX-II.
- $Na_V1.5$ ΔKPQ biophysical and pharmacological properties were reliably and efficiently determined for this optimised APC HTS cardiac safety assay.

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References

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