Application Report

$Na_V1.5$(Late) cardiac safety assay on QPatch

As an alternative to standard pharmacological procedures for $Na_V1.5$(Late) assays, we present a more reliable and accurate $Na_V1.5$(Late) assay on QPatch that removes the requirement for activators like veratridine and ATX-II and delivers improved cardiac safety screening reliability and cost.

Summary

- High fidelity QPatch recordings of small amplitude $Na_V1.5$ (Late) currents from Long QT3 syndrome mutant channels.
- Stable recordings without pharmacological activators allow more reliable drug potency assessment.
- Pharmacologically validated with sodium channel blockers with a preference for the $Na_V1.5$(Late).

Introduction

Cardiac safety side-effects remain the major cause of new compound attrition during the drug discovery process. This suggests that more robust preclinical *in vitro*, *ex vivo* and *in vivo* assays and models are required to predict clinical risk in humans. Currently the industry is moving away from an over-reliance on the human *ether-a-go-go* related gene (hERG) potassium channel (also known as $K_V11.1$) and QT prolongation readouts by developing new initiatives that provide a more balanced assessment of patient risk, focusing in particular on proarrhythmic liability. The FDA's Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative aims to more accurately model and predict proarrhythmic risk by including data from six *in vitro* ion channels (hERG, $Ca_V1.2$, $Na_V1.5$ (Peak and Late), $K_{ir}2.1$, K_VLQT1 and $K_V4.3$) in sophisticated *in silico* models of human cardiac action potentials (AP). Recently, the FDA has demonstrated that removal of certain currents from the model affects the accuracy of predicting proarrhythmia more than others. Mean prediction error increased to approximately 0.25, 0.38 and 0.75 when $hCa_V1.2$, $Na_V1.5$(Late) and hERG were removed, respectively (Chang *et al.*, 2017).

The amplitude of the $Na_V1.5$(Late) current, also known as the persistent current, is a small percentage (<1 %) of the peak $Na_V1.5$ current which undergoes bursts of channel openings during prolonged depolarisations, such as during the adult human cardiac AP (Chandra *et al.*, 2018). Due to the small amplitude of $Na_V1.5$(Late) currents, it is extremely difficult to record in recombinant cells expressing $Na_V1.5$. A common solution to this problem is to use $Na_V1.5$(Late) current enhancers, such as veratridine or ATX-II. However, the binding sites and mechanism-of-action of these openers are different and their efficacy can also vary, leading to large variations in inhibition values of known $Na_V1.5$(Late) current modulators. For example, the IC_{50} of ranolazine is 90.8 µM in the presence of veratridine compared with 5.4 µM when using ATX-II (Fisher *et al.*, 2018). Both veratridine and ATX-II are also non-selective and will enhance currents through endogenous $Na_V1.x$ channels, which are known to be present in CHO and HEK cells (West *et al.*, 1992; Lalik *et al.*, 1993; He and Soderlund, 2010), further limiting their pharmacological specificity for eliciting $Na_V1.5$(Late).

Due to the small amplitude of native $Na_V1.5$(Late) currents and the variability, non-selectivity and costs associated with using toxin enhancers, we created a $Na_V1.5$(Late) cell line utilising the $Na_V1.5$ LQT3 syndrome KPQ deletion mutant.

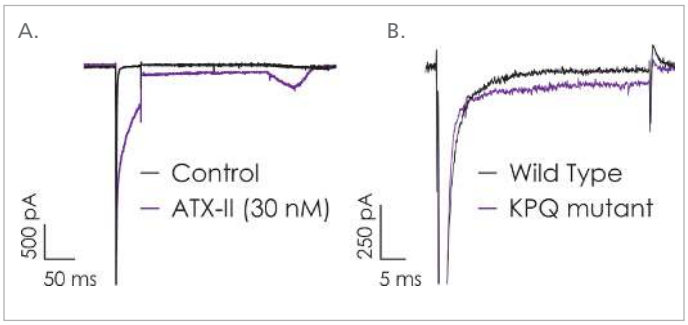

Fig. 1: **ATX-II increases the $Na_V1.5$(Late) current. A)** ATX-II addition (30 nM) to WT $Na_V1.5$ channels inhibits current inactivation to create persistent currents suitable for APC screening **B)** Comparison of ΔKPQ mutant and WT $Na_V1.5$ currents on the same QPlate using the QPatch cell clone facility to illustrate differences in kinetics in the same experiment. ΔKPQ mutant currents show increased fast inactivation but reduced slow inactivation during the persistent $Na_V1.5$(Late) phase (Spencer, 2009).

Results and discussion

HEK vs. CHO $Na_V1.5$ (Late) cell lines

We created polyclonal populations of ΔKPQ $Na_V1.5$ expressing HEK and CHO cells to compare levels of endogenous vs exogenous current expression. The transfected CHO cells showed minimal expression with negligible peak inward currents under standard cell culture conditions, and < -2.0 nA of peak $Na_V1.5$ current after low temperature preincubation (Fig. 2). In contrast, large inward sodium currents of ≥ -5.0 nA could be evoked from transfected HEK cells (Fig. 2), so these were used to develop the $Na_V1.5$(Late) assay on QPatch.

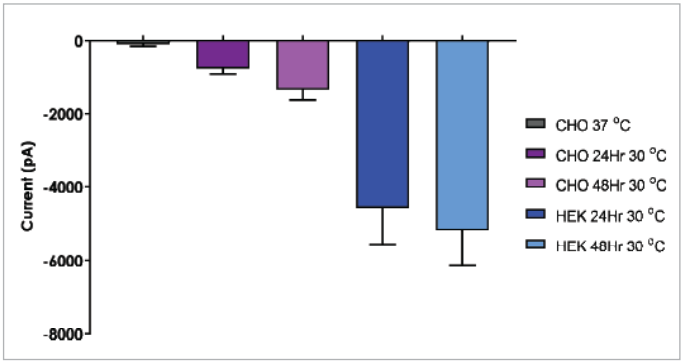

Fig. 2: **Comparison of $Na_V1.5$ ΔKPQ peak current expression in CHO and HEK cells.** CHO cells failed to express sufficient peak inward current (purple bars) to resolve sufficient late currents, but robust expression of LQT3 mutant $Na_V1.5$ currents in HEK cells (blue bars) enabled further assay development.

Choosing the optimal voltage protocol

A number of voltage protocols have been used to evoke $Na_V1.5$(Late) currents, including CiPA protocols, action potential-like waveform and step-ramp protocols (Fig. 3). Metrion compared each of these voltage protocols on the HEK $Na_V1.5$ ΔKPQ cell line on QPatch. The CiPA step-ramp voltage protocol produced small currents during the "ramp" phase, but there was evidence of persistent current after the initial depolarisation to -15 mV. Action potential-like waveforms produced currents with a large peak current followed by a persistent current that increased during the "repolarisation" phase of the simulated action potential voltage command. Finally, we tested a simple step-ramp protocol and further optimised it to create a stable $Na_V1.5$(Late) current assay for pharmacological validation.

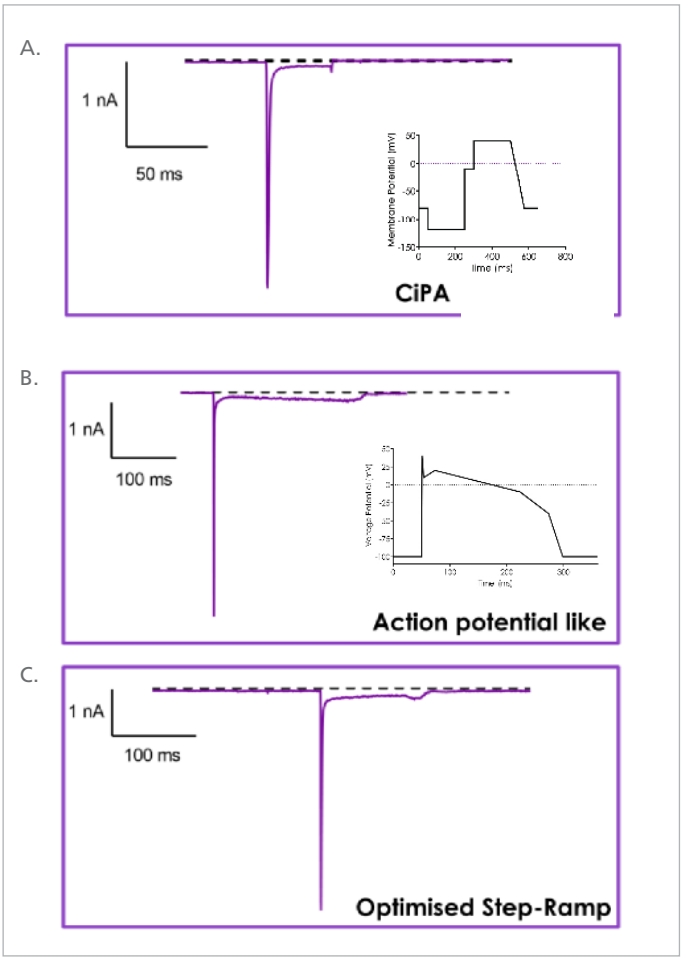

Fig. 3: **Assessment of various voltage protocols in $Na_V1.5$ ΔKPQ.** The CiPA ion channel working group (ICWG) voltage protocol (A) was used as the benchmark and compared with an AP-like waveform (B) and a step-ramp voltage protocol (C). Optimisation of step voltages and ramp speeds produced an optimised QPatch voltage protocol able to elicit robust and stable $Na_V1.5$(Late) currents.

Pharmacological validation of the $Na_V1.5$ ΔKPQ assay

Metrion validated the HEK-$Na_V1.5$(Late) cell line assay by testing two known $Na_V1.5$(Late) blockers using an optimised step-ramp voltage protocol. Both mexiletine and ranolazine showed a preference for inhibiting the late current compared with peak inward current (Fig. 4). Significantly, there was little difference in the $Na_V1.5$(Late) potency of each reference compound when measured during the persistent phase of the long depolarising step pulse or during the ramp command (Fig. 4).

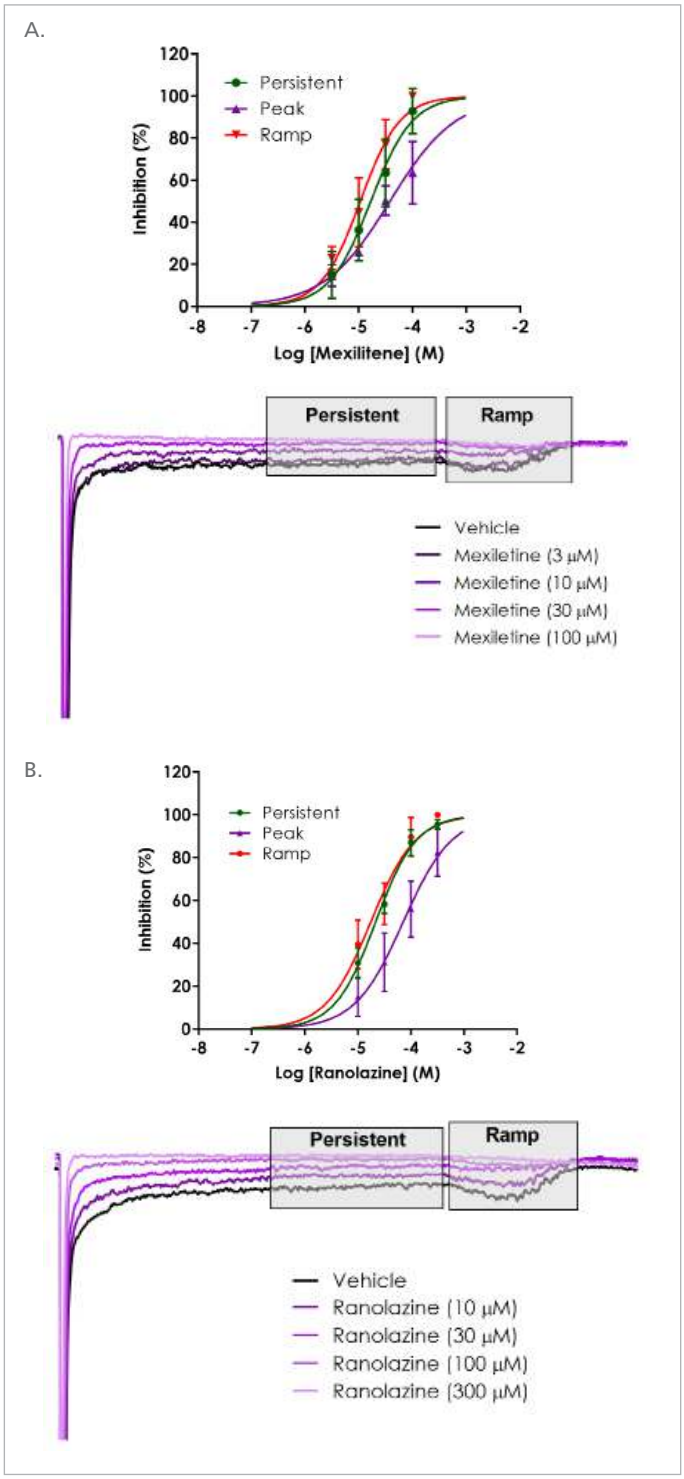

Fig. 4: **Validation of $Na_V1.5$(Late) assay with known sodium channel inhibitors.** A) Mexiletine showed a preference for inhibition of the late current (ramp =10.7 µM, persistent = 16.8 µM) compared with the peak current (38.0 µM). B) Ranolazine showed a greater preference for inhibiting the late current compared with peak current (ramp = 17.6 µM; persistent = 21.7 µM; peak = 71.7 µM).

Conclusion

A reliable, cost-effective and accurate $Na_V1.5$(Late) current assay is required on APC platforms to provide accurate cardiac safety data to support *in silico* models of proarrhythmic risk. $Na_V1.5$(Late) assays that employ non-selective activators, such as veratridine or ATX-II, produce unreliable IC_{50} values, poor stability and can be extremely expensive (ATX-II). We have used a pathophysiological ΔKPQ LQT3 mutant to create and validate a $Na_V1.5$(Late) assay that should remove the requirement for pharmacological enhancers of $Na_V1.5$(Late) and, thereby, deliver improve cardiac safety screening reliability and cost.

Methods

CHO and HEK293 cells obtained from ATCC/ECACC were transfected with a vector containing verified human LQT3 mutant $Na_V1.5$ ΔKPQ sequence using a liposomal based transfection methodology. Cells were cultured and harvested using Metrion's optimised QPatch protocols. Standard QPatch cell suspension, sealing and whole-cell protocols were utilized, with minor adjustments to obtain a high proportion of gigaohm seals and acceptable amplitude whole-cell sodium current amplitudes.

References:

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