Application Report

K_V7.4 channel assay for QPatch®: Biophysics and Pharmacology

A K_V7.4 channel assay with high success rates for biophysical evaluation and pharmacological screening, with relevance across all K_V7 subtypes.

Summary

Studies of the K_V7.4 ion channel using the automated patch clamp platform QPatch with the focus on:

- High success rates: 71% ± 6% (avg ± SD)
- K_V7.4 ion channel biophysics:
  - Voltage-dependent current and conductance curves
  - Voltage-dependent tail currents
- Pharmacological characterization of known activators and inhibitors
- Pharmacological tool compounds with different mechanisms of action, both activators and inhibitors, were profiled in concentration-response curves.

Introduction

The five members of the K_V7 voltage-gated potassium channel family (from K_V7.1 to K_V7.5) have distinct expression patterns and functional roles: K_V7.1 is primarily expressed in the heart, whereas K_V7.2-5 are expressed in neural tissue[1, 2]. K_V7.2-5 ion channels are involved in regulating excitability, action potential duration and transport processes in neurons and a variety of cell types[1, 2]. Indeed, K_V7.4 channels are specifically expressed in the inner ear[2-5].

Modulating K_V7.4 channels can stabilize electric activity, and this modulation holds great potential in the treatment of epilepsy. K_V7.4 agonists have also been shown to protect hearing function and prevent age-related hearing loss[6].

Here we employ K_V7.4 ion channels to demonstrate a robust and reproducible K_V7 assay, suitable for biophysical characterization and fast and reliable compound testing, using Sophion QPatch automated patch clamp (APC) platform.

Results and discussion

K_V7.4 biophysics and pharmacology were electrophysiologically characterized as presented in the following two sections:

- Biophysical characterization shows good assay performance with robust current amplitudes and $V_{½}$ of activation.

Biophysical properties of K_V7.4

First, we describe a biophysical characterization, showing currents in response to increasing voltage steps (Fig. 1), current- and conductance-voltage relationship (Fig. 2B-C) and tail currents (Fig. 2D). In the figures, one compound (Retigabine) has been included in one concentration (33 µM) to demonstrate how biophysical properties such as voltage sensitivity, gating kinetics and current size can be pharmacologically modulated (Fig. 2).

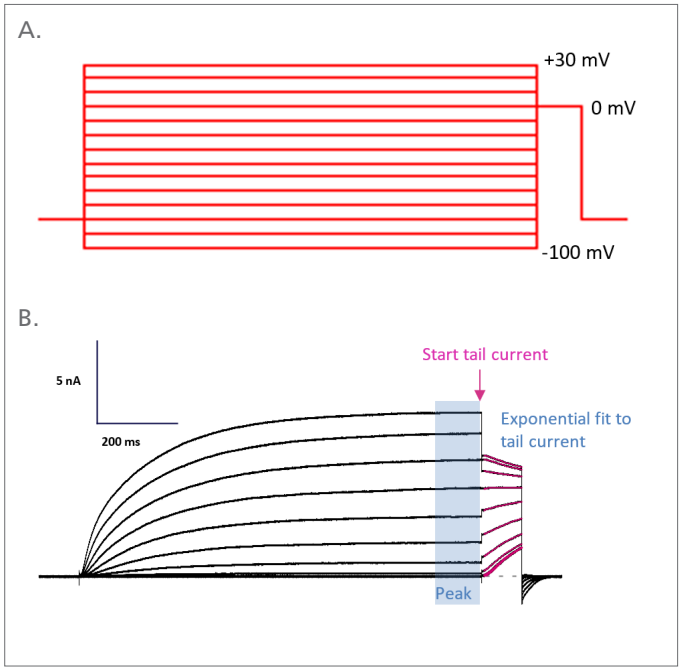

Fig. 1: Evaluation of K_V7.4 currents on QPatch. A) Voltage protocol applied for biophysical evaluation of K_V7.4 channels. B) Representative K_V7.4 currents in response to a step protocol of 1s long with, +10 mV increment steps from -100 mV to +30 mV (activation peak, blue cursor), followed by a 100 ms step to -10 mV (tail current).

The voltage protocol, illustrated in Figure 1.A, allowed the evaluation of different parameters:

- Peak current, measured during the last 50 ms, was used to plot the current-voltage (Fig. 2B) and conductance-voltage (Fig. 2C) relationship.
- Start tail current (Fig. 1B, pink arrow), was estimated from an exponential fit to the tail current trace and used to plot the tail current-voltage relationship (Fig 2D). This method was used to overcome the small capacitive current, arising in the shift from the activation voltage step protocol to the tail current.

To assess the effect of a compound on different biophysical properties of $K_V7.4$ channels, we used 33 µM retigabine, a channel opener (Fig. 2).

The K_V7 channel activator, retigabine, was developed as an antiepileptic drug. However, retigabine was withdrawn from the market due to safety concerns, including a possible blue discoloration of the skin and eye abnormalities[5]. Nowadays, there is a focus on developing safer and more effective alternatives.

As illustrated in Fig. 2, retigabine modulates the activation properties of $K_V7.4$ by shifting both the current-voltage and conductance-voltage relationships to the left (Fig. 2B and 2C). The $V_{½}$ activation went from -25.2 mV to -39.4 mV in the presence of the $K_V7.4$ activator, 33 µM retigabine. As for the activation curves, retigabine shifted the $K_V7.4$ tail current (Fig. 2D) towards more negative voltages ($V_{½}$ from -19.5 mV in saine to -35.3 mV in the presence of compound). The current level at the start of the tail was determined by extrapolating an exponential fit to the tail current.

The biophysical parameters are summarized in Table 1.

Table 1: Group statistics on the Biophysical properties of $K_V7.4$. Shown values are average values ± SD.

Biophysical properties	Avg. ± SD
Well QC pass rate (%)	71% ± 6
Peak current at 20 mV (nA, fig. 1)	3.6 ± 0.3
$V_{½}$ (peak current, mV, fig. 3)	-25.3 ± 1.0
V_{Rev} (mV, fig. 3)	-42.9 ± 0.8
$V_{½}$ tail (mV, fig. 5)	-19.5 ± 1.4

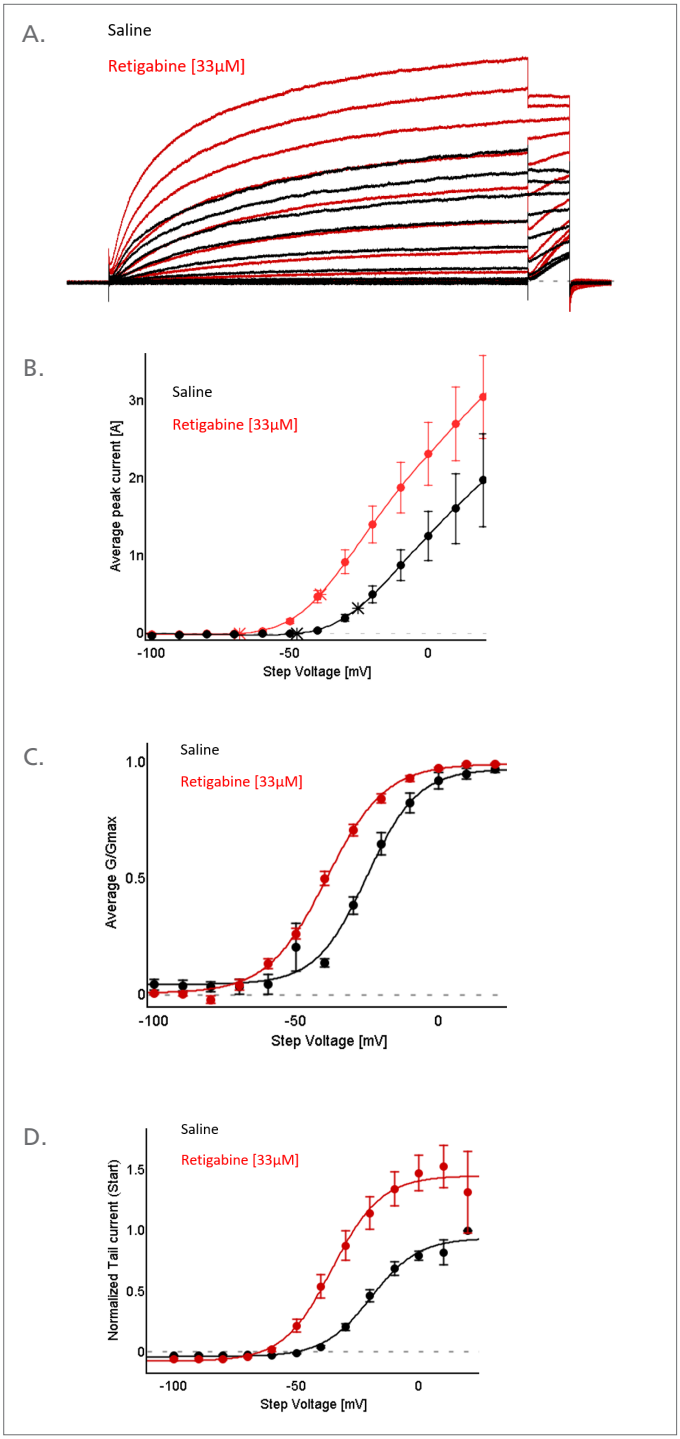

Fig. 2: Pharmacological modulation of $K_V7.4$ biophysics by retigabine. A) Representative $K_V7.4$ current traces under saline conditions (black) and after application of 33 µM retigabine (red). B) Average current from the last 50 ms of the activation step plotted against voltage, showing the voltage-dependent activation of the $K_V7.4$ channel in saline (black, n=14) and retigabine (red, n=14) conditions. $V_{½}$ and V_{Rev} are indicated with stars on both curves. C) Relative conductance (normalized within each cell to max conductance) plotted against voltage in saline (black) and in the presence of retigabine (red). D) Tail current-voltage relationship with (red) and without (black) retigabine. Error bars represent mean values +/- SD. The fitted curves represent standard (Fig 2B) or transformed (Fig. 2C-D) Boltzmann fits.

K_V7.4 pharmacology

Supported by both functional and genetic evidence, K_V7 channels are currently considered primary pharmacological targets for several human diseases: K_V7 blockers have a demonstrated positive effect on cognitive performance whereas K_V7 activators provide a therapeutic potential controlling neuronal hyperexcitability observed in several diseases, including epilepsy, migraine, and chronic pain[1].

Biophysical properties such as voltage sensitivity, gating kinetics and current size can be both positively and negatively modulated. Hence, we evaluate the effect of different compounds on biophysical properties such as voltage sensitivity/gating (Delta $V_{½}$) and current amplitude, and the following figure and tables will summarize data from:

- reference activators: flupirtine maleate, ICA 069673, and retigabine,
- reference inhibitions: linopirdine, XE 991 dihydrochloride and ML 252.

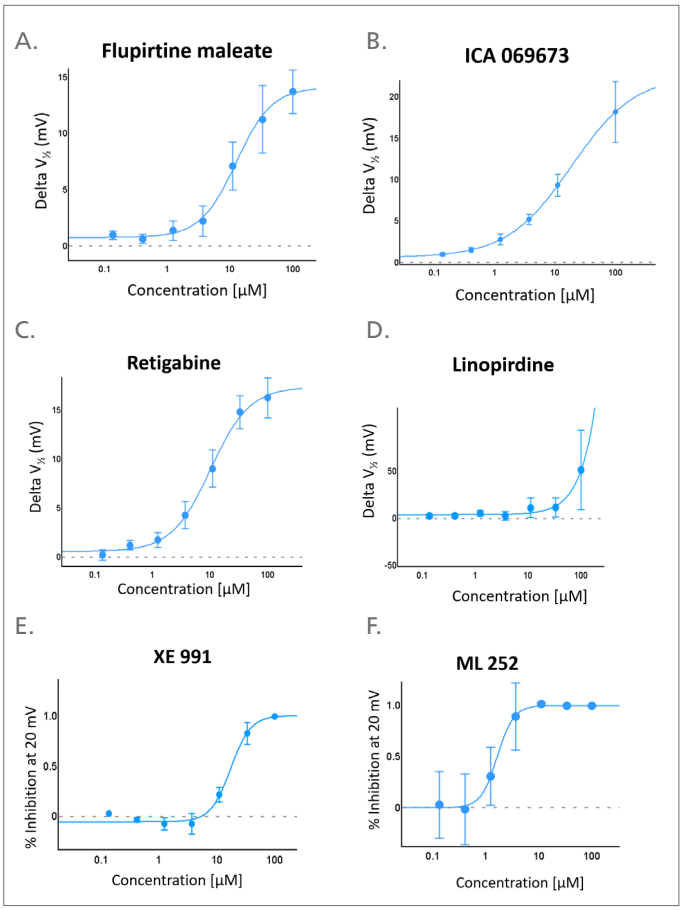

Fig. 3: Pharmacological evaluation of reference compound profiles on K_V7.4 channels. Cumulative concentration-response curves representing [Delta $V_{½}$] for K_V7.4 activators flupirtine maleate (A), ICA 069673 (B), and retigabine (C). Effects of K_V7.4 inhibitors such as linopirdine (D), XE 991 dihydrochloride (E) and ML 252 (F) are represented by cumulative concentration-response curves plotting the current amplitude at +20 mV.

Data from Fig. 3 are summarized in Tables 2 and 3.

Table 2: Summary table showing EC_{50} values from fig. 3A-C.

	EC_{50} for [Delta $V_{½}$]	EC_{50} for I_{-40mV}
Flupirtine maleate	12.6 µM	15.9 µM
ICA 069673	18.7 µM	8.9 µM
Retigabine	10.3 µM	9.5 µM

Table 3: Summary table showing IC_{50} values from fig. 3D-F

	EC_{50} for [Delta $V_{½}$]	IC_{50} for I_{+20mV}
Linopirdine	> 30 µM	5.1 µM
ML 252	> 30 µM	1.5 µM
XE 991	> 30 µM	14.4 µM

Methods

HEK-K_V7.4 were kindly supplied by Saniona and cells were cultured according to the supplier's description. All experiments were carried out at ambient temperature (controlled at 22°C ± 1°C) using QPatch single-hole consumables and patched using a standard HEK whole-cell protocol and physiological solutions.

$V_{½}$ and V_{Rev} were estimated using the transformed Boltzmann fit:

The method fits the data from the plot to the formula:

$$I_V=(V - V_{Rev}) \times \frac{G_{Max}}{\left(1+e^{\frac{(V-V_{½}(G))}{aV}}\right)}$$

where

V_{Rev} is the reversal potential

$V_{½}$(G) is the center potential

G_{Max} is the maximal conductance

dV is a voltage constant

To get the full QPatch K_V7 example assay, contact your application scientist or info@sophion.com.

Quality filters:

C_{Slow}: 4 pF

$R_{Membrane}$: 200 MΩ

References

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