High-throughput compound screening of K_V7.2/7.3 using the Automated Patch Clamp platform Qube 384

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Introduction

The heteromeric potassium channel KCNQ2 (K_V7.2)/ KCNQ3 (K_V7.3) contributes to the subthreshold M-current in many neurons, helping regulate excitability and stabilize the membrane potential near rest. Upon hK$_V$7.2/7.3 channel opening, the excitability of neurons is decreased. Loss-of-function mutations have been found to underlie a spectrum of neurological diseases such as neonatal-onset epilepsy, and epileptic encephalopathy (Biervert *et al.*, 1998; Jentsch, 2000; Maljevic *et al.*, 2008; Weckhuysen *et al.*, 2012; Kato *et al.*, 2013), which makes the channel an interesting drug target.

In 2011, the hK$_V$7.2/7.3 channel opener retigabine was approved as an antiepileptic drug. Due to its adverse effects and lack of specificity, like activation of the hK$_V$7.4 channel, it was withdrawn from the market. However, retigabine validated opening of the hK$_V$7.2/7.3 channel as an antiepileptic strategy and thereby further put a spotlight on the development of drugs aiming at this channel.

Material and methods

Cell culture and preparation: Experiments in this study were performed on HEK-hK$_V$7.2/7.3 cells, which were kindly provided by Saniona. The cells express concatenated cDNA for K_V7.2 (KCNQ2) and K_V7.3 (KCNQ3). Cell culture HEK-hK$_V$7.2/7.3 cells were cultured and harvested according to Sophion standard procedures. After being exposed at maximum 5 minutes to detachin, the cells were harvested in 5 ml serum-free media and gently transferred to the Qube, where the cells were prepared for experiments using the automatic cell preparation unit (200 seconds centrifugation at 50g).

Patch clamp experiment: All patch clamp experiments were carried out using the Qube 384 platform (Sophion Bioscience A/S, Denmark).

Experimental setup: For worktable, cell preparation and cleanup, Qube 384 default protocols were used.

Whole-cell protocol: A two-second suction pulse from -10 mbar to -250 mbar was followed by 10 seconds at -10 mbar and thereafter a two-second suction pulse from -10 mbar to -350 mbar was applied. The cell was then clamped at -100 mV.

Voltage protocol: Cells were held at a -100 mV holding potential and were depolarized for 200 ms to +20 mV. For the IV relationship studies, a 10 mV voltage step protocol up to +30 mV was used.

Sophion's automated patch clamp system **Qube 384** for high performance and high-throughput ion channel characterization and screening.

Results

Hallmarks of K_V7.2/7.3 on Qube 384

Cells were clamped to -100 mV and potassium currents were evoked by application of 1.5 seconds long depolarizations up to +30 mV in 10 mV steps. The cells were subsequently clamped to -30 mV for 1.5 seconds (Fig.1B, C). All experiments were conducted on multihole QChips – meaning 10 patch holes per well.

With success criteria of > 100 MΩ per cell > 6 pF per cell > 500 pA the success rates were at least 86% for each experiment. On average, the current size was 1.35 ± 0.03 nA per cell and the resistance was 590 ± 20 MΩ (± SEM). The tail current at -30 mV was plotted against the applied step-voltage protocol up to 30 mV (see Fig. 2A) and fitted to a Boltzmann curve (Fig.2B).

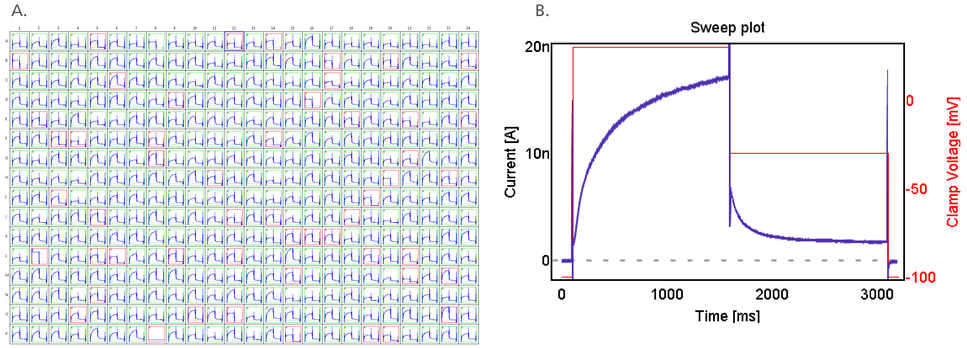

Fig. 1: Raw data traces of a multi-hole QChip (10 patch holes/well) and voltage protocol. **A**: Plate view of the potassium currents after depolarization to 30 mV. Red squares indicate a site that has failed the success criteria, green sites have passed all criteria. **B:** Recording of a potassium current (blue) following a depolarization from -100 mV to +30 mV for 1.5 s (red) in K_V7.2/7.3-expressing cells. The left y-axis displays current and the right y-axis displays the holding potential.

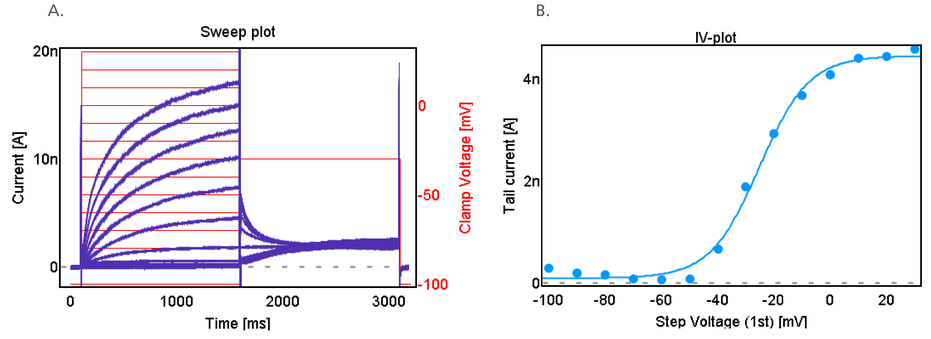

Fig. 2: A: Recording of a potassium current (purple) following a depolarization step protocol from -100 mV to +30 mV in 10 mV intervals for 1.5 s, stepping back to -30 mV (1.5 s) (red). The left y-axis displays current and the right y-axis displays the holding potential. **B:** The tail current, resulting from a step to -30 mV after applying conditioning voltage steps, was fitted to a Boltzmann curve.

Stable $V_{½}$ values for K_V7.2/7.3 on Qube 384

In order to evaluate a compound effect on the activation of K_V7.2/7.3, a stable baseline is crucial. To test the $V_{½}$ stability over time and across liquid additions, the step protocol from Fig. 2 was executed once in extracellular saline, 3 times within an application of extracellular saline, and once again after further addition of extracellular saline over a period of 19.5 minutes in total. The average shift of the $V_{½}$ value was -1.26 ± 0.19 mV when comparing the average value of the second and third liquid addition and -3.02 ± 0.27 mV when comparing the first to the third liquid addition (± SEM). The average $V_{½}$ value of all successful experiments before compound addition was -22.2 ± 0.3 mV.

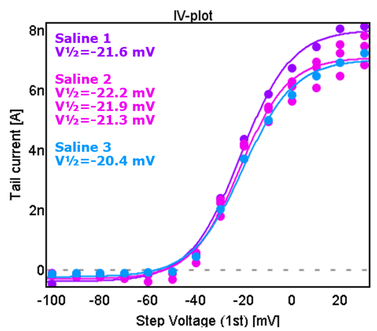

Fig. 3: The tail currents resulting from a step to -30 mV after applying conditioning voltage steps were fitted to a Boltzmann curve. The curves are derived from 3 liquid additions and 5 voltage step protocols in total.

Retigabine reliably potentiates K_V7.2/7.3 currents

The anticonvulsant retigabine acts as a positive allosteric modulator on K_V7.2-7.5 channels. In our experiments, we first established baseline current and $V_{½}$ values by applying saline, followed by consecutive applications of 3 µM and 10 µM retigabine (Fig. 3). On average, 3 µM retigabine shifted the $V_{½}$ value -8.2 mV and 10 µM retigabine caused a shift of -16.3 mV, which is in accordance with literature values (Tatulian and Brown, 2003).

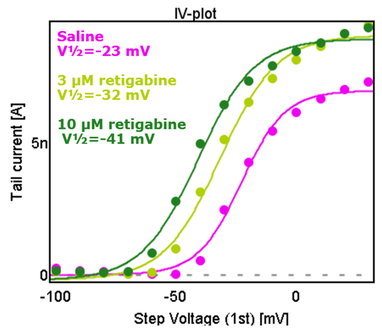

Fig. 4: The tail currents in saline and retigabine (3 µM / 10 µM) resulting from a step to -30 mV after applying conditioning voltage steps were fitted to a Boltzmann curve.

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