SOPHION BIOSCIENCE

Application Report

Stable $V_{½}$ values and reliable potentiation of K_V7.3/7.2 currents on Qube 384

High-throughput measurements of the M-current on the automated patch clamp platform Qube

Summary

The potassium channel K_V7.3/7.2 is a popular drug target for neurological disorders. We demonstrate that the automated patch clamp platform Qube 384 can successfully obtain K_V7.3/7.2 currents and its biophysical properties. Currents and half-activation potentials are stable over time, while the addition of retigabine reliably potentiates the current in agreement with the literature. The system thus provides an ideal mean of characterizing possible drug effects on this channel.

Introduction

The non-inactivating voltage-gated potassium M-current is based on heteromultimeric hK_V7.3/7.2 subunits in many neurons and has been found responsible for setting the resting membrane potential. Upon hK_V7.3/7.2 channel opening, the excitability of neurons is decreased, while 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_V7.3/7.2 channel opener retigabine was approved as an antiepileptic drug. But due to its adverse effects like its channel-opening effect on the hK_V7.4 channel, it was withdrawn from the market. However, retigabine validated opening of the hK_V7.3/7.2 channel as an antiepileptic strategy and thereby further put a spotlight on the development of drugs aiming at this channel.

Results

Hallmarks of K_V7.3/7.2 on Qube

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).

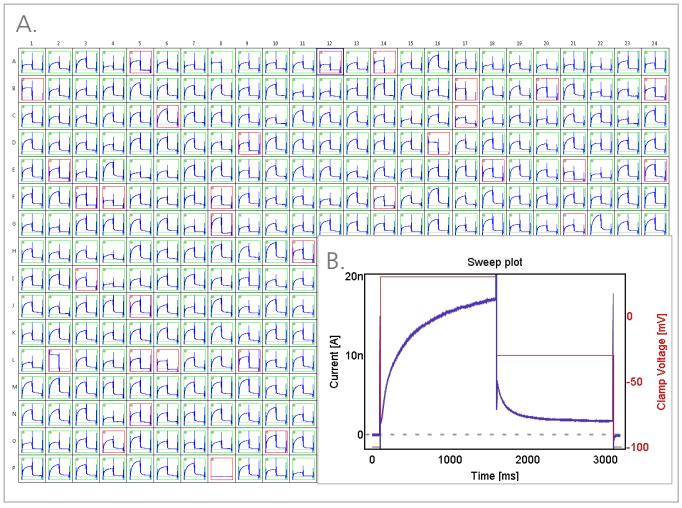

Fig. 1: Raw data traces of a multihole QChip (10 patch holes/well) and voltage protocol. A: Plate view of the potassium currents after a depolarization to 30 mV. Red squares indicate a site that has failed the success criteria, green sites have passed all criteria. B: Recording of a potassium current (blue) following a depolarization from -100 mV to +30 mV for 1.5 s (red) in K_V7.3/7.2-expressing cells (multihole consumable = 10 patch holes/well). The left y-axis displays current and the right y-axis the holding potential.

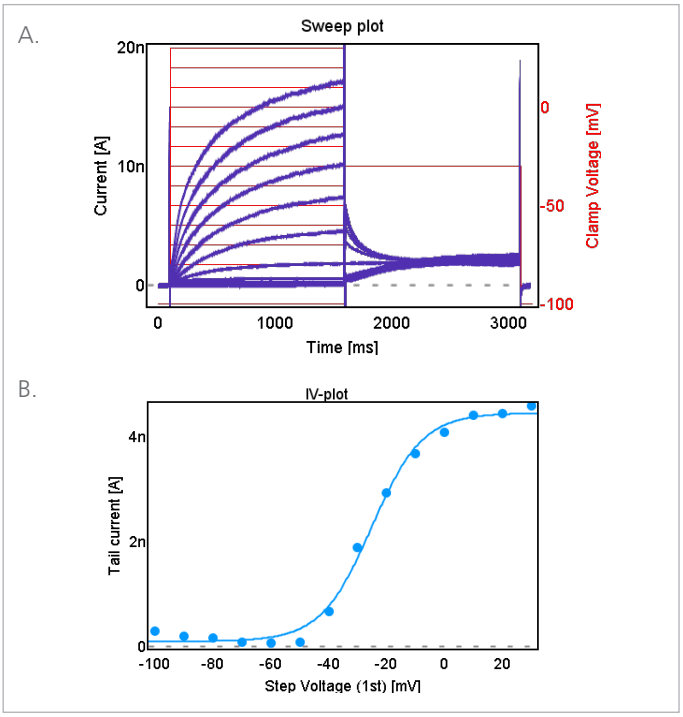

Fig. 2: A: Recording of a potassium current (purple) following a depolarization step protocol from -100 mV to +30 mV in 10 mV intervals for 1.5 s, stepping back to -30 mV (1.5 s) (red). The left y-axis displays current and the right y-axis the holding potential. B: The tail current resulting from a step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

Stable $V_{½}$ values for K_V7.3/7.2 on Qube

In order to evaluate a compound effect on the activation of K_V7.3/7.2, 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. For the entity of analyzed cells, the average shift of the $V_{½}$ value was -1.26 ± 0.19 mV when comparing the value of the average second to the value of the 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.

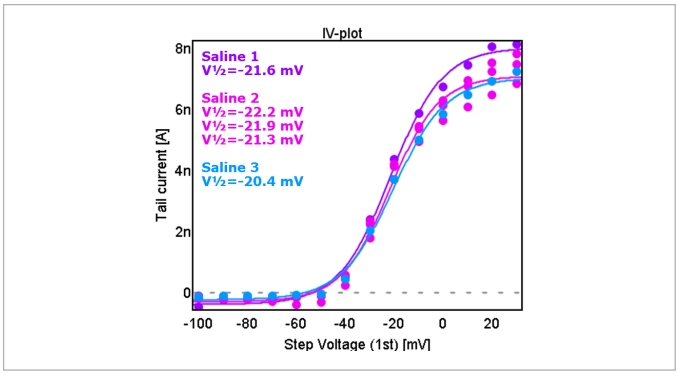

Fig. 3: The tail current resulting from a step to -30 mV after applying conditioning voltage steps was plotted against the voltage steps and fitted to a Boltzmann curve. The curves are derived from 3 liquid additions and 5 voltage step protocols in total.

Retigabine reliably potentiates K_V7.3/7.2 currents

The anticonvulsant retigabine acts as a positive allosteric modulator on K_V7.2-7.5 channels. In our experiments, we first established a baseline current/$V_{½}$ value by applying saline, followed by the application of 3 µM and then 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).

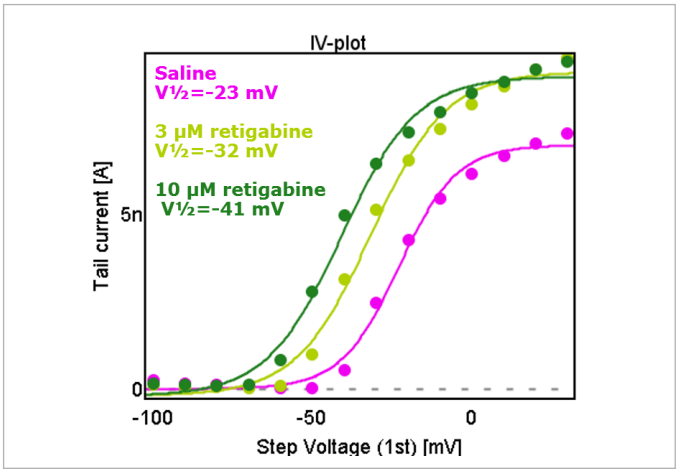

Fig. 4: The tail current in saline and retigabine (3 µM / 10 µM) resulting from a step to -30 mV after applying conditioning voltage steps was plotted against the voltage steps and fitted to a Boltzmann curve.

Methods

Experiments in this study were performed on HEK-hK_V7.3/7.2 cells, which were kindly provided by Saniona. The cells express concatenated cDNA for K_V7.3 and K_V7.2, which is selected for by 0.15 mg/ml hygromycin in culture flasks.

Cell culture

HEK-hK_V7.3/7.2 cells were cultured and harvested according to Sophion standard procedures. However, a few parameters were adjusted to take cell-specific properties into account.

After being exposed at maximum 5 minutes to detachin, the cells were harvested in 5 ml SFM and gently transferred to the Qube, where the cells were prepared for experiments using the automatic cell preparation unit (200 seconds centrifugation at 50g).

Experimental setup

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

References:

1. Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK., 1998. A potassium channel mutation in neonatal human epilepsy. Science 279 (5349) 403-6.
2. Jentsch TJ, 2000. Neuronal KCNQ potassium channels: physiology and role in disease. Nat Rev Neurosci. 1(1):21-30.
3. Kato M, Yamagata T, Kubota M, Arai H, Yamashita S, Nakagawa T, Fujii T, Sugai K, Imai K, Uster T, Chitayat D, Weiss S, Kashii H, Kusano R, Matsumoto A, Nakamura K, Oyazato Y, Maeno M, Nishiyama K, Kodera H, Nakashima M, Tsurusaki Y, Miyake N, Saito K, Hayasaka K, Matsumoto N, Saitsu H., 2013. Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation. Epilepsia, 54(7):1282-7.
4. Maljevic S, Wuttke TV, Lerche H., 2008. Nervous system K_V7 disorders: breakdown of a subthreshold brake. J Physiol. 586(7):1791-801.
5. Tatulian L, Brown DA., 2003. Effect of the KCNQ potassium channel opener retigabine on single KCNQ2/3 channels expressed in CHO cells. J Physiol 549(Pt 1):57-63.
6. Weckhuysen S, Mandelstam S, Suls A, Audenaert D, Deconinck T, Claes LR, Deprez L, Smets K, Hristova D, Yordanova I, Jordanova A, Ceulemans B, Jansen A, Hasaerts D, Roelens F, Lagae L, Yendle S, Stanley T, Heron SE, Mulley JC, Berkovic SF, Scheffer IE, de Jonghe P, 2008. KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. Ann Neurol. 71(1):15-25.

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