SOPHION BIOSCIENCE

Application Report

Reliable K_V2.1 assays and pharmacology on the QPatch

K_V2.1 currents on the QPatch are stable and pharmacology values for the tested compounds are consistent with the literature.

Summary

- K_V2.1 is a major player in diabetes, neurodegeneration and other diseases
- Reliable currents on single-hole QPlates
- Consistent half-activation potentials for activation and inactivation
- Pharmacology values in agreement with literature values

Introduction

The voltage-gated potassium channel K_V2.1 is the main contributor to the delayed rectifier potassium current in neurons and in the cortex (Du et al. 1998, Murakoshi et al. 1999).

It has been suggested that K_V2.1 activation confers neuroprotection in a state of neuronal hyperexcitability via hyperpolarizing the membrane potential (Du et al. 2000, Misonou et al. 2005, Misonou et al. 2005a). Therefore, K_V2.1 may be an attractive target in epilepsy research. Also, the ion channel has previously been shown to play a central role in neurodegeneration, ageing and pancreatic β cell signaling.

Results and discussion

Activation of K_V2.1 results in stable currents

Cells were measured on single-hole QPlates in standard solutions (see Materials & Methods). The channel's current-voltage curve was determined by applying a voltage step protocol and measuring the resulting peak current (Fig.1 & 2).

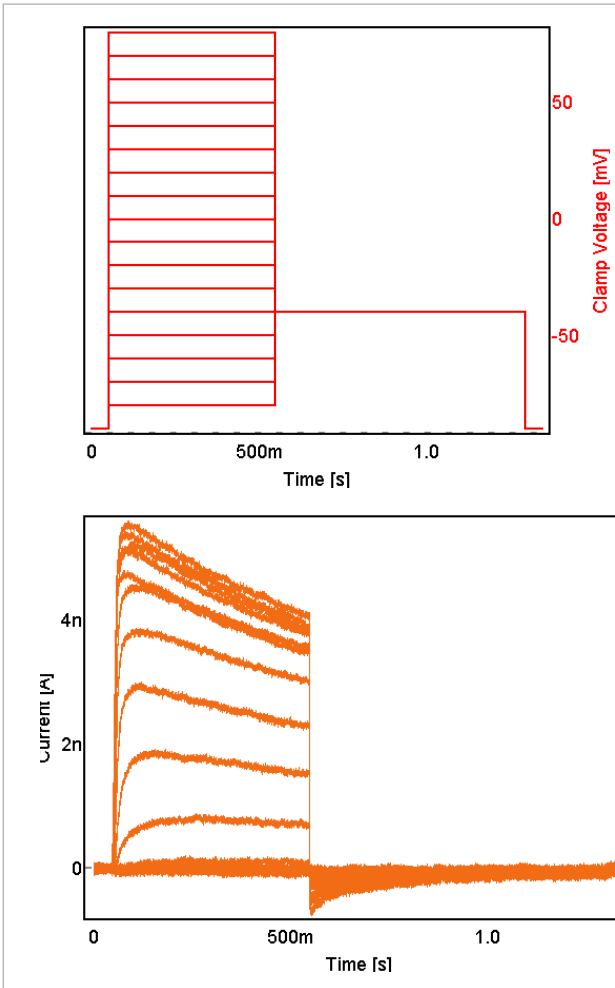

Fig. 1: CHO-K_V2.1 cells were depolarized from -90 mV to +80 mV in 10 mV steps (top). An example trace resulting from this protocol is shown in the lower figure.

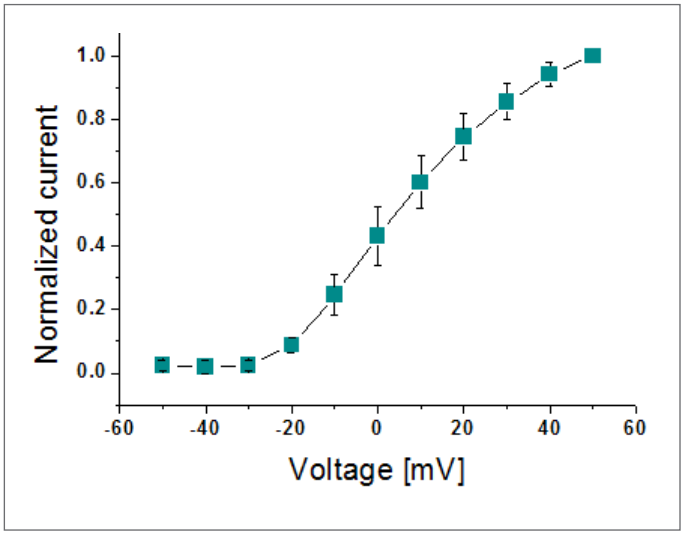

Fig. 2: Inactivation curve of $K_V2.1$. The current is normalized to the tail current amplitude at the -90 mV depolarization step. Data points represent normalized average tail current ± SD, n=18.

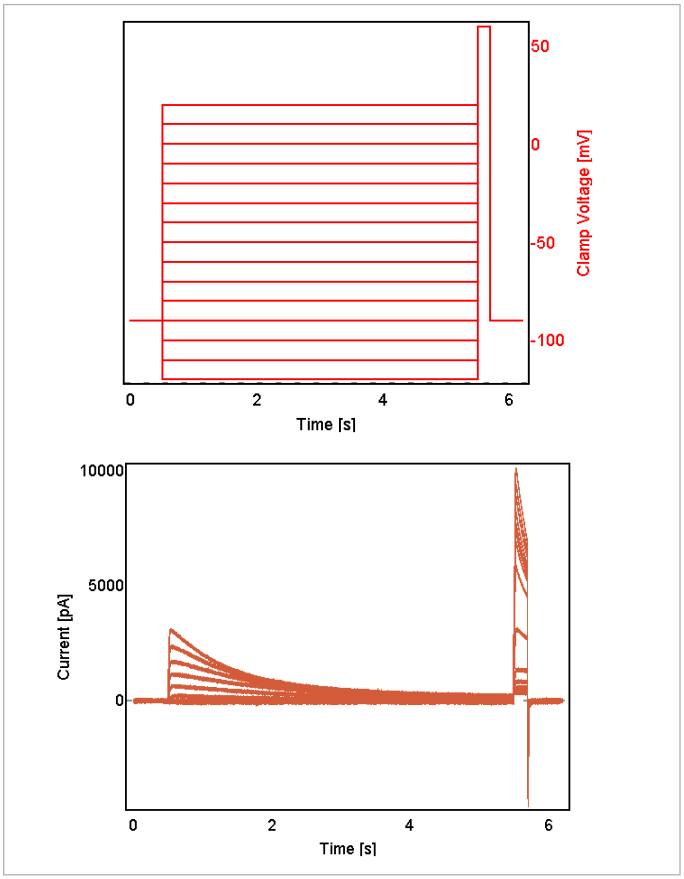

Fig. 3: CHO-$K_V2.1$ cells were clamped from -90 mV to +20 mV for 5 seconds in 10 mV increments, followed by a depolarization to +60 mV, at which the tail current was measured (top). An example trace resulting from this protocol is shown in the lower figure.

Inactivation of $K_V2.1$

As before, CHO $K_V2.1$ cells were measured on single-hole QPlates in standard solutions (see Materials & Methods). A voltage step protocol was applied and a tail current was evoked by a subsequent depolarization step to +60 mV, where the resulting current was measured (Fig. 3 & 4). The average half-maximum inactivation potential ($V_{½inact}$) was -33 mV.

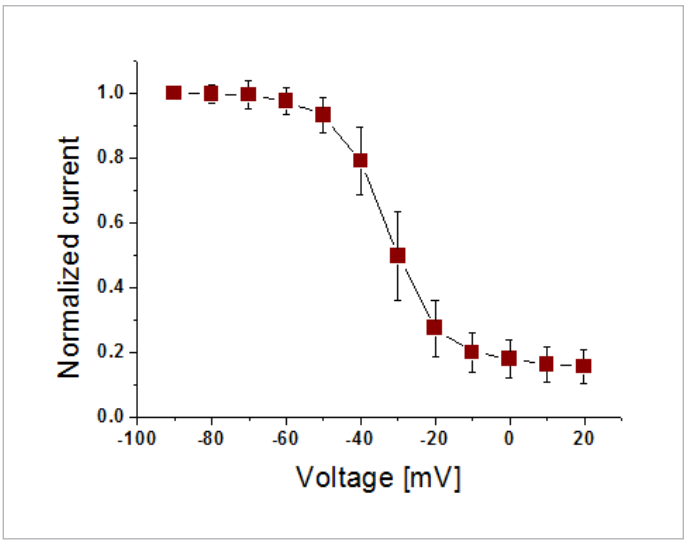

Fig. 4: Inactivation curve of $K_V2.1$. The current is normalized to the tail current amplitude at the -90 mV depolarization step. Data points represent normalized average tail current ± SD, n=18.

Pharmacology

CHO $K_V2.1$ cells were measured on single-hole QPlates in standard solutions (see Materials & Methods) with osmolarities adapted to the highest concentration of compound-containing solution (~350 mOsm). A depolarization from -80 mV to +20 mV was applied in saline solution, followed by cumulative addition of tetraethylammonium (TEA), nifedipine or saline. The lowest concentration of nifedipine was 1.2 µM, followed by 3.7, 11.1, 33.3 and 100 µM. The TEA solutions contained 0.37, 1.1, 3.3, 10 or 30 mM TEA. IC_{50} values of nifedipine (24 µM) and TEA (17mM) (Fig. 5) are in agreement with literature values (Taglialatela et al. 1991, He et al 2006, Xian-Tao Li et al. 2015).

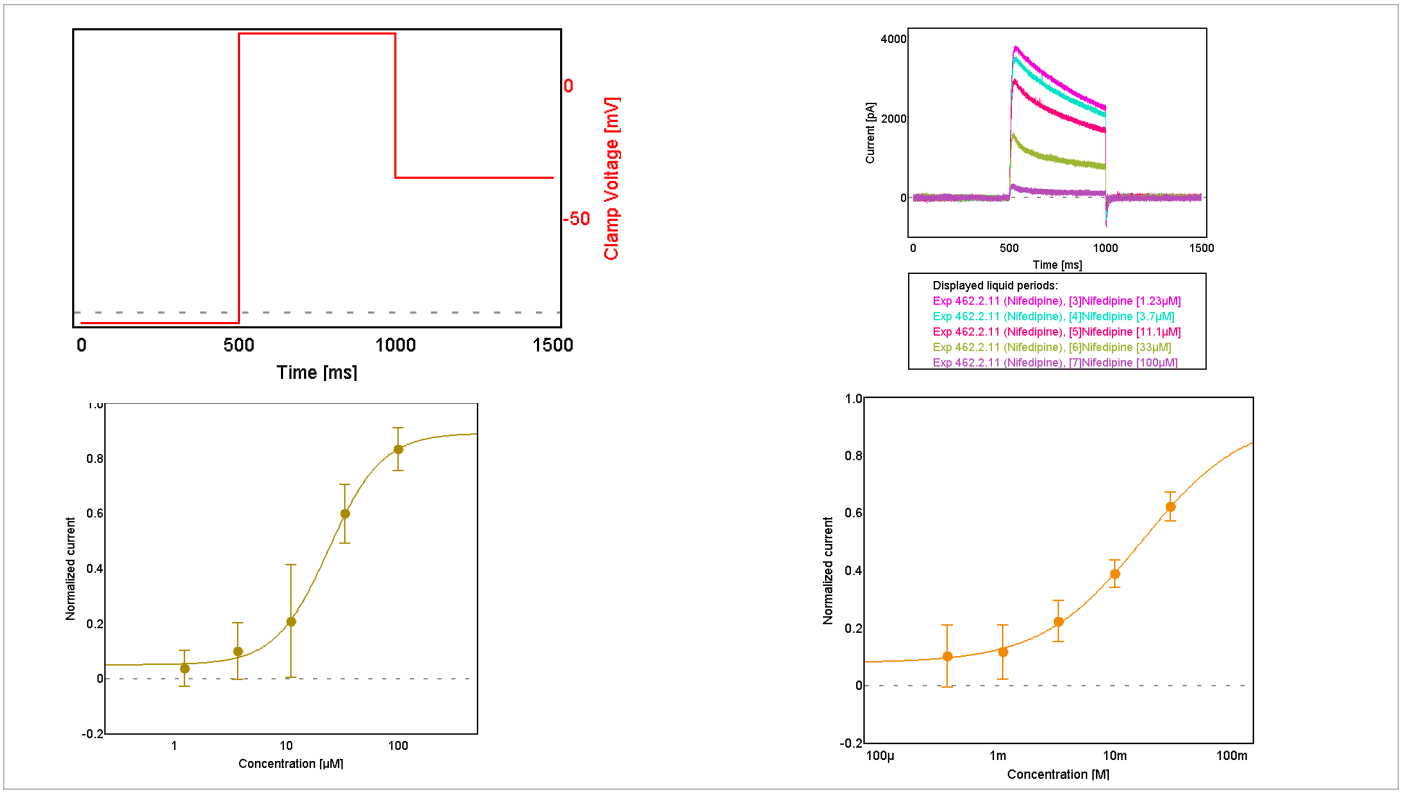

Fig. 5: Pharmacological inhibition of $K_V2.1$: Compound effects were assessed using a single depolarization step to +20 mV (left top). Both nifedipine (raw data example right top) and TEA inhibited $K_V2.1$ currents with IC_{50} values as displayed in the concentration-inhibition curve. A Hill equation was used to fit the data (solid lines). Data points represent normalized average current ± SD, n = 6 (nifedipine) / 9 (TEA).

Methods

Cell culture for CHO cells, constitutively expressing human $K_V2.1$ (NP_004966.1), was performed according to B'SYS recommendations. Seeding and harvesting was done following Sophion's standard procedures.

For electrophysiological recordings, the extracellular solution contained (in mM): NaCl 145, HEPES 10, Glucose 10, KCl 4, $CaCl_2$ 2, $MgCl_2$ 2. pH=7.4 with NaOH, Osmolarity: 290 mOsm.

The intracellular solution was (in mM): Choline chloride 120, KOH/EGTA 31.25/10, HEPES 10, $CaCl_2$ 5.374, Na-ATP 4, $MgCl_2$ 1.75. pH=7.2 with KOH, Osmolarity: 280 mOsm.

All measurements were conducted with single-hole QPlates. The whole-cell was reached with a CHO standard protocol (1 second suction from -20 to -250 mbar with a subsequent pressure increment of -50 mbar until the whole-cell state is formed).

Conclusion

The depolarization of $K_V2.1$ cells, kindly provided by B'SYS, reliably resulted in potassium currents on the QPatch. The current-voltage relationship for activation, as well as inactivation showed consistent values with minimal deviations. The QPatch is perfectly suited for the application of pharmacological compounds and the IC_{50} values for the drugs used in this assay align with literature values.

References:

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