Application Report:

CHO-K_V1.5

Voltage gated potassium channels

The voltage gated potassium channel K_V1.5 is a homotetrameric protein present in the heart. It is a delayed rectifier, participating in the early phase of the heart action potential. This report shows data from CHO cells stably expressing K_V1.5 tested on the QPatch platform. The cells are obtained through a collaboration with STZ (Germany).

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Introduction

The voltage gated potassium channel $K_V1.5$ is a homotetrameric protein present in the heart. It is a delayed rectifier, participating in the early phase of the heart action potential. This report shows data from CHO cells stably expressing $K_V1.5$ tested on the QPatch platform.

Materials & Methods

Intracellular saline (in mM): 5.374 CaCl2, 1.75 MgCl2, 3.125/10 KOH/EGTA, 10 Hepes, 120 KCl, 4 Na2-ATP, pH 7.2 with KOH, 285-296 mOsm. Extracellular saline (in mM): 2 CaCl2, 1 MgCl2, 10 Hepes, 4 KCl, 145 NaCl, 10 Glucose, pH 7.4 with NaOH, ~305 mOsm.

Cells: CHO cells stably expressing $K_V1.5$ were obtained from STZ (Mannheim, Germany). Cells were cultured and harvested for QPatch experiments as described in the Sophion SOP. Data shown here is from STZ CHO-$K_V1.5$ clone 16.

Results

Experiments were conducted to evaluate the IV-relationship of $K_V1.5$ as well as dose-response for inhibitors.

Figure 1 shows the currents elicited at potentials ranging from -90 mV to +50 mV in a representative experiment with CHO-KV1.5. The corresponding IV plot for both maximum and steady-state current is shown in Figure 2.

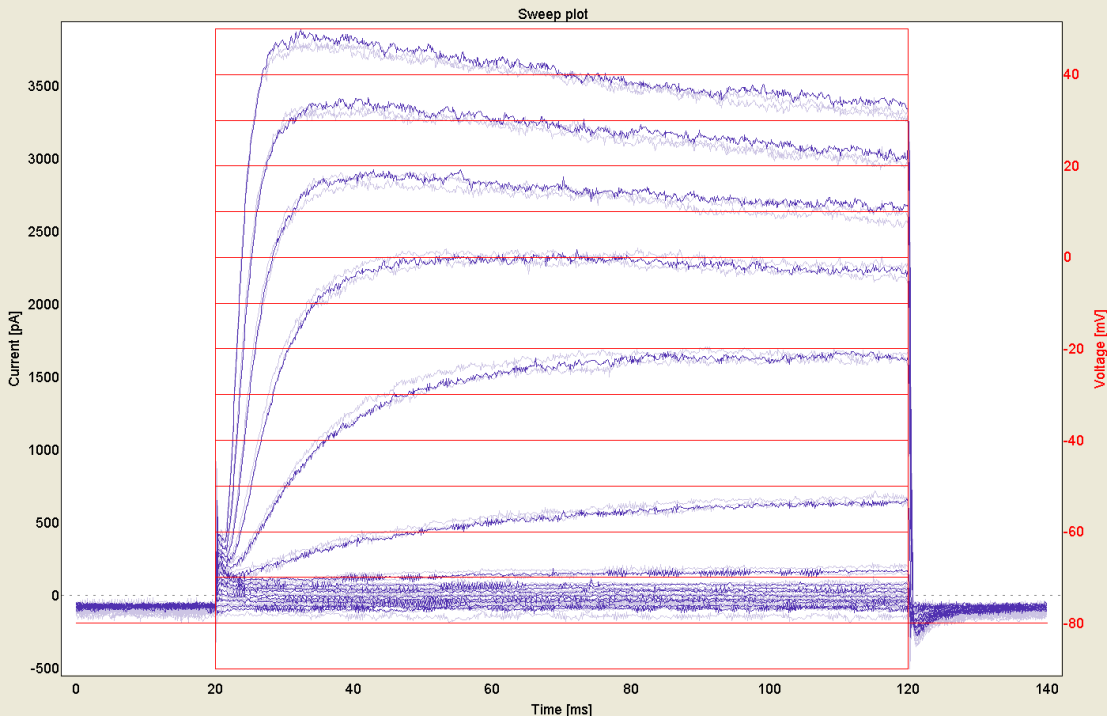

Figure 1. $K_V1.5$ raw data sweeps elicited in an IV-protocol with steps ranging from -90 to +50 mV.

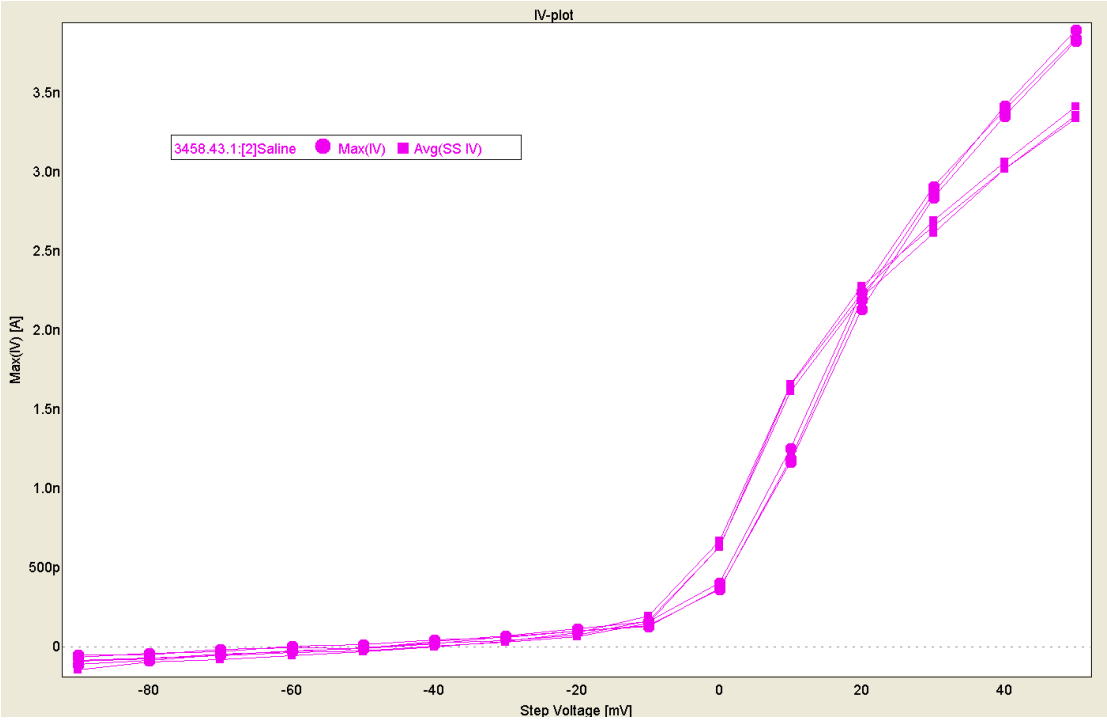

Figure 2. Current-voltage relationship (IV plot) of the data shown in Figure 1. Circles show the maximum elicited current, squares show the steady-state current.

The response of $K_V1.5$ to a known blocker was also tested. Figure 3 shows the raw data traces of the steady-state response to six different concentrations of 4-aminopyridine. Figure 4 and Figure 5 show the corresponding current versus time (IT) plot and Hill fit, respectively. The resulting IC_{50} for 4-aminopyridine is 63.8 µM.

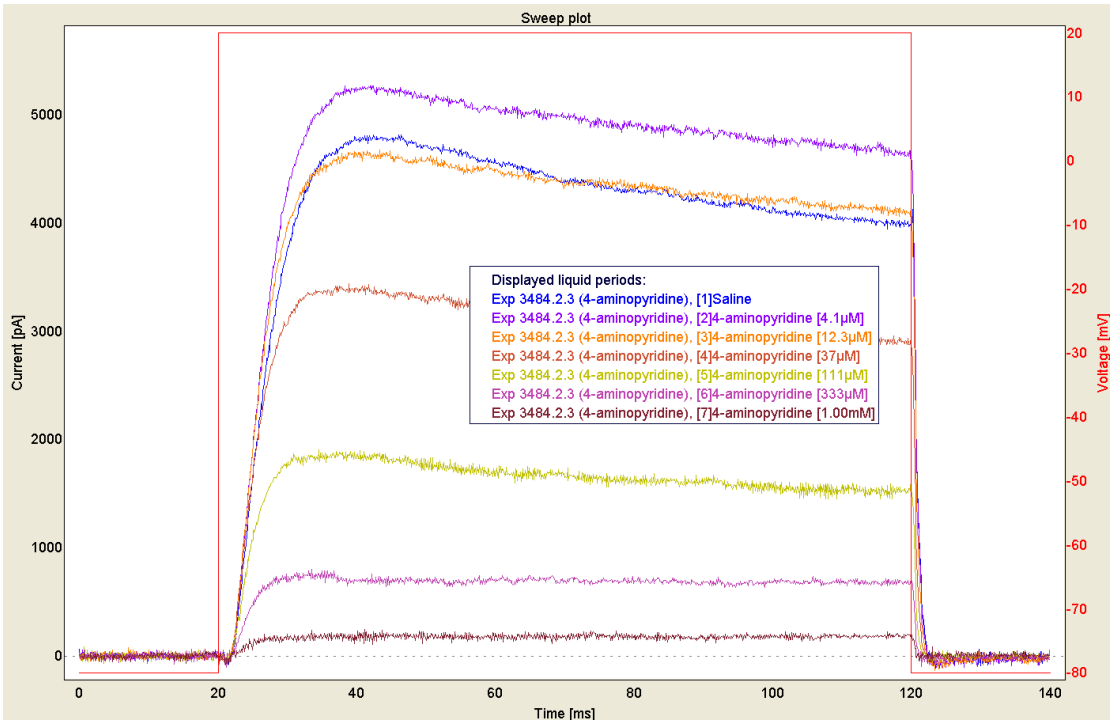

Figure 3. Six point cumulative dose-response experiment with 4-aminopyridine.

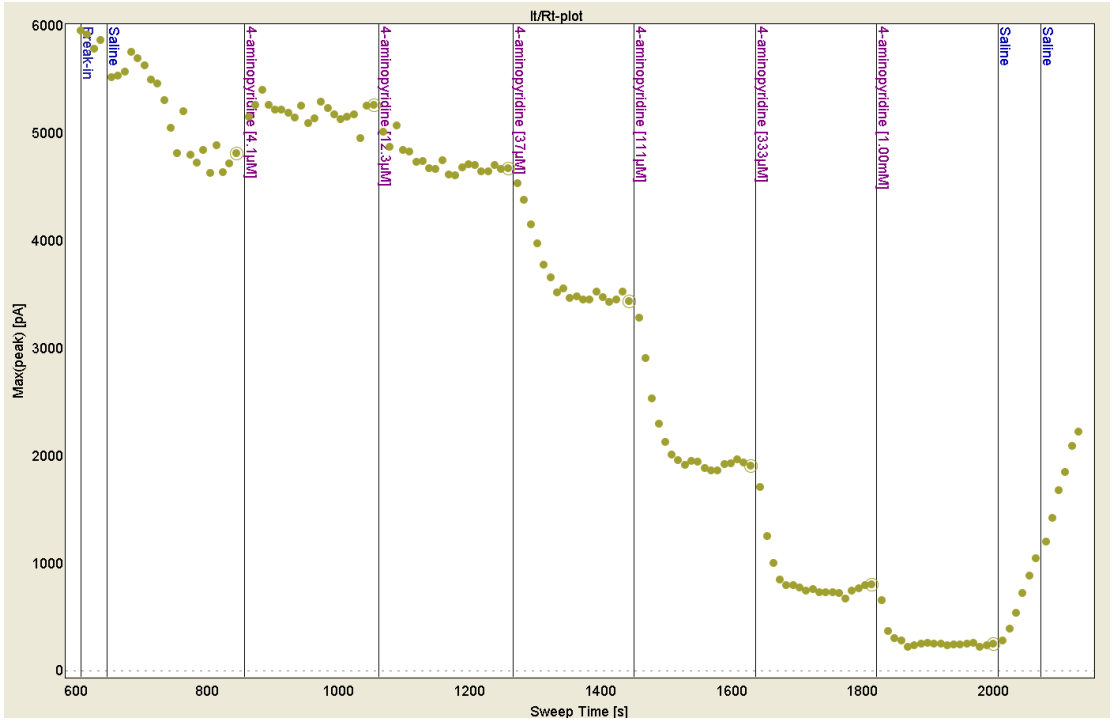

Figure 4. IT plot of KV1.5 channel response to increasing concentrations of 4-aminopyridine.

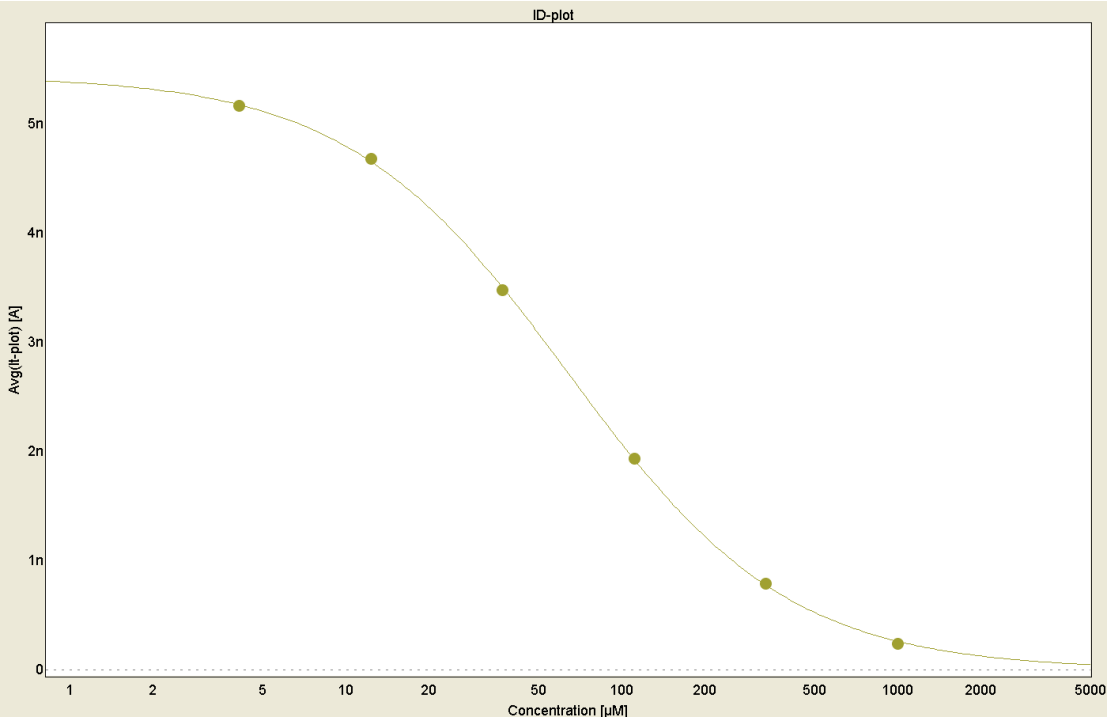

Figure 5. Dose-response plot, with Hill fit, of steady-state current level at six concentrations of 4-aminopyridine.

Conclusion

IV characteristics and dose-response experiments with a $K_V1.5$ channel blocker was successfully obtained using QPatch. $K_V1.5$ shows its characteristic outward rectification and an IC_{50} for 4-aminopyridine within range of reported literature values (e.g. Gutman et al., Pharmacological Reviews 57:473-508, 2005, 270 µM).