B'SYS GmbH

CHO K_V Cell Lines

Specification Sheet

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1. INTRODUCTION

The K_V family encodes voltage-gated potassium channels that are phylogenetically related to the Drosophila Shaker channel. The encoded proteins have six putative transmembrane segments (S1-S6), and the loop between S5 and S6 forms the pore and contains the conserved selectivity filter motif (GYGD).
They are widely expressed throughout the nervous system. The functional channel is a tetramer, while most of them have been shown to form heteromultimers in the CNS. Most of these multimers contain at least one K_V1.1 and/or K_V1.3 subunit. K_V1.x isoforms were also found in peripheral tissues, like in the heart, the cardiovascular and immune system (K_V1.5 / K_V1.3).
B'SYS K_V1.x cell lines express robust levels of these currents for more then 20 passages. The expressed K_V channels were verified by RT PCR. All B'SYS cell lines are periodically tested for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

2. MATERIAL AND METHODS

2.1. General

CHO K_V1.x cells are incubated at 37°C in a humidified atmosphere with 5% CO_2 (rel. humidity > 95%). The cells are continuously maintained and passaged in sterile culture flasks containing F12 (HAM) medium supplemented with 9% fetal bovine serum, 0.9% Penicillin/Streptomycin solution and 100 µg/mL Hygromycin. The CHO K_V1.x cells are passaged at a confluence of about 80%. For electrophysiological measurements the cells are seeded onto e.g. 35 mm sterile culture dishes containing complete medium.

2.2. Electrophysiology

K_V1.x currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, CaCl2 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. For automated patch-clamping the following solutions were used: K_V1.1, K_V1.2, K_V1.4: CsF 67.5, KCl 65, NaCl 5, $MgCl_2$ 0.5, $CaCl_2$ 0.1, Mg-ATP 2.5, HEPES 10, EGTA 5, pH (KOH) 7.2; K_V1.3, K_V1.5, K_V1.6: CsF 121.5, KCl 13, NaCl 9.0, $MgCl_2$ 0.1, Mg-ATP 0.5, HEPES 10, EGTA 5, pH (KOH) 7.2After formation of a GΩ seal between the patch electrodes and individual K_V1.x stably transfected CHO cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature.

2.2.1 Patch-clamp Success Rates

The patch-clamp properties of the CHO K_V1.x cell line were elucidated at typical working passage numbers (passage ≤ 20). More than 10 cells were analyzed per isoform. Success for establishment of on-cell configuration was defined as follows: > 1 GΩ. The whole-cell configuration was not accepted if the membrane resistance was below 1 GΩ. A successful recording had to be free of rundown effects and variations in series resistance.

- On-cell successful: **>95%**
- Whole-cell successful: **>85%**
- Recording (20-30 min) successful: **>75%**

2.3 Accession Numbers

K_V1.1 / KCNA1	BC_101733
K_V1.2 / KCNA2	NM_004974.2
K_V1.3 / KCNA3	NM_002232
K_V1.4 / KCNA4	NM_002233.2
K_V1.5 / KCNA5	NM_002234.2
K_V1.6 / KCNA6	NM_002235.3

3. K_V CELLS LINES

3.1. Introduction

K_V1.x channels are expressed in various tissues and fulfil several physiological important functions. K_V1.x can coassemble with other K_V1 family members in heteromultimers but not with members of other K_V families. Unique in that they have an intronless coding region.

K_V1.1 is expressed in CNS, the node of Ranviera and kidney. Missense mutations can cause episodic ataxia and primary hypomagnesaemia.

K_V1.2 was mostly found in CNS. There are now channelopathies known that are related to mutations within this ion channel.

K_V1.3 was found in T and B cells, macrophages, microglia, osteoclasts, platelets, CNS and testis. K_V1.3 expression is increased in activated effector memory T-cells and class-switched CD27+ memory B-cells. In contrast naive and memory T-cells and IgD^+ B-cells, up-regulate KCa3.1 following activation. Up-regulation of K_V1.3 has no real pathophysiological effect, but allows to selectively target effector memory T-cells. One single nucleotide polymorphism (T1645C) is associated with impaired glucose tolerance and lower insulin sensitivity.

K_V1.4 is expressed in CNS, heart, skeletal and smooth muscle and in pancreatic islets. It is expressed less after myocardial infarction, but until now, there is no channelopathy reported related to K_V1.4.

K_V1.5 has properties similar to the ultrarapidly activating I_{Kur} current in the heart. It was also found in vascular smooth muscle, CNS, microglia and Schwann cells. There were no mutations detected, but the expression level of K_V1.5 decreases during atrial fibrillation.

K_V1.6 was found in spinal cord, CNS, oligodendrocyte progenitor cells, astrocytes and pulmonary artery smooth muscle. No mutations were found causing channelopathies.

For more detailed information, please click:
http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=81

3.2. Representative Currents

K_V1.1

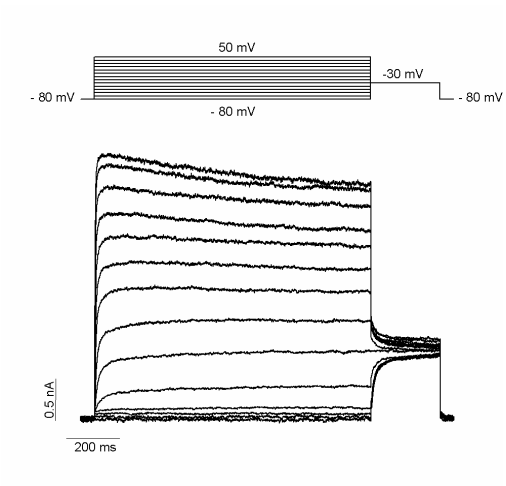

Fig. 1.1.1: Representative K_V1.1 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -80 mV to voltages between -80 mV and +50 mV in 10 mV increments (1000 ms). Voltage pulses to -300 mV (300 ms) elicited K_V1.1 tail currents.

K_V1.2

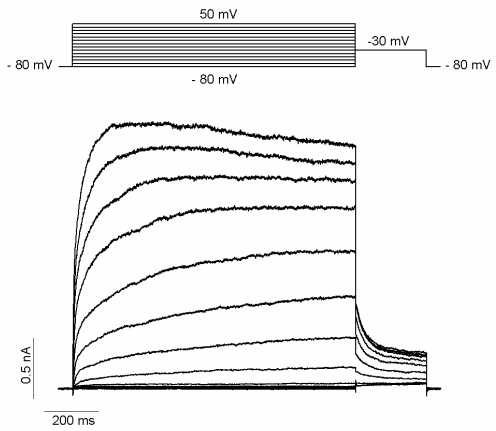

Fig. 1.1.2: Representative K_V1.2 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -80 mV to voltages between -80 mV and +50 mV in 10 mV increments (1000 ms). Voltage pulses to -30 mV (300 ms) elicited K_V1.2 tail currents.

K_V1.3

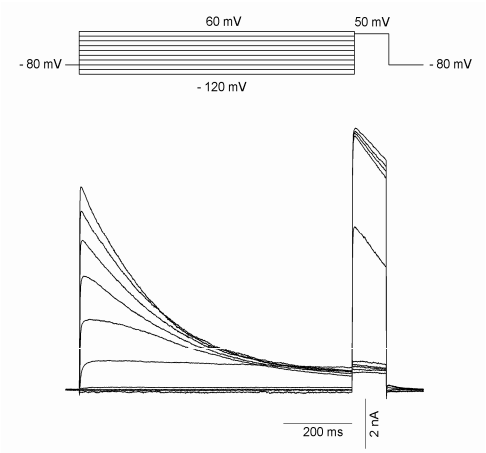

Fig. 1.1.3: Representative K_V1.3 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -80 mV to voltages between -120 mV and +60 mV in 20 mV increments (800 ms). Voltage pulses to 50 mV (100 ms) elicited K_V1.3 tail currents.

K_V1.4

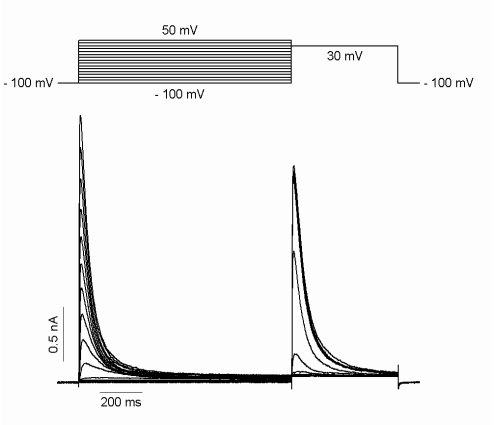

Fig. 1.1.4: Representative K_V1.4 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -100 mV to voltages between -100 mV and +50 mV in 10 mV increments (500 ms). Voltage pulses to -30 mV (250 ms) elicited K_V1.4 tail currents.

K_V1.5

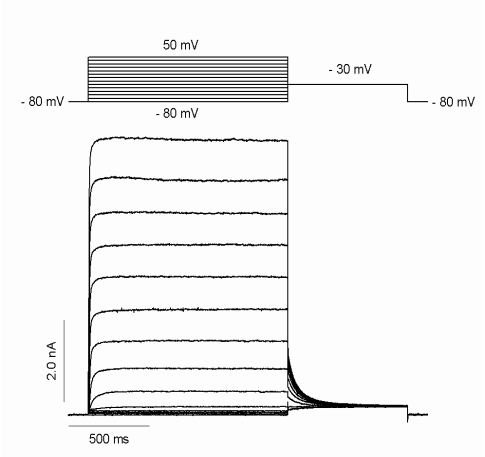

Fig. 1.1.5: Representative K_V1.5 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -80 mV to voltages between -100 mV and +50 mV in 10 mV increments (2000 ms). Voltage pulses to -30 mV (250 ms) elicited K_V1.5 tail currents.

K_V1.6

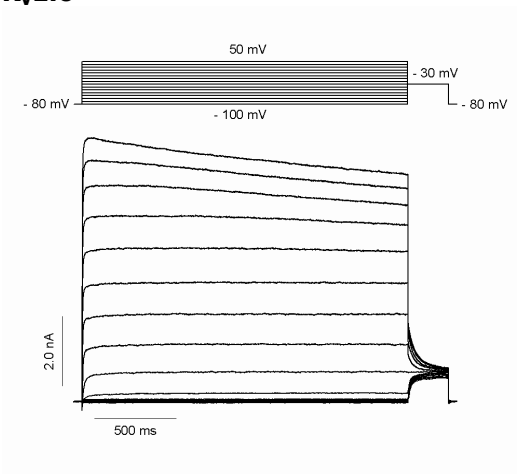

Fig. 1.1.6: Representative K_V1.6 outward currents recorded upon depolarization of the CHO membrane from a holding potential of -80 mV to voltages between -100 mV and +50 mV in 10 mV increments (2000 ms). Voltage pulses to -30 mV (250 ms) elicited K_V1.6 tail currents.

3.3. Current Activation

The K_V1.x channels are known as an outwardly rectifying potassium channels. To verify the functional properties of B'SYS's cloned CHO K_V1.x cells, both the activation plot and IV curve were constructed. The activation or inactivating plot of K_V1.x peak currents were best fitted with a Boltzmann function shown in the figures below:

K_V1.1

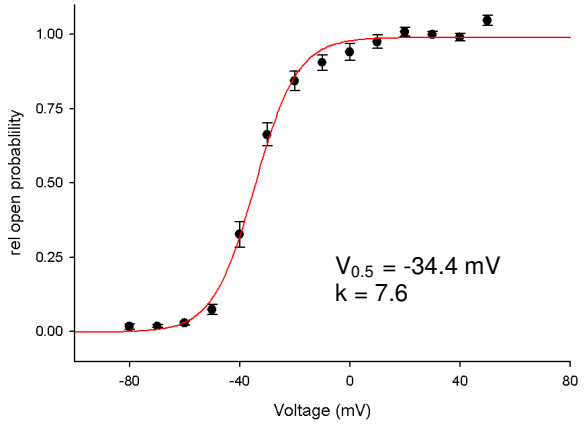

Fig. 2.1.1.1: Activation plot of K_V1.1 tail currents fitted to Boltzmann function with $V_{0.5}$ = -34.4 mV, k = 7.6 (n= 6 cells).

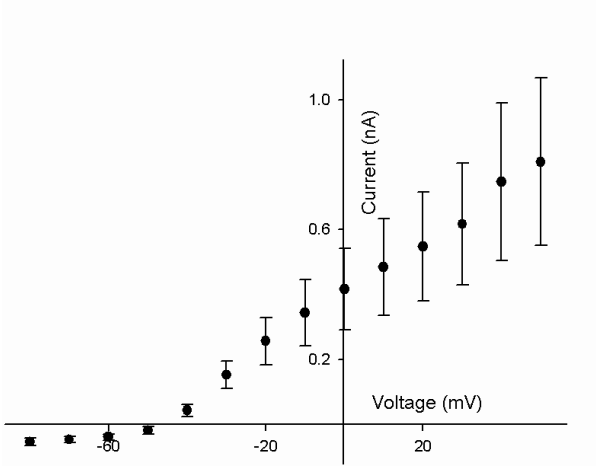

Fig. 2.1.1.2: IV curve of K_V1.1 activation currents. Voltages between -120 and +70 mV were applied (n=11).

K_V1.2

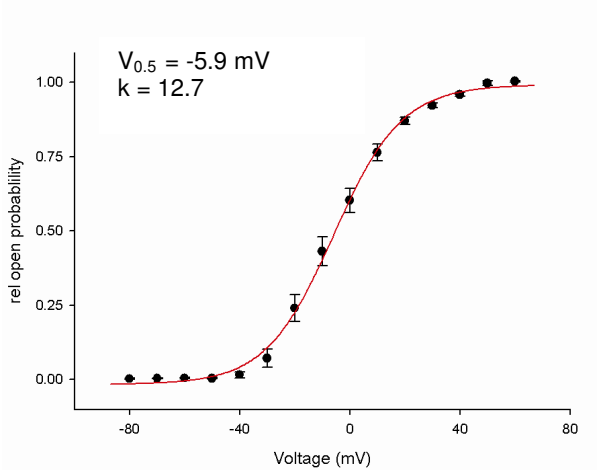

Fig. 2.1.2.1: Activation plot of K_V1.2 tail currents fitted to Boltzmann function with $V_{0.5}$ = -5.9 mV, k = 12.4 (n= 6 cells).

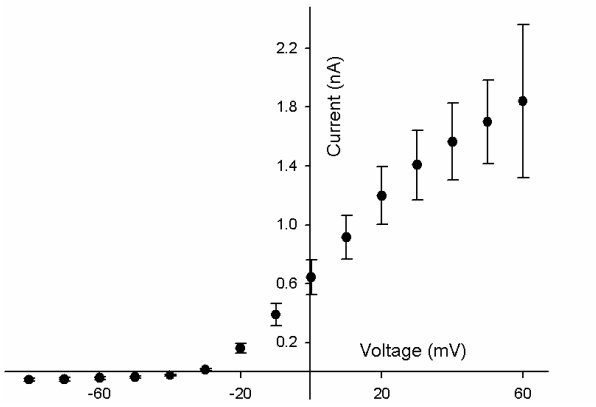

Fig 2.1.2.2: IV curve of K_V1.2 activation currents. Voltages between -80 and +60 mV were applied.

K_V1.3

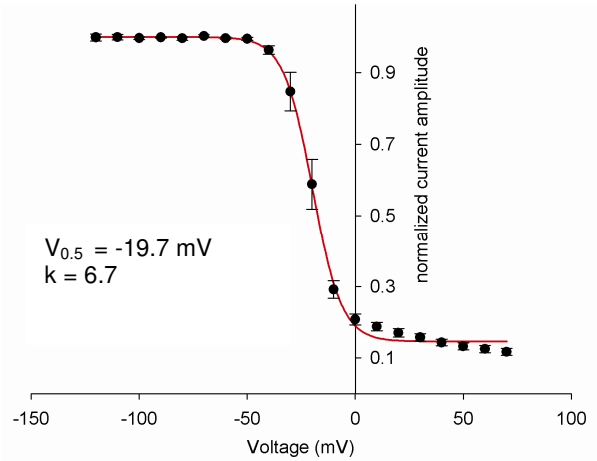

Fig. 2.1.3.1: Inactivation plot of K_V1.3 tail currents fitted to Boltzmann function with $V_{0.5}$ = -19.7 mV, k = 6.7 (n= 4 cells).

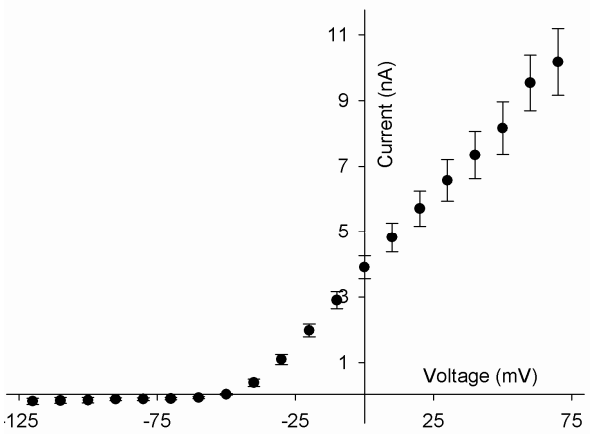

Fig. 2.1.3.2: IV curve of K_V1.3 activation currents. Voltages between -120 and +70 mV were applied.

K_V1.4

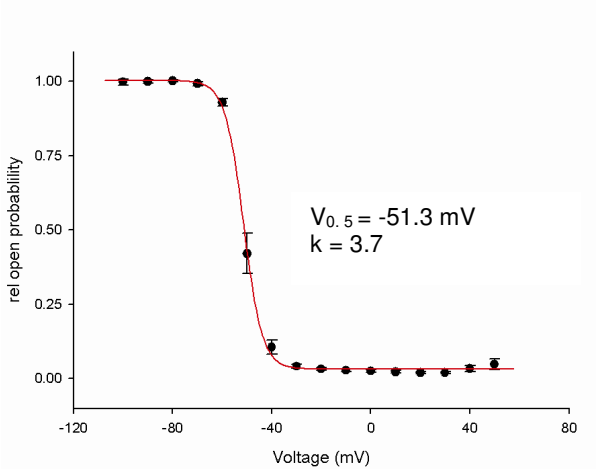

Fig. 2.1.4.1: Inactivation plot of K_V1.4 tail currents fitted to Boltzmann function with $V_{0.5}$ = -51.3 mV, k = 3.7 (n= 6 cells).

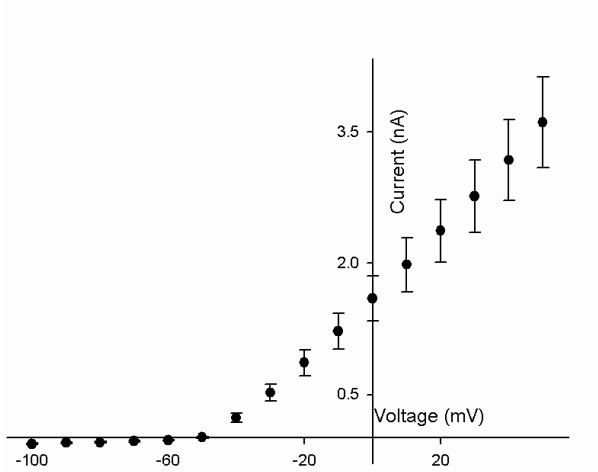

Fig. 2.1.4.2: IV curve of K_V1.4 activation currents. Voltages between -100 and +50 mV were applied.

K_V1.5

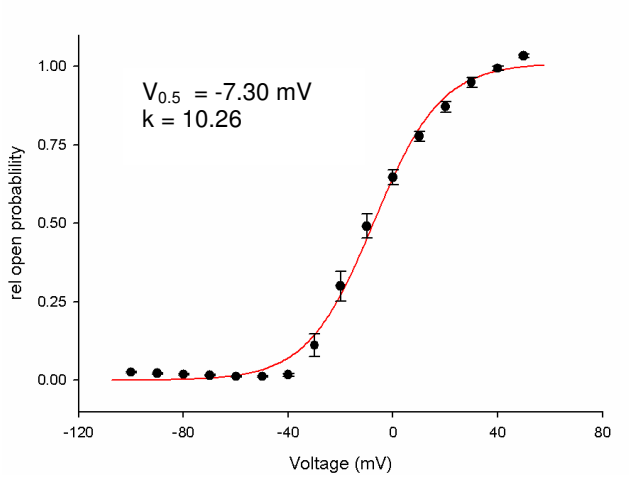

Fig. 2.1.5.1: Activation plot of K_V1.5 tail currents fitted to Boltzmann function with $V_{0.5}$ = -7.30 mV, k = 10.26 (n= 4 cells).

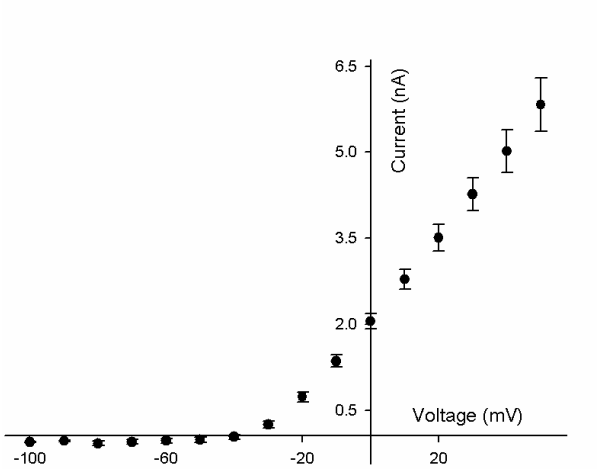

Fig. 2.1.5.2: IV curve of K_V1.5 activation currents. Voltages between -100 and +50 mV were applied.

K_V1.6

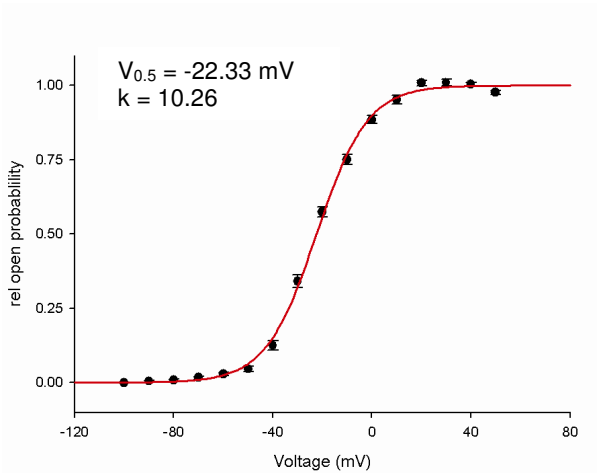

Fig. 2.1.6.1: Activation plot of K_V1.6 tail currents fitted to Boltzmann function with $V_{0.5}$ = -22.33 mV, k = 10.26 (n= 9 cells).

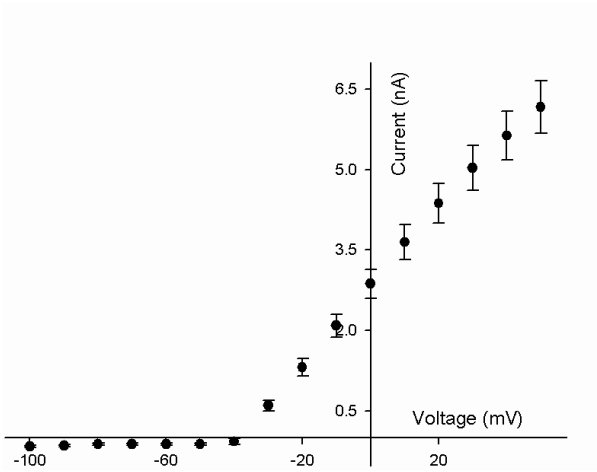

Fig. 2.1.6.2: IV curve of K_V1.6 activation currents. Voltages between -100 and +50 mV were applied.

3.4. Specific K_V1.x channel blocker

K_V1.1 Channel Blocker Nifedipine

Nifedipine, which is known as a K_V1.1 blocker, was used as positive control to validate the pharmacological profile of the CHO K_V1.1 cells. The K.$_V$1.1 cells were exposed to 3.0, 10.0, 30.0, 100.0 µM Nifedipine, the steady-state level relative peak current amplitudes revealed values of 98.45 ± 1.61%, 89.31 ± 3.26%, 61.97 ± 4.15%, 31.94 ± 2.24%, respectively (mean ± SEM of 3 cells). The inhibition curve (Fig. 3.1.1.2) was best fitted with an IC_{50} value of 49.89
µM, Hill Coefficient: 1.19.

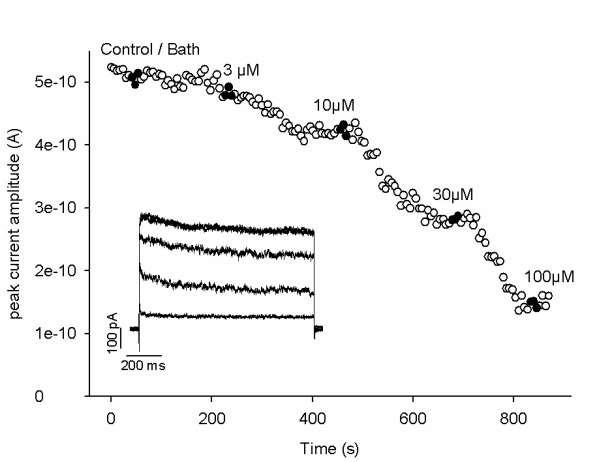

Fig. 3.1.1.1: Long time course of a CHO K_V1.1 cell. Peak current amplitude was reduced by increasing concentrations of Nifedipine.

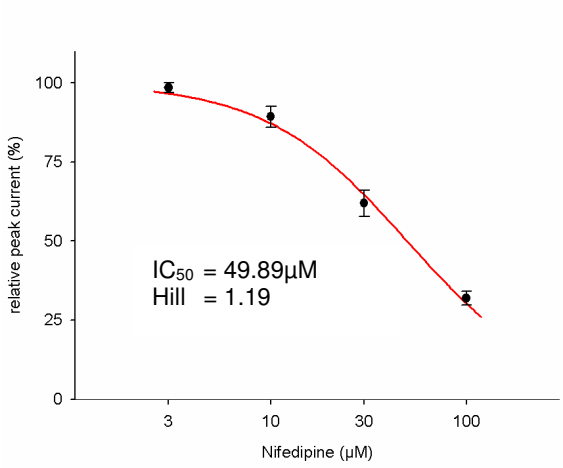

Fig. 3.1.1.2: K_V1.1 peak current inhibition curve of Nifedipine. The inhibition curve was best fitted with an IC_{50} value of 49.89 µM, Hill Coefficient: 1.19 (n=3)

CHO K_V1.1 cells were tested with automated patch-clamping (Q-Patch). Hongotoxin was used as a K_V1.1 specific blocker. The K.$_V$1.1 cells were exposed to 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0 and 10 nM. The inhibition curve (Fig. 3.1.1.4) was best fitted with an IC_{50} value of 0.20 nM, Hill Coefficient: 1.41 and a remaining current amplitude of 44.70% for the instantaneous current. For the late current amplitude an IC_{50} of 0.27 nM, Hill coefficient 0.83 was determined.

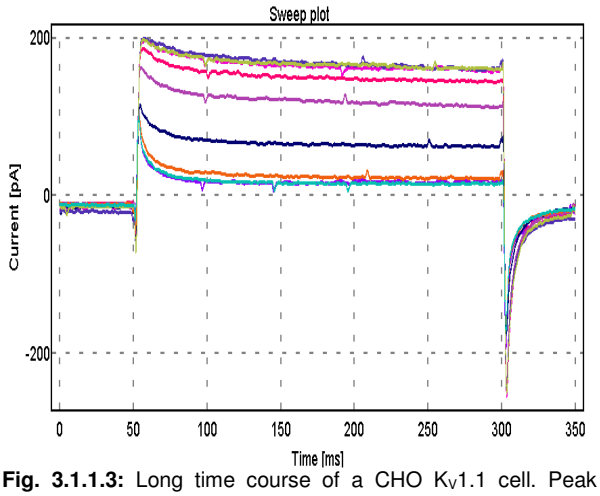

Fig. 3.1.1.3: Long time course of a CHO K_V1.1 cell. Peak current amplitude was reduced by increasing concentrations of Hongotoxin.

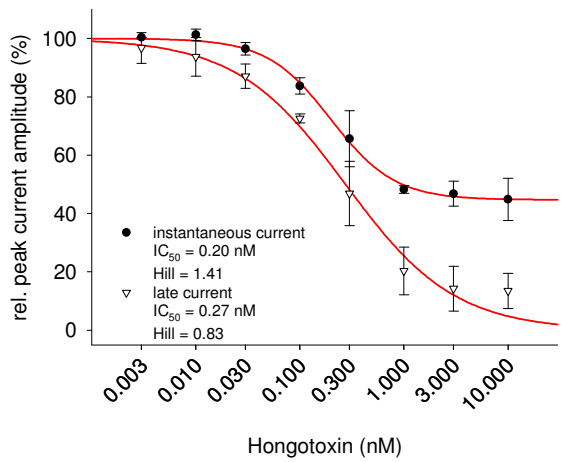

Fig. 3.1.1.4: K_V1.1 peak current inhibition curve of Hongotoxin. The inhibition curve was best fitted with an IC_{50} value of 0.02 nM, Hill Coefficient: 1.41 for the instantaneous current

amplitude (black circles) and 0.27 nM, Hill Coefficient 0.83 for the late current amplitude (white triangles).

K_V1.2 Channel Blocker Nifedipine

Nifedipine, which is known as a K_V1.2 blocker, was used as positive control to validate the pharmacological profile of the CHO K_V1.2 cells. The K_V1.2 cells were exposed to 3.0, 10.0, 30.0 and 100.0 µM Nifedipine, the steady-state level relative peak current amplitudes revealed values of 85.38 ± 0.90%, 68.89 ± 1.91%, 36.38 ± 2.35%, 8.52 ± 0.97%, respectively (mean ± SEM of 4 cells). The inhibition curve (Fig. 3.1.2.2) was best fitted with an IC_{50} value of 18.04 µM, Hill Coefficient: 1.18.

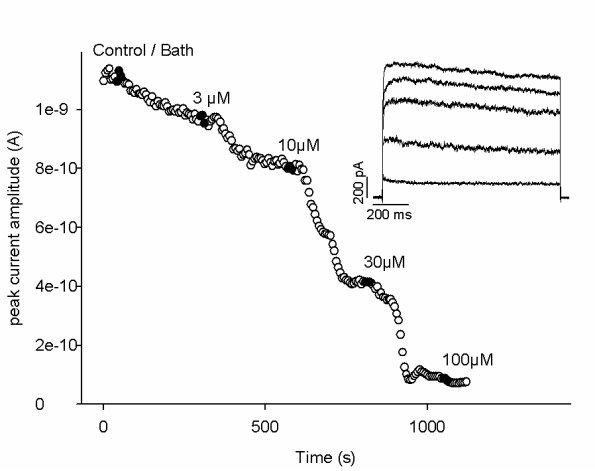

Fig. 3.1.2.1: Long time course of a CHO K_V1.2 cell. Peak current amplitude was reduced by increasing concentrations of Nifedipine.

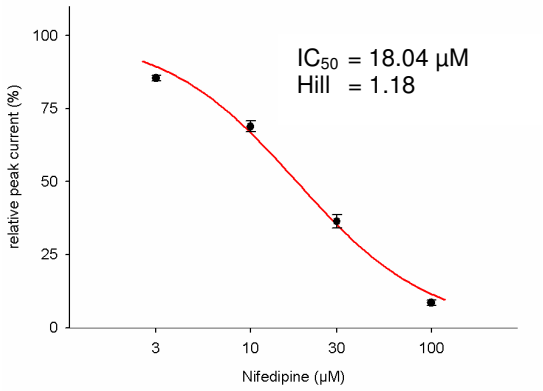

Fig. 3.1.2.2: K_V1.2 peak current inhibition curve of Nifedipine. The inhibition curve was best fitted with an IC_{50} value of 18.04 µM, Hill Coefficient: 1.18 (n=4)

CHO K_V1.2 cells were tested with automated patch-clamping (Q-Patch). Hongotoxin was used as a high affinity blocker of K_V1.2. The K.$_V$1.2 cells were exposed to 0.03, 0.1, 0.3, 1.0, 3.0 and 10 nM. The inhibition curve (Fig. 3.1.2.4) was best fitted with an IC_{50} value of 0.81 nM, Hill coefficient 1.08 for the instantaneous current and 0.48 nM, Hill coefficient 0.93 for the late current amplitude.

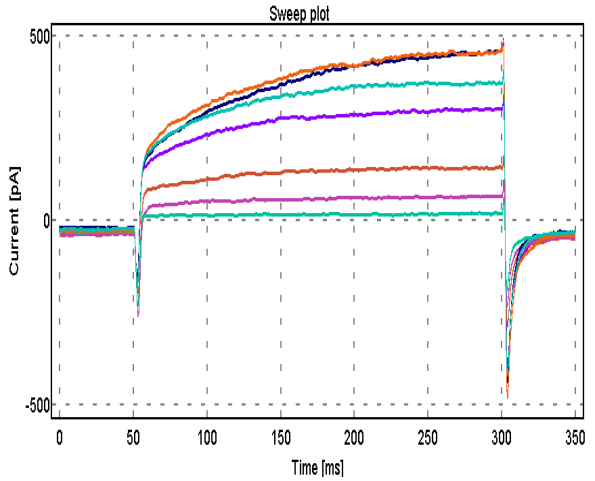

Fig. 3.1.2.3: Long time course of a CHO K_V1.2 cell. Peak current amplitude was reduced by increasing concentrations of Hongotoxin.

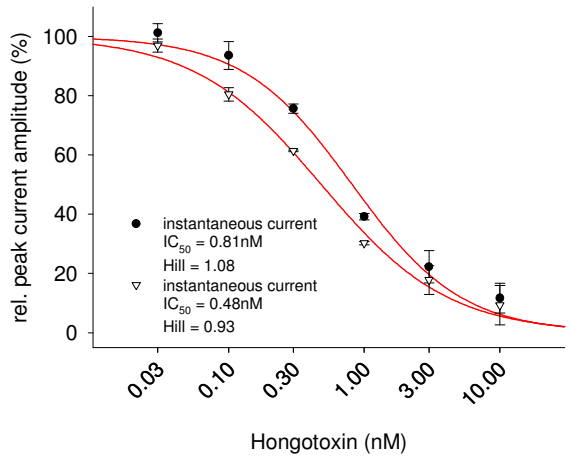

Fig. 3.1.2.4: K_V1.2 peak current inhibition curve of Hongotoxin. The inhibition curve was best fitted with an IC_{50} value of 0.81 nM, Hill Coefficient: 1.08 for the instantaneous current amplitude (black circles) and 0.48 nM, Hill Coefficient 0.93 for the late current amplitude (white triangles).

K_V1.3 Channel Blocker Margatoxin

Margatoxin, which is known as a selective K_V1.3 blocker, was used as positive control to validate the pharmacological profile of the CHO K_V1.3 cells. The effect of Margatoxin was first measured at 1nM, which blocked K_V1.3 peak currents almost completely (24.32 % relative peak current amplitude 1 cells). When the cells were exposed to 0.1, 0.3, 1.0 and 3.0 nM Margatoxin, the steady-state level relative peak current amplitudes revealed values of 77.65 ± 3.98%, 44.03 ± 3.82%, 20.48 ± 2.98%, 5.63 ± 0.90%, respectively (mean ± SEM of 3 cells). The inhibition curve (Fig. 3.1.3.2) was best fitted with an IC_{50} value of 268.7 pM, Hill Coefficient: 1.15.

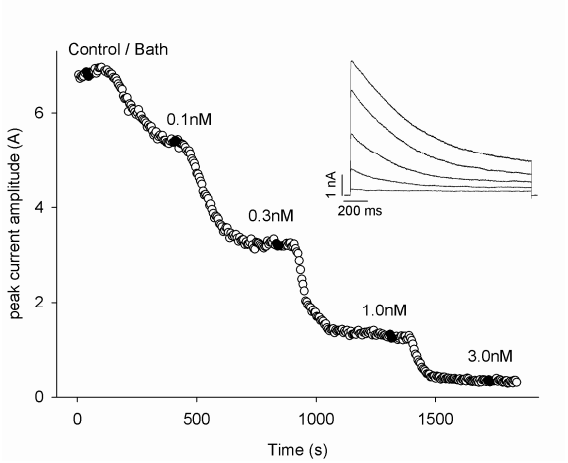

Fig. 3.1.3.1: Long time course of a CHO K_V1.3 cell. Peak current amplitude was reduced by increasing concentrations of Margatoxin.

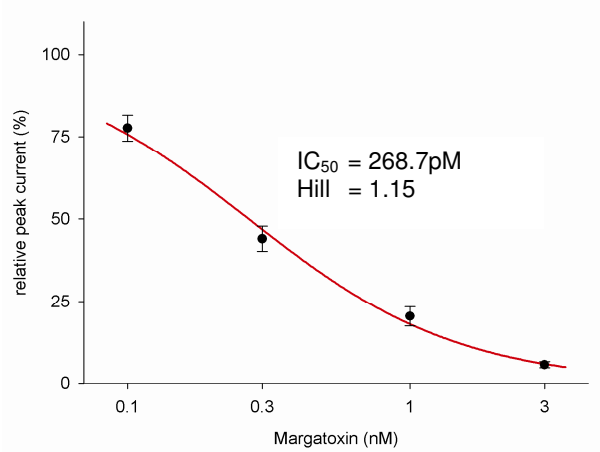

Fig. 3.1.3.2: K_V1.3 peak current inhibition curve of Margatoxin. The inhibition curve was best fitted with an IC_{50} value of 268.7 pM, Hill Coefficient: 1.15 (n=4)

CHO K_V1.3 cells were tested with automated patch-clamping (Q-Patch). Margatoxin was used as a high affinity blocker of K_V1.3. The K_V1.3 cells were exposed to 0.08, 0.16, 0.31, 0.63, 1.25, 2.50, 5.00 and 10 nM. The inhibition curve (Fig. 3.1.3.4) was best fitted with an IC_{50} value of 1.39 nM, Hill coefficient 0.85 for the peak current.

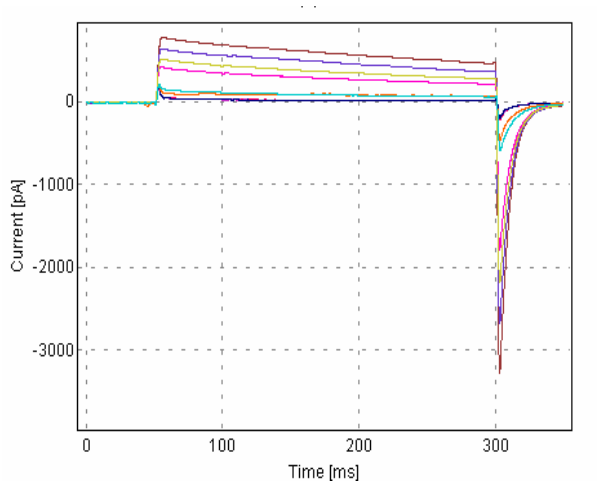

Fig. 3.1.3.3: Long time course of a CHO K_V1.3 cell. Peak current amplitude was reduced by increasing concentrations of Margatoxin.

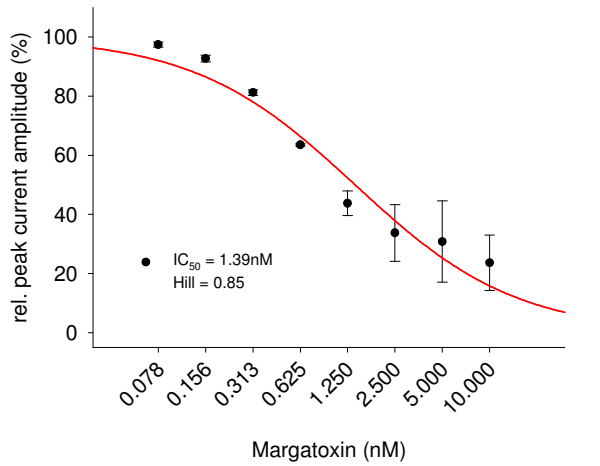

Fig. 3.1.3.4: K_V1.3 peak current inhibition curve of Margatoxin. The inhibition curve was best fitted with an IC_{50} value of 1.39 nM, Hill Coefficient: 0.85 for the instantaneous current amplitude.

K_V1.4 Channel Blocker Quinidine

Quinidine, which is known as a K_V1.4 blocker, was used as positive control to validate the pharmacological profile of the CHO K_V1.4 cells. The K_V1.4 cells were exposed to 3.0, 10.0, 30.0, 100.0 and 300.0 µM Quinidine, the steady-state level relative peak current amplitudes revealed values of 84.44 ± 1.24, 64.67 ± 3.43, 44.69 ± 3.05, 23.31 ± 3.44, 8.08 ± 1.44%, respectively (mean ± SEM of 6 cells). The inhibition curve (Fig. 3.1.4.2) was best fitted with an IC_{50} value of 22.13 µM, Hill Coefficient: 0.83.

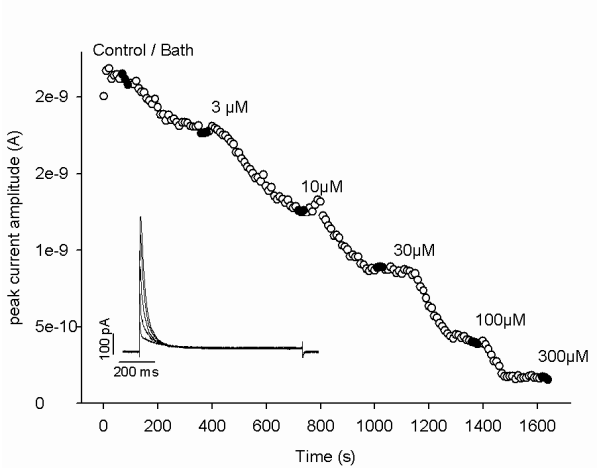

Fig. 3.1.4.1: Long time course of a CHO K_V1.4 cell. Peak current amplitude was reduced by increasing concentrations of Quinidine.

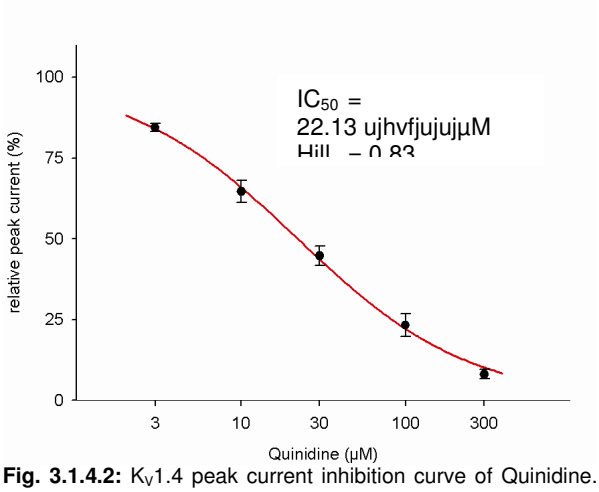

Fig. 3.1.4.2: K_V1.4 peak current inhibition curve of Quinidine. The inhibition curve was best fitted with an IC_{50} value of 22.13 µM, Hill Coefficient: 0.83 (n=6)

CHO K_V1.4 cells were tested with automated patch-clamping (Q-Patch). Quinidine was used as reference compound. The K.$_V$1.4 cells were exposed to 0.03, 0.10, 0.30, 1.00, 3.00, 10, 30, 100 and 300 µM. The inhibition curve (Fig. 3.1.4.4) was best fitted with an IC_{50} value of 8.84 µM, Hill coefficient 0.69 for the peak current.

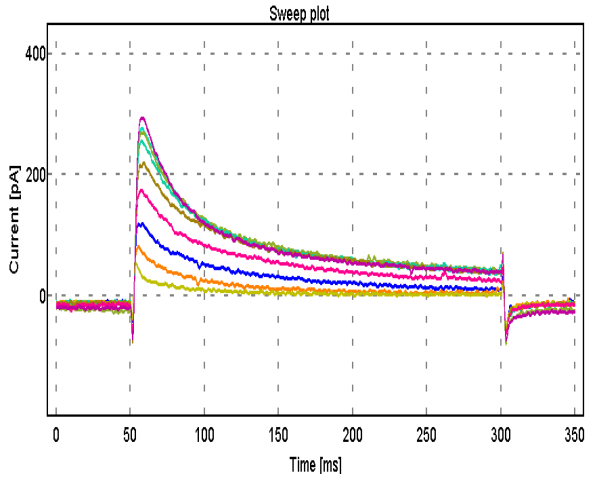

Fig. 3.1.4.3: Long time course of a CHO K_V1.4 cell. Peak current amplitude was reduced by increasing concentrations of Quinidine.

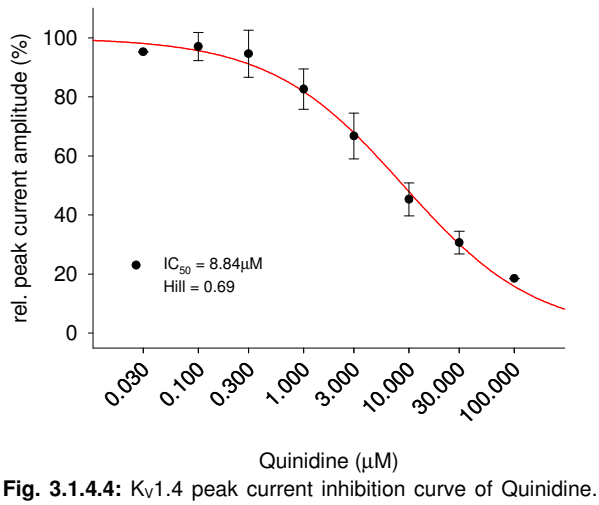

Fig. 3.1.4.4: K_V1.4 peak current inhibition curve of Quinidine. The inhibition curve was best fitted with an IC_{50} value of 8.84 µM, Hill Coefficient: 0.69 for the instantaneous current amplitude.

K_V1.5 Channel Blockers Nifedipine, Terfenadine and Verapamil

Nifedipine and Terfenadine, which are known as a K_V1.5 blockers, were used as control to validate the pharmacological profile of the CHO K_V1.5 cells. The K_V1.5 cells were exposed to 3.0, 10, 30 and 100 µM Nifedipine or 0.3, 1.0, 3.0 and 10 µM Terfenadine, the steady-state level relative peak current amplitudes revealed values of 94.17 ± 2.58, 81.28 ± 4.11, 61.13 ± 3.71, 30.73 ± 4.14%, respectively (mean ± SEM of 8 cells) for Nifedipine and 96.60 ± 2.26, 76.74 ± 2.74, 50.11 ± 3.65, 17.99 ± 5.03%, respectively (mean ± SEM of 3 cells) for Terfenadine. The inhibition curve (Fig. 3.1.5.2) was best fitted with an IC_{50} value of 45.46 µM, Hill Coefficient: 1.01 for Nifedipine and an IC_{50} value 2.92 µM, Hill coefficient: 1.22 for Terfenadine.

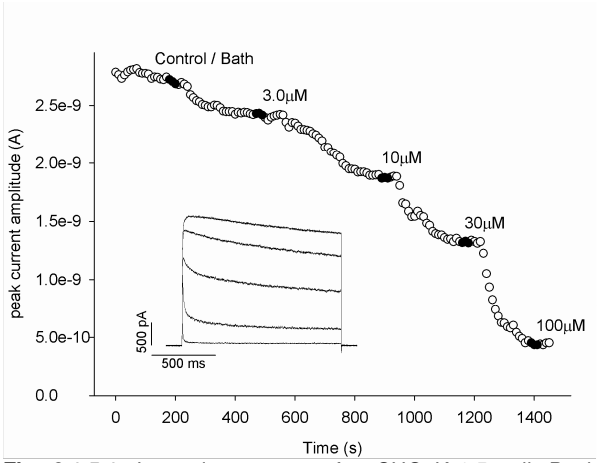

Fig. 3.1.5.1: Long time course of a CHO K_V1.5 cell. Peak current amplitude was reduced by increasing concentrations of Nifedipine.

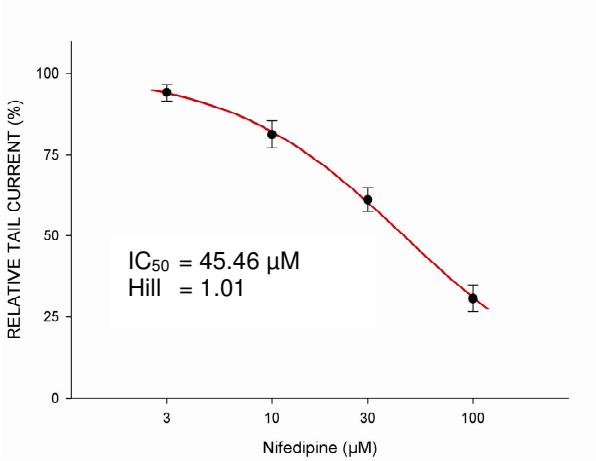

Fig. 3.1.5.2: K_V1.5 peak current inhibition curve of Nifedipine. The inhibition curve was best fitted with an IC_{50} value of 45.46 µM, Hill Coefficient: 1.01 (n=8)

On the QPatch automate experiments with the specific KV1.5 blocker S9947 and verapamil were carried out. Verapamil was tested at concentrations of 0.1, 1, 10, 30 and 100 µM, S9947 at concentrations of 10, 30, 100, 300, 1000, 3000, 100000 and 300000 while channels were activated by pulses to +40 mV from a holding potential of -80 mV. The IC_{50} for verapamil was determined to as 6.87 µM (Hill coefficient: 0.90). For S9947 an IC_{50} of 12.05 µM (Hill coefficient: 0.87) was found.

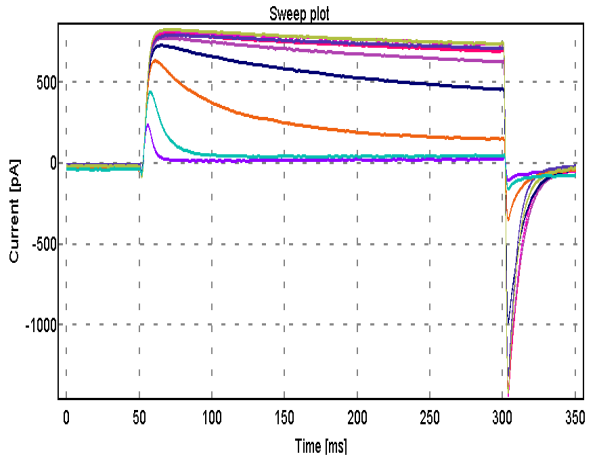

Fig. 3.1.5.3: Long time course of a CHO K_V1.5 cell. Peak current amplitude was reduced by increasing concentrations of S9947.

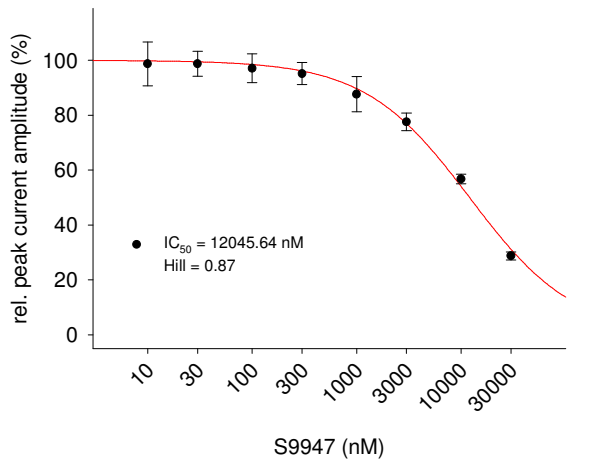

Fig. 3.1.5.4: K_V1.5 steady state current inhibition curve for S9947. The inhibition curve was best fitted with an IC_{50} value of 12045.64 nM, Hill Coefficient: 0.87

K_V1.6 Channel Blocker 4-AP

4-AP (4-Aminopyridine), which is known as a K_V1.6 blocker, was used as positive control to validate the pharmacological profile of the CHO K_V1.6 cells. The K_V1.6 cells were exposed to 0.3, 1.0, 3.0 and 10.0 mM 4-AP, the steady-state level relative peak current amplitudes revealed values of 64.63 ± 1.30, 51.86 ± 1.51, 39.96 ± 3.33, 18.18 ± 1.52%, respectively (mean ± SEM of 5 cells). The inhibition curve (Fig. 3.1.6.2) was best fitted with an IC_{50} value of 1.06 mM, Hill Coefficient: 0.56.

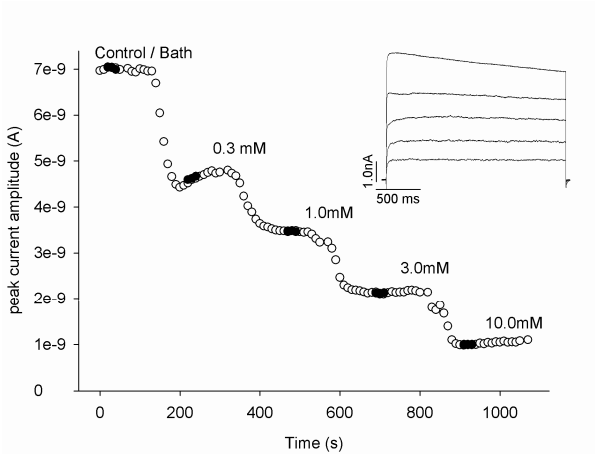

Fig. 3.1.6.1: Long time course of a CHO K_V1.6 cell. Peak current amplitude was reduced by increasing concentrations of 4-AP.

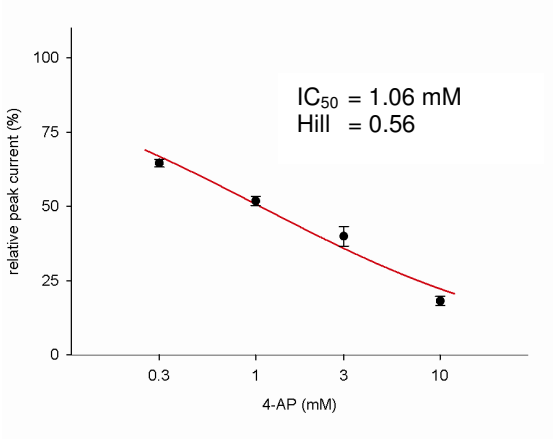

Fig. 3.1.6.2: K_V1.6 peak current inhibition curve of 4-AP. The inhibition curve was best fitted with an IC_{50} value of 1.06 mM, Hill Coefficient: 0.56 (n=5)

CHO K_V1.6 cells were tested with automated patch-clamping (Q-Patch). 4-AP was used as reference compound. The K_V1.4 cells were exposed to 10, 30, 100, 300, 1000, 3000 and 10000 µM. The inhibition curve (Fig. 3.1.6.4) was best fitted with an IC_{50} value of 276.41 µM, Hill coefficient 0.87 for the peak current.

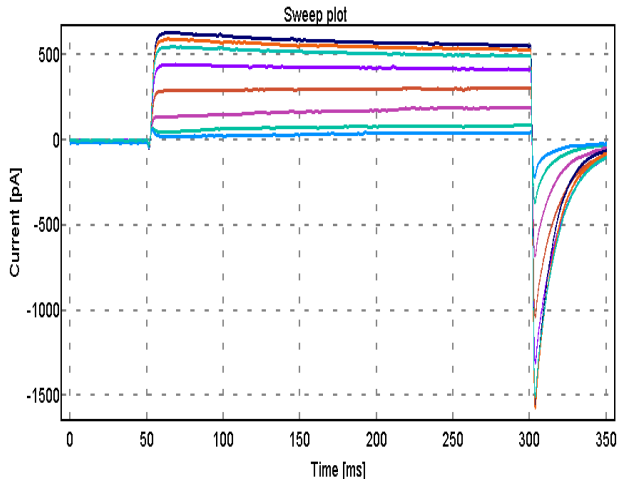

Fig. 3.1.6.3: Long time course of a CHO K_V1.6 cell. Peak current amplitude was reduced by increasing concentrations of 4-AP.

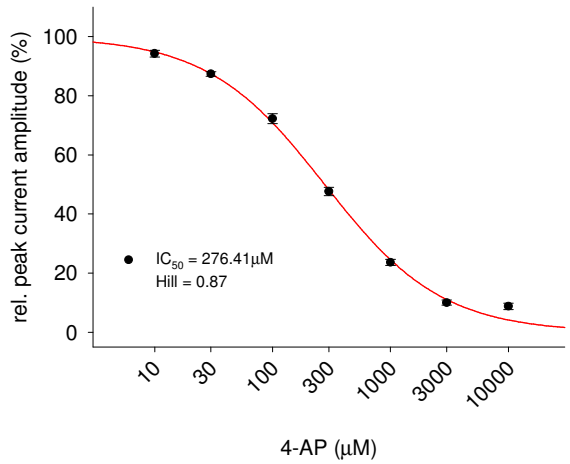

Fig. 3.1.6.4: K_V1.6 peak current inhibition curve of 4-AP. The inhibition curve was best fitted with an IC_{50} value of 276.41 µM, Hill Coefficient: 0.87 for the instantaneous current amplitude.

4. SUMMARY

Isoform	Accession	$I_{(50mV)}$ mean ± SEM (nA)	$V_{0.5}$ (mV)	Pharmacology (IC_{50})	
				Manual PC	Automated PC
K_V1.1 / KCNA1	BC_101733	0.81 ± 0.26	-34.4	Nifedipine 49.89 µM	Hongotoxin 0.20 nM
K_V1.2 / KCNA2	NM_004974.2	1.70 ± 0.28	-5.9	Nifedipine 18.04 µM	Hongotoxin 0.81 nM
K_V1.3 / KCNA3	NM_002232	8.16 ± 0.81	-19.7	Margatoxin 268.7 pM	Margatoxin 1.39 nM
K_V1.4 / KCNA4	NM_002233.2	3.61 ± 0.52	-51.3	Quinidine 22.13 µM	Quinidine 8.84 µM
K_V1.5 / KCNA5	NM_002234.2	5.83 ± 0.47	-7.3	Nifedipine 45.46 µM	Verapamil 6.87 µM S9947 12.05 µM
K_V1.6 / KCNA6	NM_002235.3	6.17 ± 0.49	-22.3	4-AP 1.06 mM	4-AP 276.41 µM

5. CONTACT INFORMATION

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