Validation of B'SYS K_V3.x cell lines using automated and manual patch-clamp electrophysiology

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Introduction

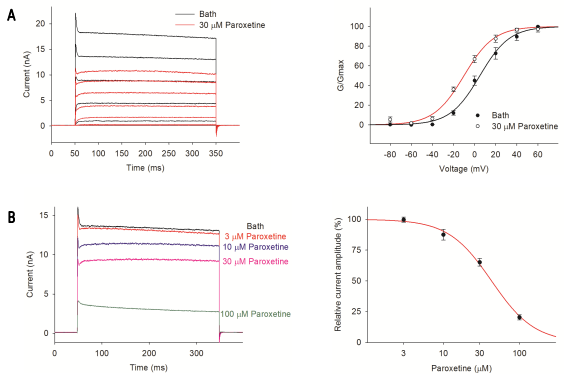
K_V3.x receptors have recently emerged as potential targets for treatment of a variety of CNS disorders, including epilepsy, ataxias, hearing disorders, schizophrenia and cognitive impairments. K_V3.1–K_V3.4 protein isoforms contribute to the high-frequency firing of neurons such as auditory brain stem neurons, fast-spiking cortical and hippocampal GABAergic interneurons, and Purkinje cells of the cerebellum, and play an important role in the regulation of intrinsic excitability and neurotransmitter release at presynaptic terminals of many neurons.

B'SYS has generated a panel of cell lines stably transfected with K_V3.1a/b, K_V3.2, K_V3.3 and K_V3.4 subunits. Using automated (Qpatch™) as well as manual patch-clamp recordings, the aim of this validation study was to characterize these cell lines pharmacologically using the selective serotonin reuptake inhibitor paroxetine. In addition, the effect of K_V modulator 1 was investigated on K_V3.1b.

Materials & Methods

Sequences of K_V3.1a (NP_001075651.1), K_V3.1b (NP_001106212.1), K_V3.2 (NM_139137.4), K_V3.3 (NP_004968.2) and K_V3.4 (NP_004969.2) were cloned into suitable expression vectors and verified by sequencing. Chinese hamster ovary (CHO) cells were stably transfected and selected by antibiotic selection and FACS sorting. Clones were tested electrophysiologically, and after biophysical characterization, the assay was optimized for automated patch-clamping (Q-Patch™). For both manual and automated whole cell patch-clamp recordings the extracellular solution contained (in mM) NaCl (137), KCl (4), $CaCl_2 \cdot 2H_2O$ (1.8), $MgCl_2 \cdot H_2O$ (1), HEPES (10), Glucose (10); intracellular solution: KCl (130), $MgCl_2 \cdot 6H_2O$ (1), Mg-ATP (5), EGTA (5), HEPES (10). For K_V3.1a/b and K_V3.2 the mean current at the end of the depolarizing pulse and for K_V3.3 and K_V3.4 the peak current was used for analysis. $V_{0.5}$ values were determined in SigmaPlot by fitting the voltage-current relationship to a Boltzmann equation, EC_{50}/IC_{50} values were determined by fitting the data to a logistic equation. All chemicals and paroxetine hydrochloride were purchased from Sigma. All experiments were carried out at room temperature.

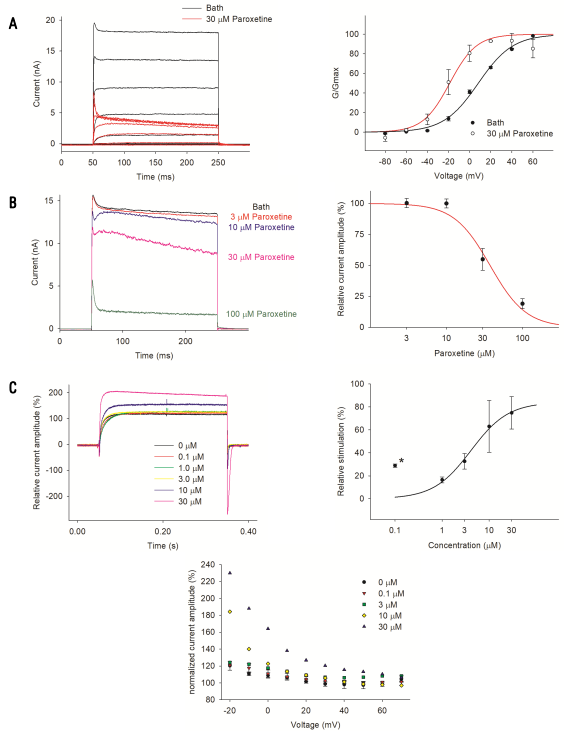
Biophysical and pharmacological characterization of K_V3.1a



A) Cells expressing K_V3.1a were voltage clamped from -80 to +60 mV for 300 ms in 20 mV increments. In the presence of 30 µM paroxetine, the voltage dependence of activation was shifted to more negative potentials: $V_{0.5}$: 4.5 mV (control) to -10.08 mV (30 µM paroxetine).

B) Concentration-response curve showing the effect of paroxetine at +60 mV. Paroxetine inhibited K_V3.1a currents in a concentration-dependent manner with an IC_{50} of 42.93 µM.

Biophysical and pharmacological characterization of K_V3.1b

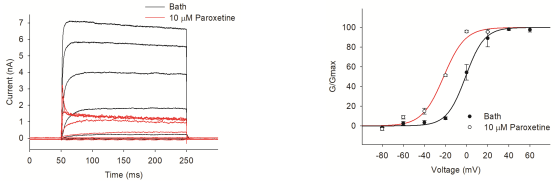


A) Cells expressing K_V3.1b were voltage clamped from -80 to +60 mV for 200 ms in 20 mV increments. In the presence of 30 µM paroxetine, the voltage dependence of activation was shifted to more negative potentials: $V_{0.5}$: 8.23 mV (control) to -18.88 mV (30 µM paroxetine).

B) Concentration-response curve showing the effect of paroxetine at +60 mV. Paroxetine inhibited K_V3.1b currents in a concentration-dependent manner with an IC_{50} of 37.45 µM.

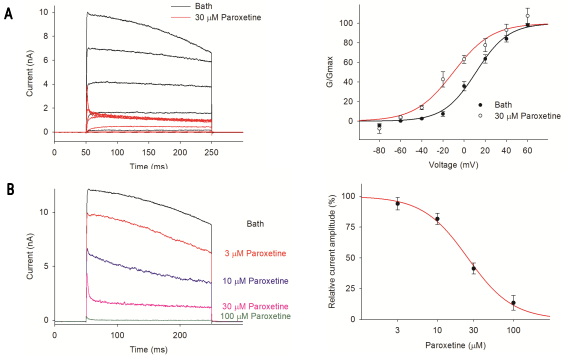
C) Representative traces and concentration-response curve showing the effect of K_V3 modulator 1 on K_V3.1b-mediated currents at -10 mV. The compound stimulated currents in a concentration-dependent manner with an EC_{50} of 4.1 µM, Hill slope: 1.1. The normalized IV plot shows the effect of the compound across different holding potentials.

Biophysical characterization of K_V3.2



Cells expressing K_V3.2 were voltage clamped from -80 to +60 mV for 200 ms in 20 mV increments. In the presence of 10 µM paroxetine, the voltage dependence of activation was shifted to more negative potentials : $V_{0.5}$: -0.98 mV (Control) to -21.78 mV (10 µM paroxetine).

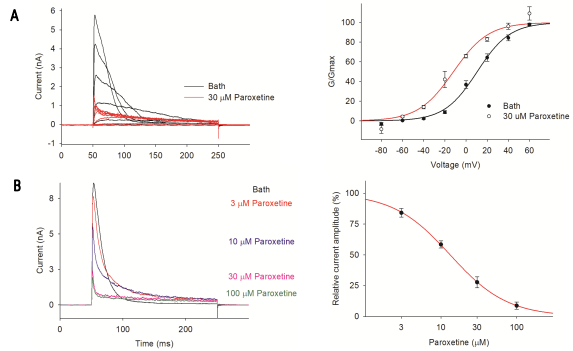
Biophysical and pharmacological characterization of K_V3.3



A) Cells expressing K_V3.3 were voltage clamped from -80 to +60 mV for 200 ms in 20 mV increments. In the presence of 30 µM paroxetine, the voltage dependence of activation was shifted to more negative potentials: $V_{0.5}$: 11.21 mV (control) to -10.11 mV (30 µM paroxetine).

B) Concentration-response curve showing the effect of paroxetine at +60 mV. Paroxetine inhibited K_V3.3 currents in a concentration-dependent manner with an IC_{50} of 25.05 µM.

Biophysical and pharmacological characterization of K_V3.4



A) Cells expressing K_V3.4 were voltage clamped from -80 to +60 mV for 200 ms in 20 mV increments. In the presence of 30 µM paroxetine, the voltage dependence of activation was shifted to more negative potentials: $V_{0.5}$: 10.59 mV (control) to -11.83 mV (30 µM paroxetine).

B) Concentration-response curve showing the effect of paroxetine at +60 mV. Paroxetine inhibited K_V3.4 currents in a concentration-dependent manner with an IC_{50} of 13.24 µM.

Summary & Conclusions

- B'SYS K_V3.x cell lines are suitable test systems for the screening and profiling of compounds

	Paroxetine	
	IC_{50} (µM)	Hill Slope
K_V3.1a	42.93	1.53
K_V3.1b	37.45	1.83
K_V3.3	25.05	1.47
K_V3.4	13.24	1.15

	Control		Paroxetine	
	$V_{0.5}$ (mV)	Slope (k)	$V_{0.5}$ (mV)	Slope (k)
K_V3.1a	4.50	13.33	-10.08	15.25
K_V3.1b	8.23	16.36	-18.88	12.65
K_V3.2	-0.98	8.87	-21.78	10.18
K_V3.3	11.21	15.18	-10.11	18.18
K_V3.4	10.59	15.44	-11.83	16.54