B'SYS GmbH

CHO $Na_V1.7$

Specification Sheet

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1. BACKGROUND

1.1 Voltage-gated Sodium channel $Na_V1.7$

Human $Na_V1.7$ (SCN9A) is a voltage-gated Na+ channel preferentially expressed in sensory neurones, which plays a key role in the depolarisation phase (upstroke) of the action potential. Mutations in this gene have been associated with primary erythermalgia, channelopathy-associated insensitivity to pain, and paroxysmal extreme pain disorder. $Na_V1.7$ is of interest as a target for novel analgesics.

2. VALIDATION OF CHO $NA_V1.7$ CELLS

2.1 Biophysical characterization

$Na_V1.7$ currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137 mM KCl 4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM, HEPES 10 mM, D-Glucose 10 mM, pH (NaOH) 7.4. The pipette solution consisted of (in mM) KCl 120 mM, NaCl 10 mM, $MgCl_2$ 6 mM, HEPES 10 mM, EGTA 5 mM, pH (KOH) 7.2. After formation of a GΩ seal between the patch electrodes and individual $Na_V1.7$ 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. As soon as a stable seal could be established fast deactivating sodium currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to -50 mV up to +50 mV in 10 mV increments of 10 ms duration (Fig. 1 A, B). The voltage pulses were run at intervals of 5 s.
For the IV curve the peak current amplitude of the pulses between -50 mV and +50 mV was plotted versus the applied voltage. This resulted in a bell shaped curve. The minimum of the IV curve was between -20 mV and -10 mV (Fig 1 B).

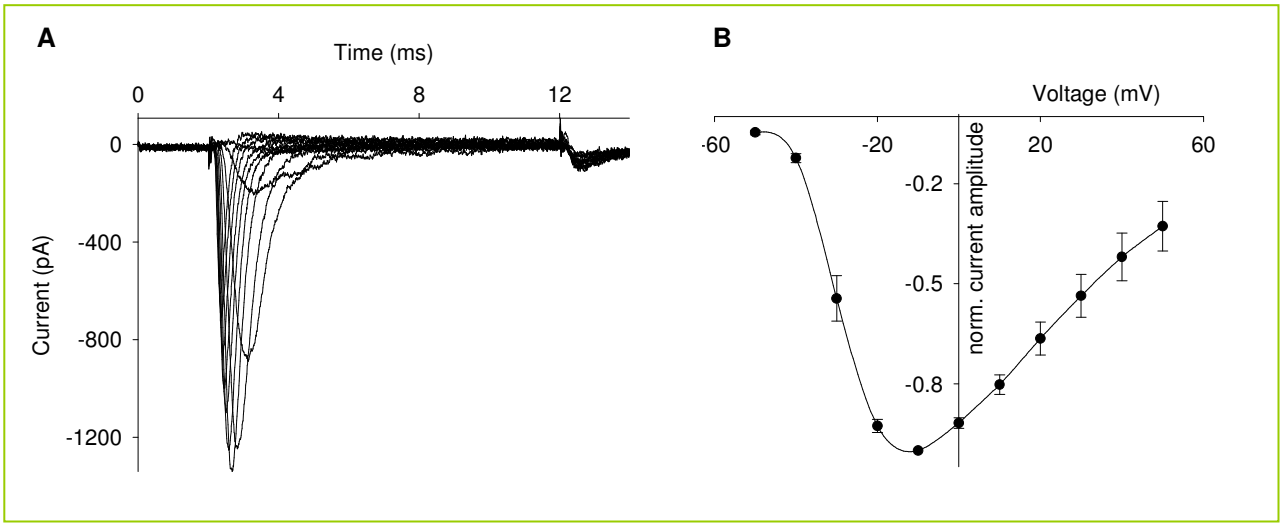

Fig. 1: IV curve of stably transfected CHO NaV1.7 cells. A) representative current recording. Depolarized to potentials between -50 mV and +50 mV in 10 mV increments from a holding potential of -80 mV. B). C) IV curve if the NaV1.7 channel. Peak current were measured at the beginning of the voltage pulse (n=6). No currents were recorded in untransfected cells (data not shown).

Tab. 1: Biophysical Properties of CHO $Na_V1.7$ cells

Channel	Expression System	Averaged Current	Proposed potentials (mV)		
			Resting state	Half inactivated	Max peak
$Na_V1.7$ (SCN9A)	CHO-K1	959.70 ± 103.08pA n=50	-120mV	$V_{0.5}$= -59.10 ± 0.73mV k= -5.70 ± 0.14 (n=22)	I_{max}= -10mV (n=6)

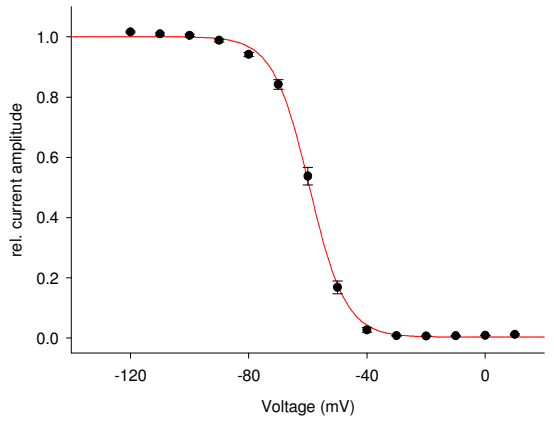

Fig. 2: Inactivation curve of $Na_V1.7$

2.2 Pharmacological characterization

For the pharmacological characterization of the $Na_V1.7$ channel Lidocaine was tested at concentrations of 30, 100, 300 and 1000 nM and TTX at concentrations of 3.0, 10, 30 and 100 nM (n≥3 cells). The dose response curves were generated (see Fig. 3) and the IC_{50} values calculated.
The $Na_V1.7$ currents were stimulated by a 10 ms pulse to -10 mV from a holding potential of -100 mV.

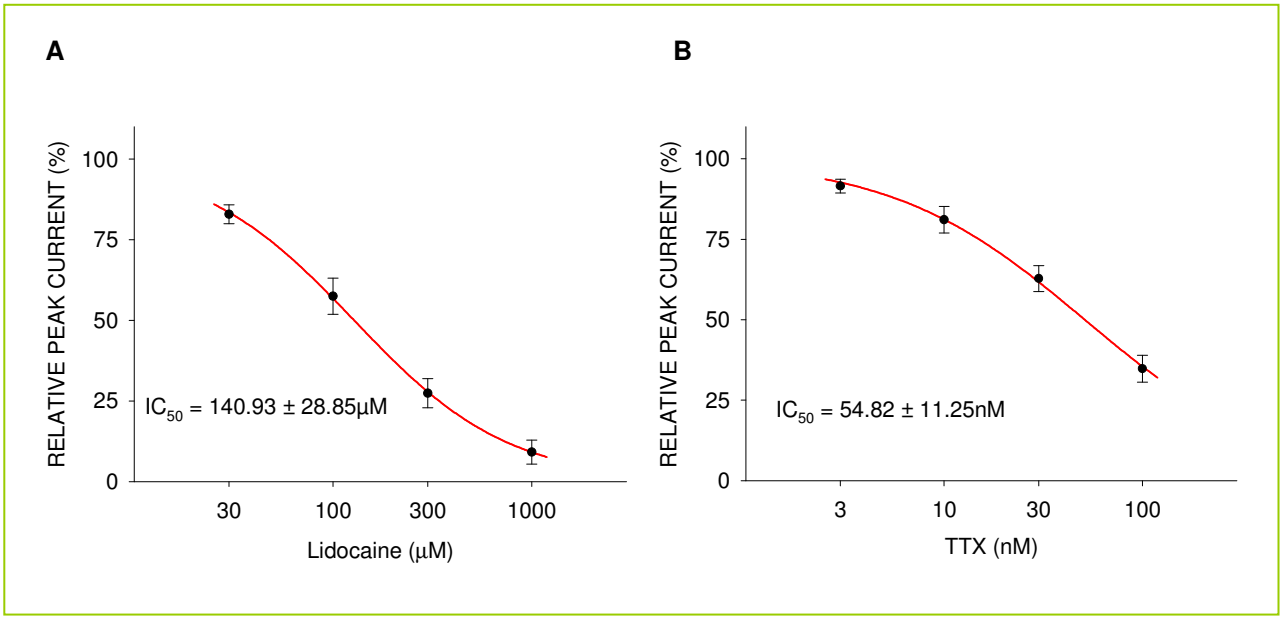

Fig. 3: Dose response curves for Lidocaine and TTX.

2.3 Automated Patch-clamp

The CHO $Na_V1.7$ cell line was validated on the automated patch-clamp system Q-Patch 16.
The bath solution contained (in mM) NaCl 137 mM KCl 4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM, HEPES 10 mM, D-Glucose 10 mM, pH (NaOH) 7.4. The intracellular solution consisted of (in mM) CsF 135 mM, NaCl 10 mM, HEPES 10 mM, EGTA 5 mM, pH (CsOH) 7.3.
To analyze the effect of the tested compound in the resting, fast and slow inactivated state the following voltage protocol was applied to the cells in the whole cell configuration.
The cells were clamped at a holding potential of -110 mV. To test the channels in the resting state a first test pulse to 0 mV was applied (10 ms). Then the cells were clamped to -80 mV for 2 s followed by the second test pulse to 0 mV (10 ms duration). This test pulse results in the current values for the fast inactivated sodium channels. For the slow inactivation, channels were depolarized to 0 mV for duration of 5 s. To recover the fast inactivated channels the cells were depolarized to -110 mV for 100 ms, followed by the third test pulse for the slow inactivated channels (0 mV, 10 ms). This voltage protocol was applied three times for each test concentration, after an incubation time of 90 s.

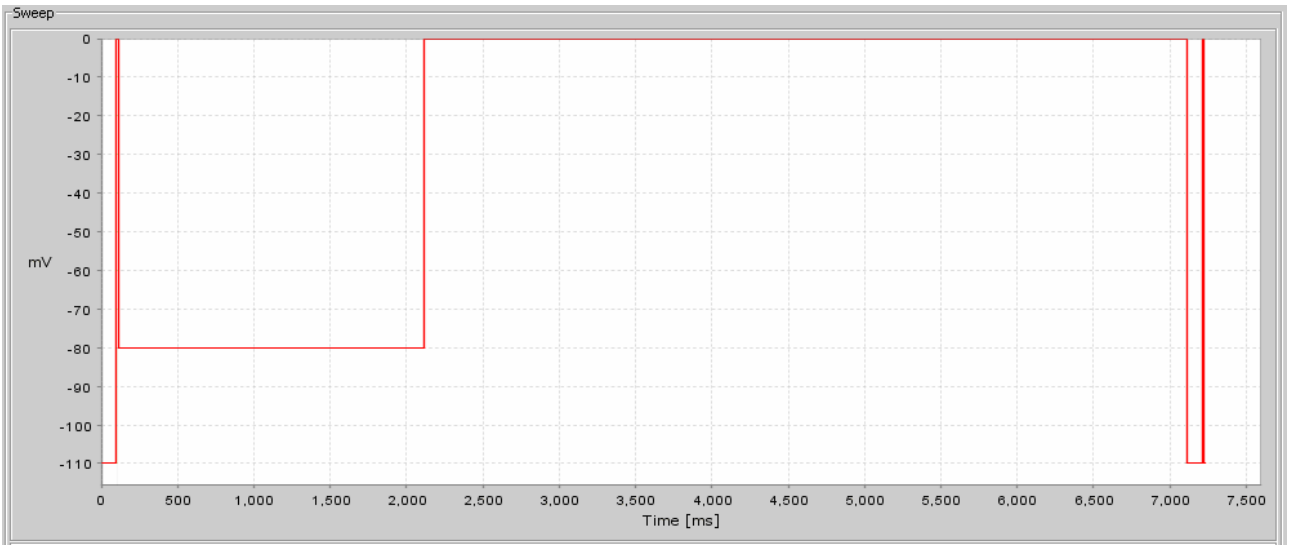

Fig. 4: Pulse protocol used to determine the affinity in the resting, fast and slow inactivated state of $Na_V1.7$.

The effect of three Lidocaine and Mexiletine concentrations on $Na_V1.7$ currents in the resting, fast and slow inactivated state was analyzed. The dose response curves of each state were generated and the IC_{50} values and the Hill coefficients were calculated.

Tab. 2: Pharmacology of CHO $Na_V1.7$ cells

State	**IC_{50}**	**Hill coefficient**	**IC_{50}**	**Hill coefficient**
Compound	Lidocaine		Mexiletine	
resting	1.15 mM	1.23	333.08 µM	1.30
Fast inactivated	0.35 mM	1.11	103.73 µM	1.05
Slow inactivated	1.10 mM	1.46	107.51 µM	0.94

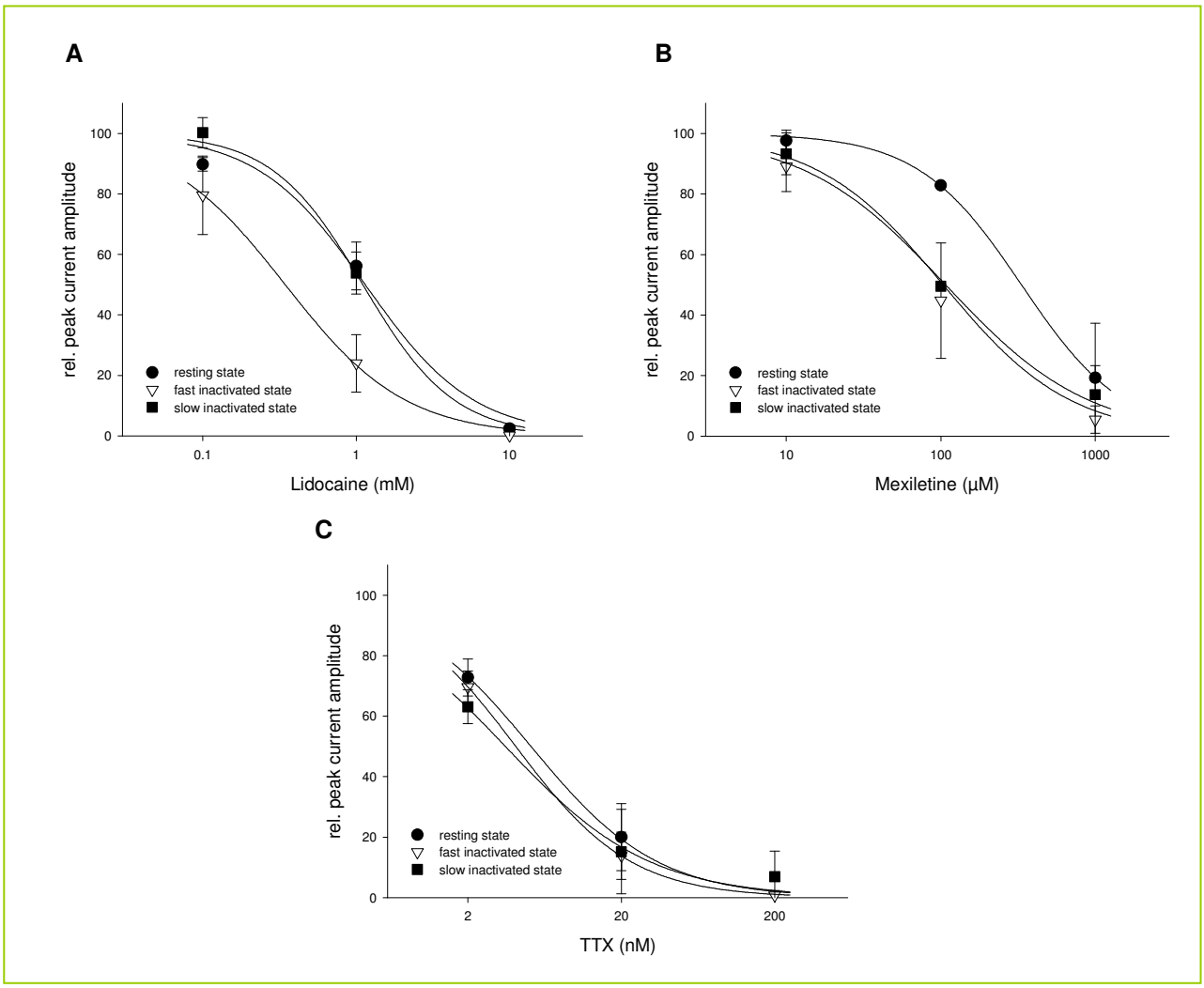

Fig. 5: Dose response curves for Lidocaine, Mexiletine and TTX for the resting, fast and slow inactivated state.

3. $NA_V1.7$ SEQUENCE

3.1 Human $Na_V1.7$ Accession Number NM_002977.1

Cloned cDNA sequence of human $Na_V1.7$ subunit was error-free and identical with NM_002977.1 sequence.

4. CONTACT INFORMATION

4.1 Contact Address for Technical Support & Ordering Information

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