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CHO $Na_V1.2$ Cell Line

Specification Sheet

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1. PRODUCT SHIPMENT

1.1. Product Format

CHO cells stably transfected with recombinant $Na_V1.2$ potassium channel:

- 0.75 mL aliquots of frozen cells at 1 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO
- Cells are frozen at passage number, see vial

Genomic integration was confirmed by PCR.

1.2. Mycoplasma Certificate

B'SYS periodically tests cells for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

1.3. Assays

CHO $Na_V1.2$ cells were validated for manual patch-clamping and automated patch-clamping (Q-Patch, Sophion)

2. SCIENTIFIC BACKGROUND

$Na_V1.2$ expression increases during the third postnatal week, but then continues to increase until reaching maximal levels during adulthood. $Na_V1.2$ is the most abundant alpha-subunit expressed in the central nervous system, comprising approximately 70% of the total rat brain Na_V alpha subunit pool. It is abundantly expressed in axons in the adult CNS, particularly in cortex, thalamus, globus pallidus and hippocampus (mossy fibers, stratum radiatum, stratum oriens). In the cerebellum $Na_V1.2$ is expressed in both Purkinje and granule cells. Early in development, $Na_V1.2$ is highly expressed in regions destined to become nodes of Ranvier and is replaced during development by $Na_V1.6$.
$Na_V1.2$ is also expressed in the utricular hair cells (only in early postnatal period) and in the corti organ.

Somatic Na^+ current in CG cells is dominated by fast gating channels, consistent with the contribution of $Na_V1.2$ and/or $Na_V1.6$. Developmentally regulated $Na_V1.2$ splicing may be one mechanism that counters the normally high excitability of neonatal neurons and helps to reduce seizure susceptibility in normal human infants.
$Na_V1.2$ are regulated by neutrotransmitters that act through G-protein coupled receptors and activate protein kinase A such dopamine, acetylcholine and serotonin.

3. VALIDATION OF CHO $Na_V1.2$ CELLS

3.1. Biophysical Characterization

$Na_V1.2$ 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) CsCl 135, NaCl 10, HEPES 10, EGTA 5, pH (KOH) 7.30. After formation of a GΩ seal between the patch electrodes and individual $Na_V1.2$ 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 $Na_V1.2$ currents were measured upon depolarization of the cell membrane from a holding potential of -110 mV to voltages between -90 mV and 0 mV in 10 mV increments of 100 ms duration (Fig. 1). $Na_V1.2$ tail currents were elicited upon a voltage step to -10 mV for 10 ms. The voltage pulses were run at intervals of 12 s.

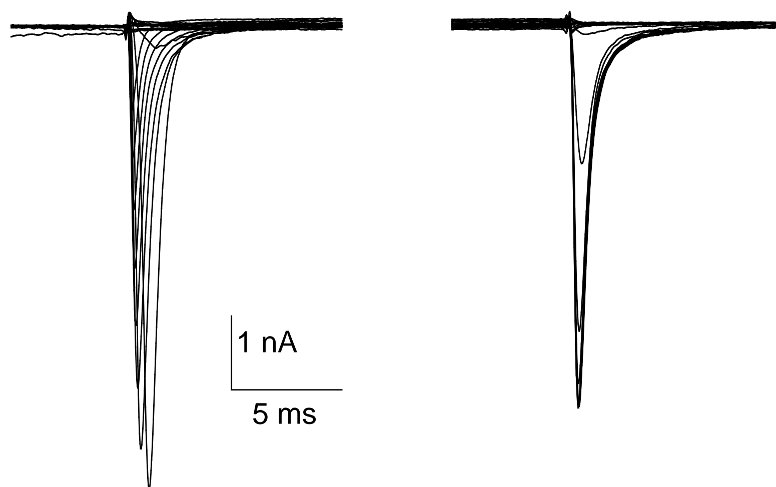

Fig. 1: Representative $Na_V1.2$ inward currents: left: recorded upon depolarization of the CHO membrane from a holding potential of -110 mV to voltages between -90 mV and +0 mV in 10 mV increments (100 ms). right: voltage pulses of the same voltage protocol to -10 mV (10 ms) elicited $Na_V1.2$ tail currents. The 90 ms between both $Na_V1.2$ peaks are not shown.

To verify the functional properties of B'SYS's cloned CHO $Na_V1.2$ cells, the IV curve, the activation curve and the inactivation curve were constructed. The voltage dependence of the activation and of the inactivation were best fitted with a Boltzmann function. The half maximal activation was found at -31.20 ± 2.83 mV, the half maximal inactivation at -48.06 ± 4.09 mV.

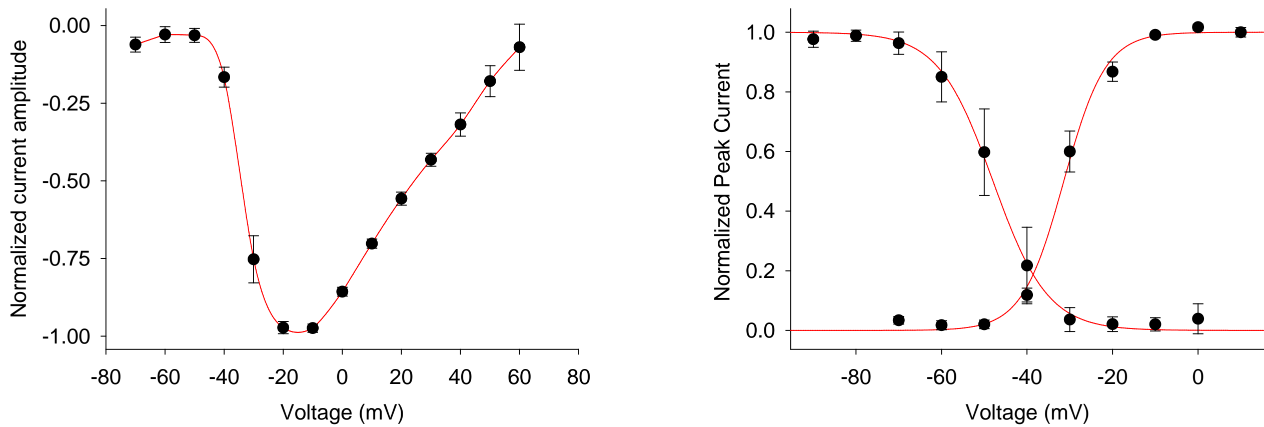

Fig. 2: Biophysical characterization of $Na_V1.2$ cells: left: IV curve, right activation and inactivation curve.fitted to a Boltzmann function.

3.2. Pharmacological Characterization

Mibefradil and Lidocaine were used as positive control to validate the pharmacological profile of the CHO $Na_V1.2$ cells. The cells were exposed to 1.0, 3.0, 10 and 30 µM Mibefradil. Instantaneous peak current amplitudes were recorded for a pulse from -80 mV to 0 mV for manual and from -110 mV to -10 mV for automated patch-clamping (Q-Patch). The IC_{50} was determined as: 3.06 µM (manual patch-clamping) and 9.63 µM for automated patch-clamping. The IC_{50} for Lidocaine was only determined using manual patch-clamping. The IC_{50} was determined as 130.20 µM.

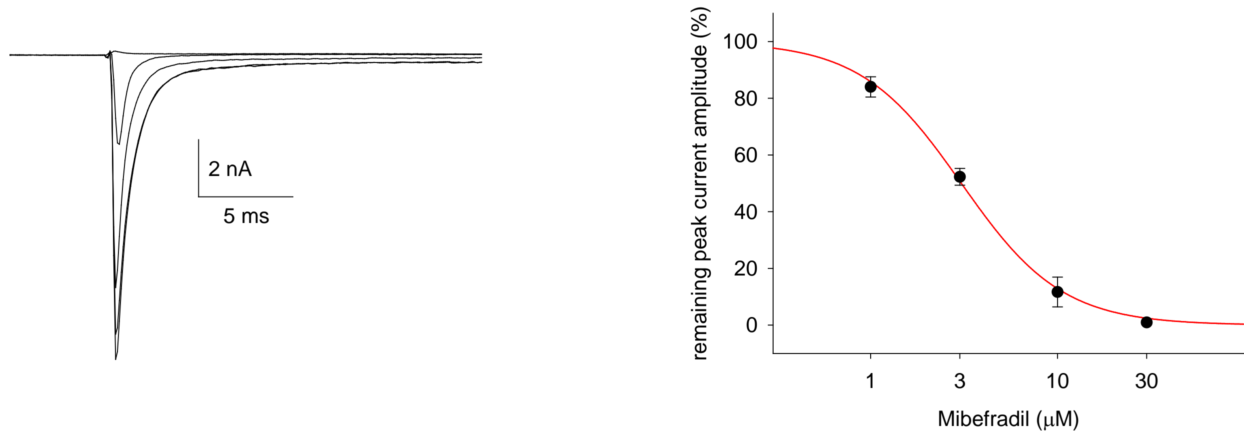

Fig. 3: Effect of Mibefradil on $Na_V1.2$ peak currents: left: representative current recording of $Na_V1.2$ peak currents in the presence of 0, 1, 3, 10 and 30 µM Mibefradil (manual patch-clamping). Right: dose response curve for Mibefradil. The IC_{50} was determined as: 3.06 µM (manual patch-clamping).

4. CELL CULTURE CONDITIONS

4.1. General

CHO $Na_V1.2$ 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 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and 200 µg/ml Hygromycine. The CHO $Na_V1.2$ 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.

- All solutions and equipment coming in contact with the cells must be sterile.
- Use proper sterile technique and work in a laminar flow hood.
- Be sure to have frozen cell stocks at hand before starting experiments.
- Cells should be split every 2-3 days at 70% - 80% confluence at 1:3 to 1:5 ratio.

4.2. Recommended Complete Medium

- F12 (HAM) with L-Glutamine or GlutaMAX I
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3. Antibiotics

- CHO $Na_V1.2$ clones were selected under 200 µg/mL Hygromycine antibiotic pressure.
- To cultivate CHO $Na_V1.2$ cells, a reduced antibiotic pressure (200 µg/mL) must be used.
- To separate CHO $Na_V1.2$ cells from untransfected cells, use 500 µg/mL Hygromycine.

Remark: The permanent application of high antibiotic pressure has no effect on current density.

4.4. Thawing Cells

- Remove vial of cells from liquid nitrogen and thaw quickly at 37°C.
- Decontaminate outside of vial with 70% ethanol.
- Transfer cells to a T-25 culture flask containing 55 mL complete medium.
- Incubate cells at 37°C for 4-6 h to allow the cells to attach to the bottom of flask.
- Aspirate off the medium and replace with 5 mL complete medium & antibiotics.
- Antibiotics: 200 µg/mL Hygromycine.
- Incubate cells and check them daily until 70% - 80% confluence is reached.

4.5. Splitting Cells

- When cells are 70% - 80% confluent remove medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
- Remove Trypsin/EDTA quickly and incubate cells for 2 min at 37°C.
- Detach cells, add complete medium and pipette up and down to break clumps of cells.
- Passage cells into new flask with complete medium and antibiotics at 1:3 to 1:5 ratio.
- Use remaining suspension for counting the cells.

4.6. Freezing Cells

- Cells should have 80% - 90% confluence prior to freezing.
- Remove the complete medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
- Remove Trypsin/EDTA quickly and incubate cells for 2 min at 37°C.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Resuspend Cells in complete medium to reach a density of approximately1.0E+06 cells per mL.
- Add sterile DMSO to reach 10% DMSO in the complete medium.
- Resuspend cells at a density of approximately 2.0 E+06 cells per mL with fresh freezing medium.
- Mix DMSO and cell suspension by pipetting up and down several times
- Aliquot 0.5 mL of cell suspension into each cryovial.
- Overnight incubate cells in a styropor box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

4.7. Stability of CHO $Na_V1.2$ cells

CHO $Na_V1.2$ cells stably express functionally active $Na_V1.2$ potassium channels over 20 passages. Under recommended cell culture conditions no variation in current density was observed during this time.

5. $Na_V1.2$ SEQUENCE

5.1. Accession Number NP_001035232

Cloned cDNA (codon optimized) encodes for the protein of the $Na_V1.2$ channel (NP_001035232):

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RESKK

6. CONTACT INFORMATION

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