B'SYS GmbH

HEK-293 HCN2 Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The HCN2 channel

HCN channels are voltage-gated ionic channels, regulated by cyclic nucleotides, such as cyclic adenosine-mono-phosphate (cAMP). In contrast to most Na^+ and K^+ ionic channels, which open when membrane potential is depolarized, they are opened when the membrane potential hyperpolarizes below -50 mV.
Recent publications show that members of the hyperpolarization-activated cyclic nucleotide-modulated (HCN) ion channel family – better known for their role in the pacemaker potential of the heart – play important roles in both inflammatory and neuropathic pain. Deletion of the HCN2 isoform from nociceptive neurons abolishes heat-evoked inflammatory pain and all aspects of neuropathic pain, but acute pain sensation is unaffected. This work shows that inflammatory and neuropathic pain have much in common, and suggests that selective blockers of HCN2 may have value as analgesics in the treatment of pain.

1.2 B'SYS' HEK-293 HCN2 cells

B'SYS has designed a new HEK-293 HCN2 cell line with Tetracycline inducible expression of human HCN2 channel. The human HCN2 cDNA (codon optimized) was cloned and transfected into HEK-293 cells by lipofection. Functional properties of the HCN2 channels were validated by means of the patch-clamp technique on the QPatch automate. Biophysical and pharmacological properties were evaluated.

2 VALIDATION OF HEK-293 HCN2 CELLS

2.1 Material and Methods

For testing HEK-293 HCN2 cells automated patch-clamping (Q-Patch from Sophion) was used. The extracellular solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 10, Hepes 10, D-Glucose 10. The pH was adjusted to 7.4 with NaOH. The intracellular solution consisted of (in mM) CsF 135, $CaCl_2$ 0.2, NaCl 10, cAMP 0.1, EGTA 5, HEPES 10. The pH was adjusted to 7.3 with CsOH.

2.2 Biophysical Validation

HCN2 channels stably expressed in HEK293 cells were clamped to test potentials between -30 mV and -120 mV in 10 mV decreasing steps for 1 s. The holding potential was -40 mV. The mean current amplitude at -120 mV was 1285 ± 770 pA, n=17. Tail currents were analyzed to calculate the voltage dependence of activation. $V_{0.5}$ was determined to be -76.77 ± 1.54 mV, k: -6.82 ± 0.77 (n=4)

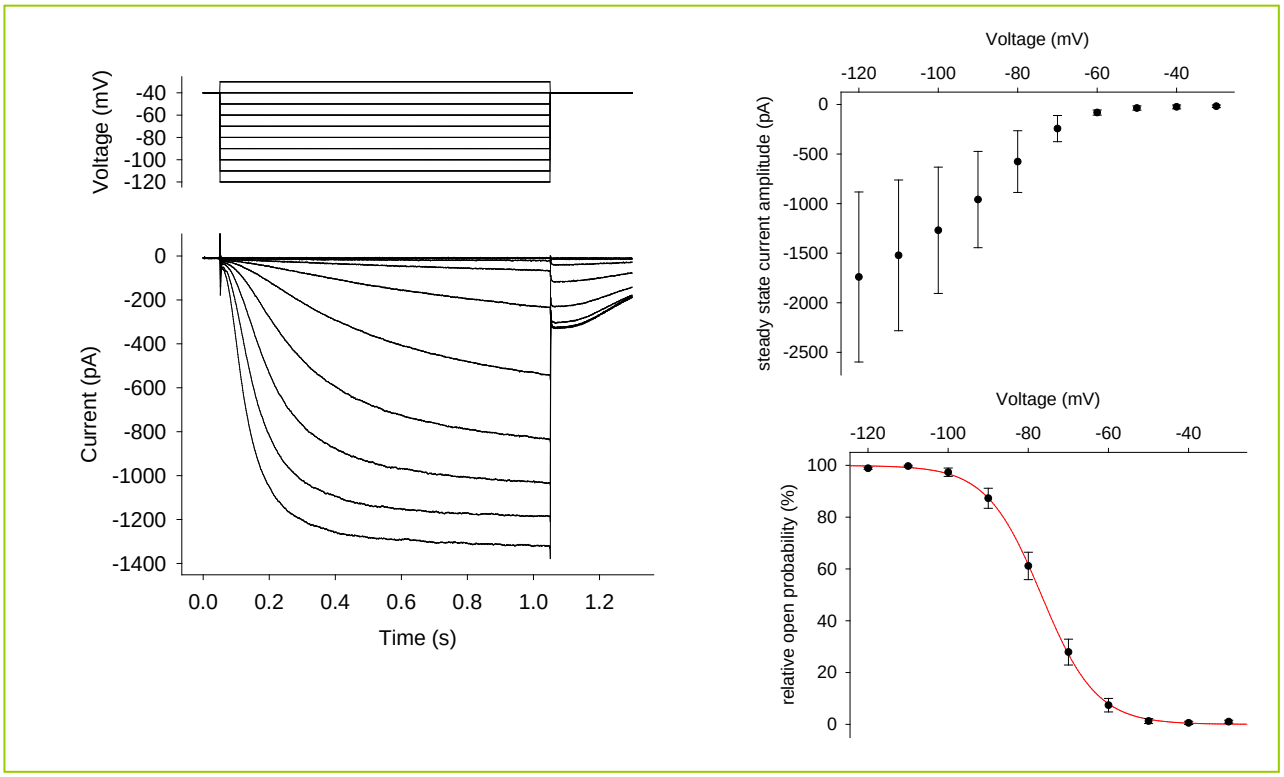

Fig. 1: Biophysical characterization of HCN2. Left top: voltage protocol to record a IV curve. Left bottom: resulting currents recorded from a stably transfected HEK-293 HCN2 cell (induced by 2.5 µg/mL Tetracycline). Right top: IV curve of current at end of variable pulse. Right bottom: activation curve derived from tail current amplitudes. Data were fit with a Boltzman equation to determine $V_{0.5}$.

2.3 Pharmacological Validation

HCN2 channels were activated by hyperpolarization to -120 mV for 1.0 s from a holding potential of -40 mV. The current amplitude at the end of this hyperpolarization was used for analysis. After stabilisation of the current amplitude under control conditions, increasing concentrations of test item were perfused. IT plots were generated and steady state current amplitudes were normalized to the initial current amplitude under control conditions. The resulting dose response curves were fit with a logistic equation assuming a_{max} of 100% and a_{min} of 0%.

2.4 ZD7288:

Concentrations of 3.0, 10, 30 and 100 µM ZD7288 were tested, the dose response curve was generated and the IC_{50} was determined to be 21.25 µM, Hill coefficient: 0.99.

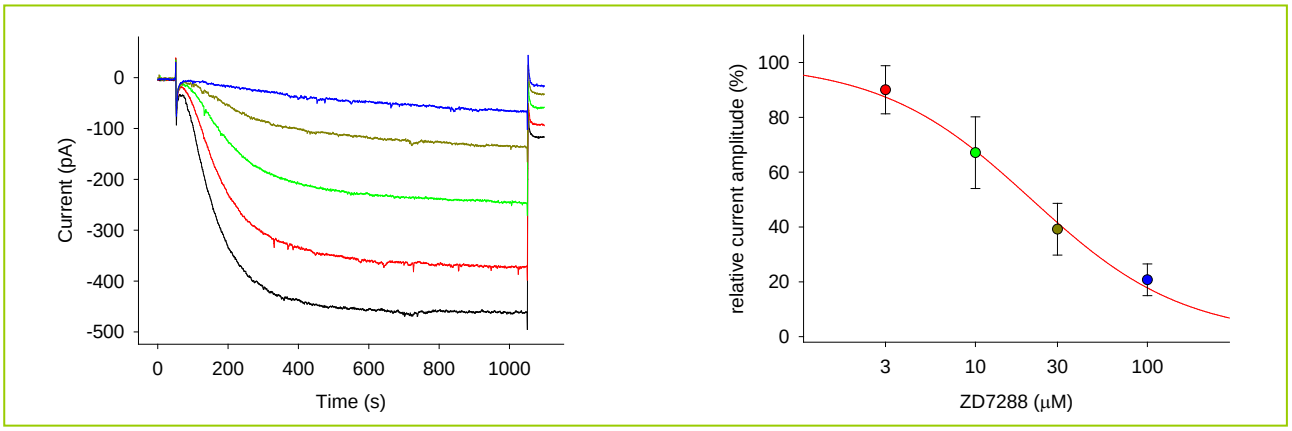

Fig. 2: Dose response curve for ZD7288: left: representative current recordings (black: 0 µM, red: 3 µM, green: 10 µM, brown: 30 µM, blue: 100 µM), right dose response curve (n=2), IC_{50}: 21.25 µM, Hill coefficient: 0.99

2.5 Ivabradine:

Six concentrations of the cardiotonic agent Ivabradine were tested on its effects on HCN2 channels. A dose response curve was generated and the IC_{50} was determined to be 15.03 µM (Hill coefficient 0.99).

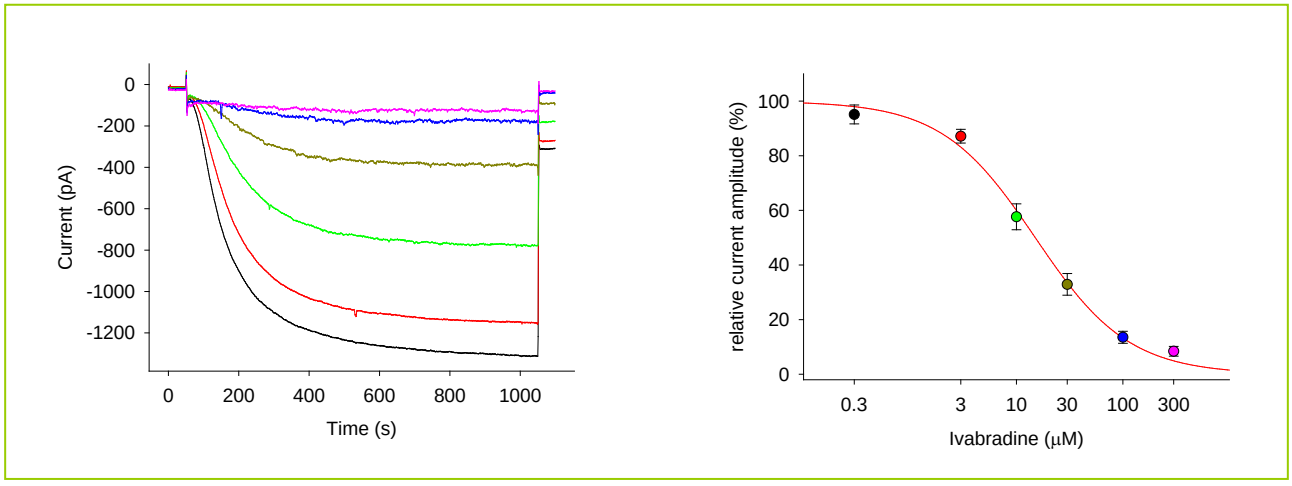

Fig. 3: Dose response curve for Ivabradine: left: representative current recordings (black: 0 µM, red: 0.3 µM, green: 3 µM, brown: 10 µM, blue: 30 µM, pink: 100 µM, cyan: 300 µM), right dose response curve (n=6), IC_{50}: 15.03 µM, Hill coefficient: 0.99

3 CELL CULTURE CONDITIONS

3.1 General

HEK-293 HCN2 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 DMEM medium supplemented with 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and 0.5 µg/mL Puromycin and 15 µg/mL Blasticidin. The HEK-293 HCN2 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% to 80% confluency at 1:3 to 1:5 ratio.

EXPRESSION OF HCN2 HAS TO BE INDUCED BY ADDING 2.5 µg/mL TETRACYCLINE AT LEAST 24 H BEFORE START OF EXPERIMENTATION

3.2 Recommended Complete Medium

- DMEM with Glutamine or GlutaMAX I
- 10% FBS
- 1.0% Penicillin/Streptomycin

3.3 Antibiotics

- HEK-293 HCN2 clones were selected under 0.5 µg/mL Puromycin / 15 µg/mL Blasticidin antibiotic pressure.
- To cultivate HEK-293 HCN2 cells, 100 µg/mL Hygromycin / 15 µg/mL Blasticidin are recommended.
- To separate HEK-293 HCN2 cells from untransfected cells, use 0.5 µg/mL Puromycin / 15 µg/mL Blasticidin

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

3.4 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-75 culture flask containing 10 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 10 mL complete medium & antibiotics.
- Antibiotics: 100 µg/mL Hygromycin / 15 µg/mL Blasticidin.
- Incubate cells and check them daily until 70% - 80% confluency is reached.

3.5 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 pipet 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.

3.6 Freezing Medium

- Mix 0.9 mL fresh complete medium (without FBS) and 0.1 mL DMSO for every 1 mL freezing medium.
- Sterilize freezing medium by means of appropriate micro filter (0.1 µm – 0.2 µm).

Alternatively sterile DMSO and medium can be used.

3.7 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% to 90% confluency 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.
- Pellet cells with centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 2.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.5 mL of cell suspension into each cryovial.
- Overnight incubate cells in a polystyrene box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

4 HCN2 SEQUENCE

4.1 Human HCN2 Accession Number NP_001185.3

Cloned cDNA sequence of human HCN2 subunit was error-free and encodes for NP_001185.3:

MDARGGGGRPGESPGATPAPGPPPPPPPAPPQQQPPPPPPPAPPPGPGPAPPQHPPRAEALPPEAADEGGPRGRLRSRDS
SCGRPGTPGAASTAKGSPNGECGRGEPQCSPAGPEGPARGPKVSFSCRGAASGPAPGPGPAEEAGSEEAGPAGEPRGSQA
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APLAGPALPARRLSRASRPLSASQPSLPHGAPGPAASTRPASSSTPRLGPTPAARAAAPSPDRRDSASPGAAGGLDPQDS
ARSRLSSNL

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