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HEK-293 HCN4 Cell Line

Specification Sheet

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1 MATERIAL AND METHODS

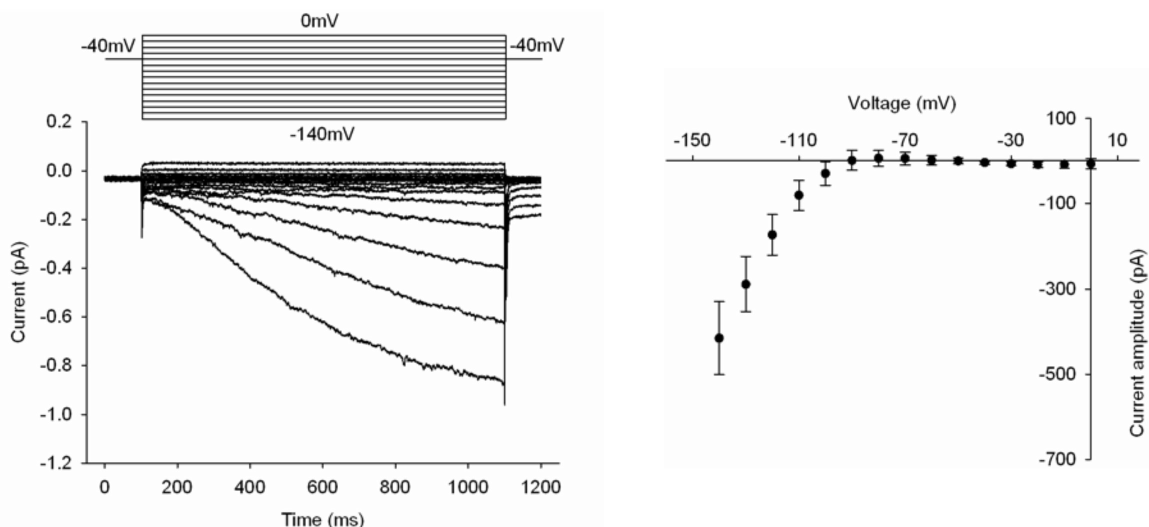
For manual patch-clamping a setup equipped with an EPC-10 amplifier and Patchmaster Software was used.

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) CsCl 135, NaCl 10, $CaCl_2$ 0.2, cAMP 10, EGTA 5, HEPES 10. The pH was adjusted to 7.3 with CsOH. After formation of a GΩ seal between the patch electrodes and individual HCN4 stably transfected HEK HCN4 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 the cells were clamped at -40 mV and the voltage stimulus was applied continuously.

For measurements with the QPatch, the same extracellular solution as for manual patch-clamping was used. It contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 10, Hepes 10, D-Glucose 10 and the pH was adjusted to 7.4 with NaOH. The intracellular solution consisted of (in mM) CsF 135, $CaCl_2$ 0.2, NaCl 10, EGTA 5, HEPES 10. The pH was adjusted to 7.3 with KOH.

2 BIOPHYSICAL VALIDATION

Manual patch-clamp recordings were performed to characterize HCN4 channels stably expressed in HEK293 cells. Cells were clamped to test potentials between 0 mV and -140 mV in 10 mV decreasing steps for 1 s. The holding potential was -40 mV.



3 PHARMACOLOGICAL VALIDATION

HCN4 channels were activated by hyperpolarization to -120 mV for 2.0 s. Increasing concentrations of the test item was perfused and steady state current amplitudes were normalized to the steady state current amplitude under control conditions.

3.1 ZD7288:

One concentration of ZD7288, a potent blocker of the HCN channels in heard and brain was tested. At a concentration of 100 µM 76.95 ± 3.41% (n=3) of the current amplitude was blocked.

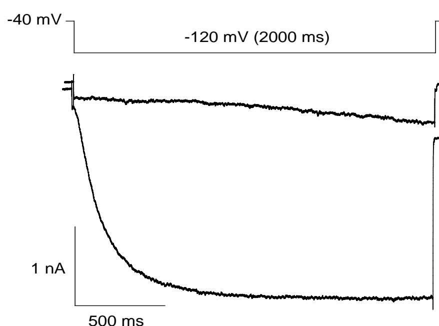


Fig.1: Representative current recordings for HEK HCN4 cells treated at 100 µM ZD7288

3.2 Ivabradine:

Four increasing concentrations of the cardiotonic agent Ivabradine were tested on its effects on HCN4 channels. A dose response curve was generated and the IC_{50} was determined to be 4.46 µM (Hill coefficient 1.47) for manual patch-clamping and 12.16 µM (Hill coefficient 0.96) for automated patch-clamping.

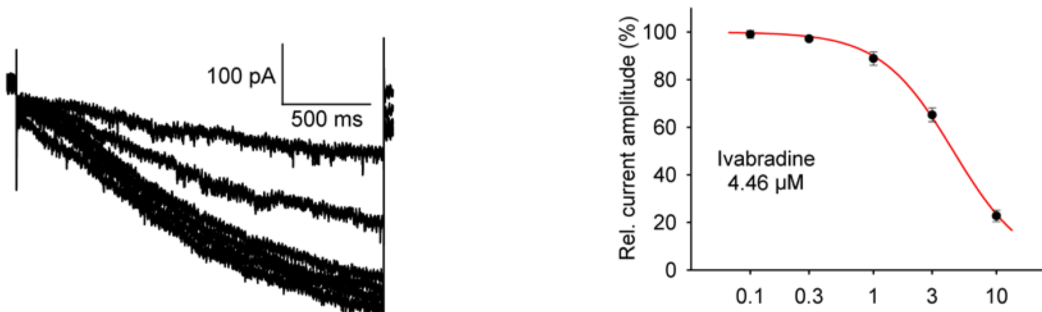


Fig.2: Dose response curve for Ivabradine with manual patch-clamping: left: representative current recordings, right dose response curve (n=3), IC_{50}: 4.46 µM, Hill coefficient: 1.47

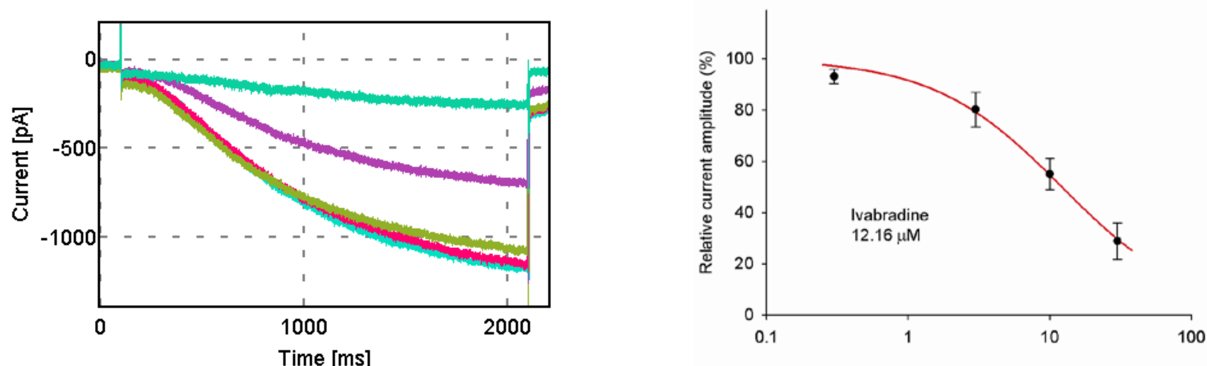


Fig.3: Dose response curve for Ivabradine with automated patch-clamping on the QPatch: left: representative current recordings, right dose response curve (n=4), IC_{50}: 12.16 µM, Hill coefficient: 0.96

4 CELL CULTURE CONDITIONS

4.1 General

HEK HCN4 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 100 µg/mL Hygromycin / 15 µg/mL Blasticidin. The HEK HCN4 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% confluency at 1:3 to 1:5 ratio.

EXPRESSION OF HCN4 HAS TO BE INDUCED BY ADDING 2.5 µg/mL TETRACYCLINE AT LEAST 24 H BEFORE START OF EXPERIMENTATION

4.2 Recommended Complete Medium

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

4.3 Antibiotics

- HEK HCN4 clones were selected under 500 µg/mL Hygromycin / 15 µg/mL Blasticidin antibiotic pressure.
- To cultivate HEK HCN4 cells, 100 µg/mL Hygromycin / 15 µg/mL Blasticidin are recommended.
- To separate HEK HCN4 cells from untransfected cells, use 500 µg/mL Hygromycin / 15 µg/mL Blasticidin

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-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.

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 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.

4.6 Freezing Medium

- Mix 0.9 mL fresh complete medium 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).

4.7 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 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 styropor box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

5 HCN4 SEQUENCE

5.1 Human HCN4 Accession Number NP_005468

Cloned cDNA sequence of human HCN4 subunit was error-free and encodes for NP_005468:

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MDKLPPSMRKRLYSLPQQVGAKAWIMDEEEDAEEEGAGGRQDPSRRSIRLRPLPSPSPSAAAGGTESRSSALGAADSEGP
ARGAGKSSTNGDCRRFRGSLASLGSRGGGSGGTGSGSSHGHLHDSAEERRLIAEGDASPGEDRTPPGLAAEPERPGASAQ
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SNL
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6 CONTACT INFORMATION

6.1 Contact Address for Technical Support and Ordering Information

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