B’SYS GmbH

HCN1 Assay

1 MATERIAL AND METHODS

For manual patch-clamping a patch-clamp rig 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 100, GTP 1000, 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 HCN1 transfected 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 to was applied continuously.

2 BIOPHYSICAL VALIDATION

HCN1 channels were activated by hyperpolarization to voltages between -40 and -160 mV for 0.5 s. The steady state current density was plotted versus the applied voltage. An inward rectifying current was found. Channels opened at voltages more negative than -80 mV. For the activation curve, the normalized conductance was plotted versus the applied voltage. The resulting curve was fit with a Boltzmann equation. The $V_{0.5}$ value was determined as: -98.9 mV, k: 5.5.

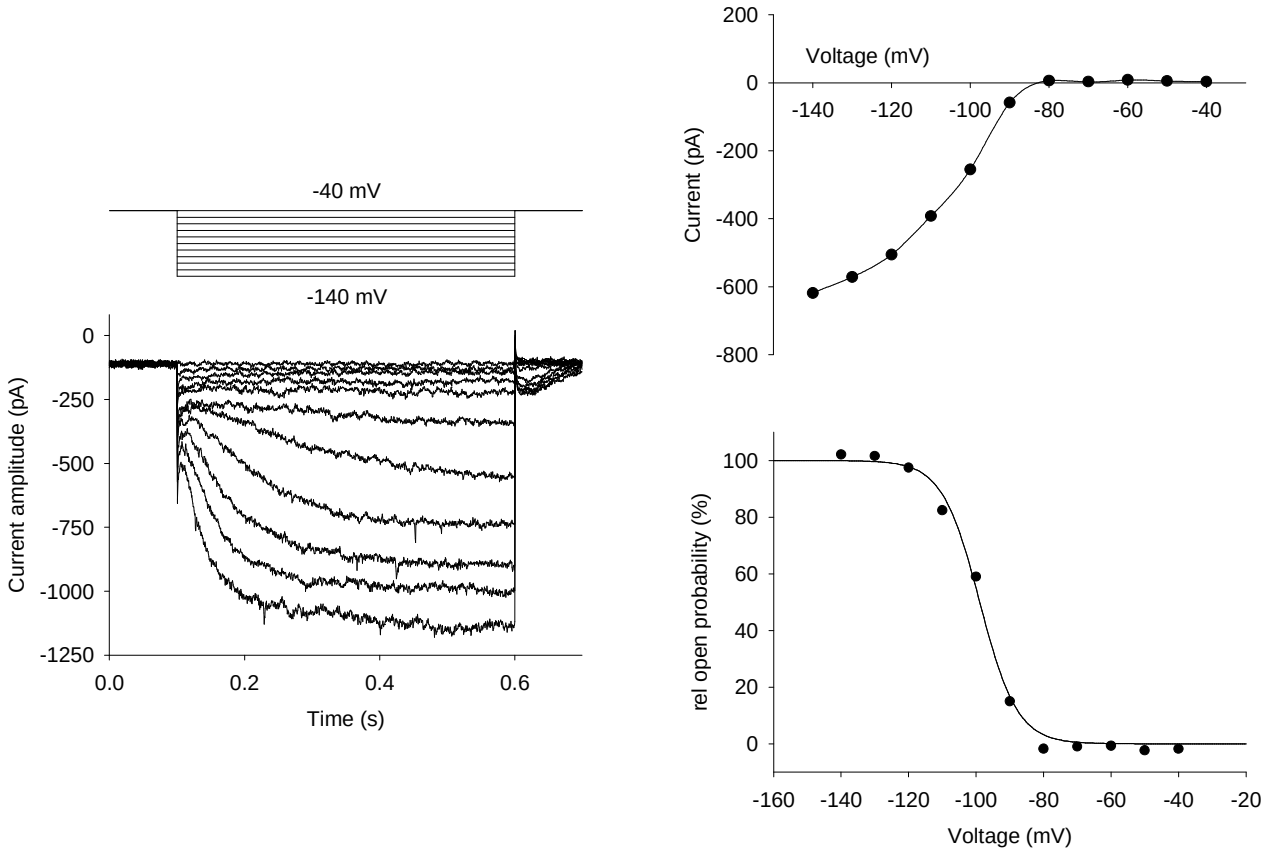

Fig.1: Biophysical Characterization of HCN1 currents: left representative current recording, right: top: IV curve obtained, right bottom: activation curve for HCN1

3 PHARMACOLOGICAL VALIDATION

HCN1 channels were activated by hyperpolarization to -120 mV for 0.5 s. Increasing concentrations of the ZD7288 were perfused and steady state current amplitudes were normalized to the steady state current amplitude under control conditions.

ZD7288, a potent blocker of the HCN channels in heart and brain was used as reference compound. Two concentrations of 100 µM and 300 µM were tested. HCN-1 currents were blocked with increasing concentration.

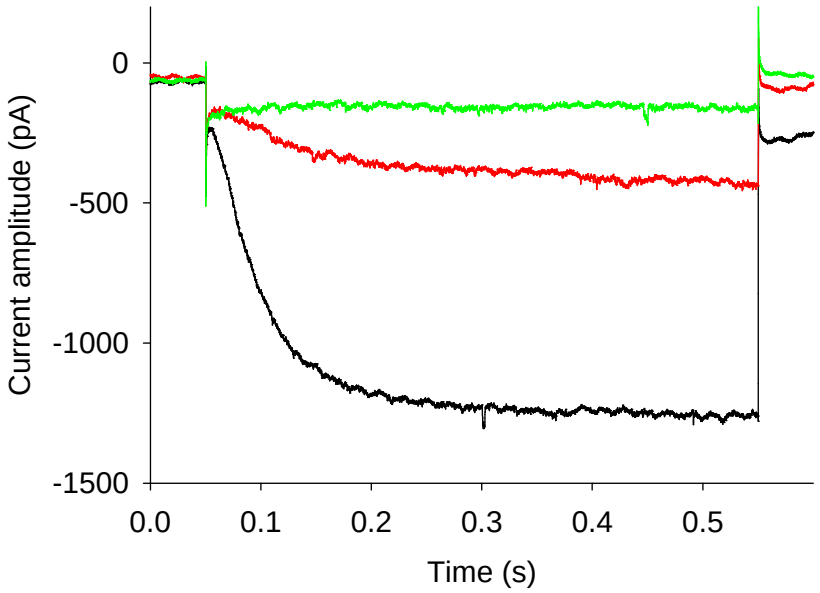

Fig.2: Representative current recordings for HCN1 currents treated with 100 µM ZD7288 (red) and 300 µM ZD7288 (green). Black: current under control conditions.

4 HCN1 SEQUENCE

Cloned cDNA sequence of human HCN1 subunit was error-free and encodes for NP_066550.2:

MEGGGKPNSSSNSRDDGNSVFPAKASATGAGPAAAEKRLGTPPGGGGAGAKEHGNSVCFKVDGGGGGGGGGGGGEEPAGG
FEDAEGPRRQYGFMQRQFTSMLQPGVNKFSLRMFGSQKAVEKEQERVKTAGFWIIHPYSDFRFYWDLIMLIMMVGNLVII
PVGITFFTEQTTTPWIIFNVASDTVFLLDLIMNFRTGTVNEDSSEIILDPKVIKMNYLKSWFVVDFISSIPVDYIFLIVE
KGMDSEVYKTARALRIVRFTKILSLLRLLRLSRLIRYIHQWEEIFHMTYDLASAVVRIFNLIGMMLLLCHWDGCLQFLVP
LLQDFPPDCWVSLNEMVNDSWGKQYSYALFKAMSHMLCIGYGAQAPVSMSDLWITMLSMIVGATCYAMFVGHATALIQSL
DSSRRQYQEKYKQVEQYMSFHKLPADMRQKIHDYYEHRYQGKIFDEENILNELNDPLREEIVNFNCRKLVATMPLFANAD
PNFVTAMLSKLRFEVFQPGDYIIREGAVGKKMYFIQHGVAGVITKSSKEMKLTDGSYFGEICLLTKGRRTASVRADTYCR
LYSLSVDNFNEVLEEYPMMRRAFETVAIDRLDRIGKKNSILLQKFQKDLNTGVFNNQENEILKQIVKHDREMVQAIAPIN
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ISRPHPTVGESLASIPQPVTAVPGTGLQAGGRSTVPQRVTLFRQMSSGAIPPNRGVPPAPPPPAAALPRESSSVLNTDPD
AEKPRFASNL*

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