B'SYS GmbH

CHO GlyRα_3 Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The GlyRα_3 Receptors

Glycine is a major inhibitory neurotransmitter in the adult vertebrate central nervous system. Glycinergic synapses have a well-established role in the processing of motor and sensory information that controls movement, vision and audition. This action of glycine is mediated through its interaction with the glycine receptor, a chloride channel opens after glycine binding. The influx of anions prevents membrane depolarisation and neuronal firing induced by excitatory neurotransmitters. Strychnine acts as a competitive antagonist of glycine binding, thereby reducing the activity of inhibitory neurons. Compounds that modulate Glycine receptors activity include zinc, some alcohols and anaesthetics, picrotoxin, cocaine and some anticonvulsants. GlyRα_3 is expressed in the cerebellum, olfactory bulb and hippocampus.

1.2 B'SYS' CHO GlyRα_3 Cells

B'SYS has designed a CHO GlyRα_3 cell line with constitutive coexpression of human Glycine receptor alpha 3. The human GlyRα_3 cDNA was cloned and transfected into CHO cells and then the functional properties of the GlyRα_3 receptors validated by means of the patch-clamp technique. Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human GlyRα_3 channel:

- 1 x 0.5 mL aliquots of frozen cells at 2.2 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO

2.2 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.

3 VALIDATION OF CHO GLYRA3 CELLS

3.1 Electrophysiology

GlyRα3 currents were measured by means of the automated or manual patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a GΩ seal between the patch electrodes and individual GlyRα3 stably transfected CHO cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. GlyRα3-mediated currents were measured at a holding potential of -80 mV. All solutions applied to cells were continuously perfused and maintained at room temperature. Glycine, or applications of glycine containing a concentration of strychnine were applied for 4 s. Between each application bath was perfused for 30s.

3.2 Activation by Glycine

Increasing cumulative concentrations of glycine were applied using manual and automated patch-clamp recordings to generate dose-response curves. For manual patch-clamp recordings the EC_{50} was 154.10 µM (Hill coefficient 4.62). For automated patch-clamp recordings the EC_{50} was 212.70 µM (Hill coefficient 2.74).

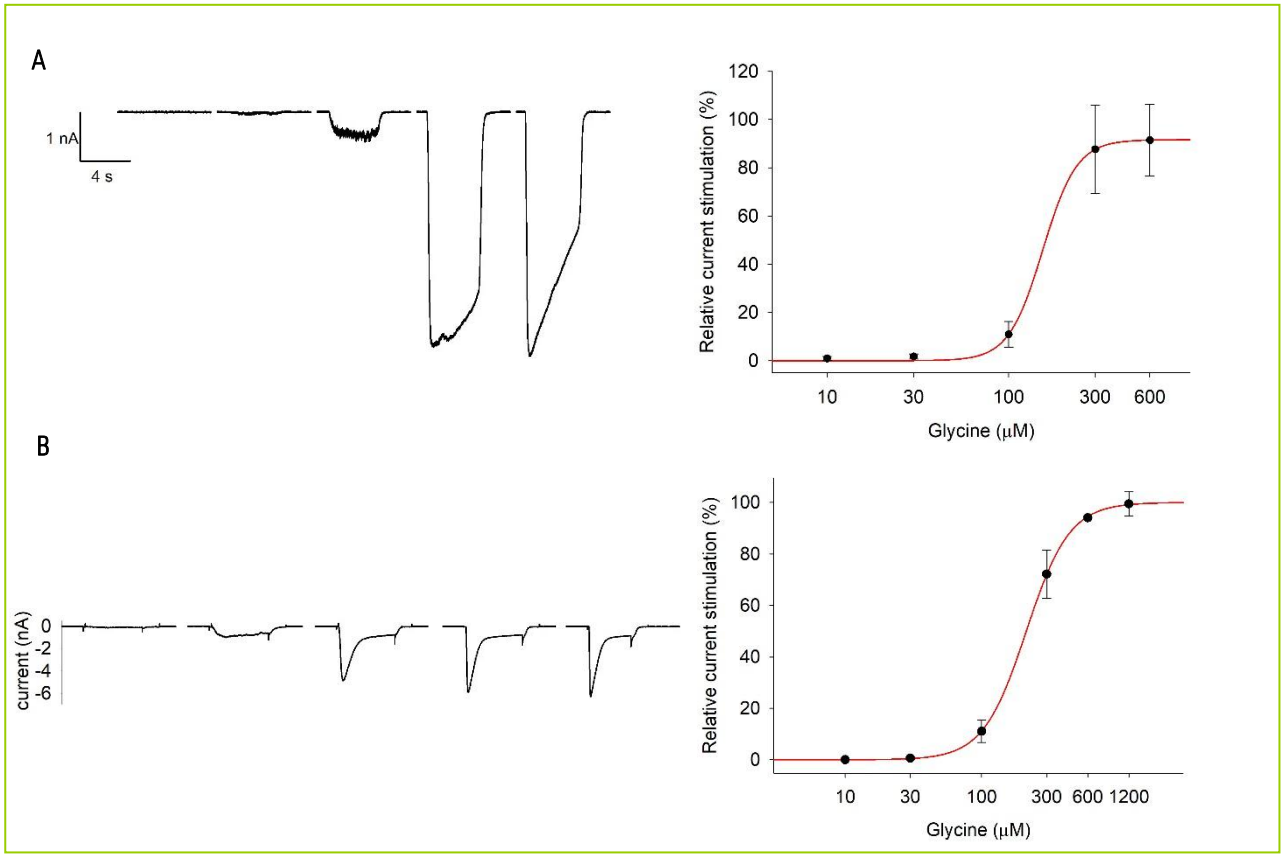

Fig.1: A) Dose-response curve to glycine using manual patch-clamp on GlyRα3. The calculated EC_{50} was 154.10 µM (Hill coefficient 4.62, n=4). **B)** Dose-response curve to glycine using manual patch-clamp on GlyRα3. The calculated EC_{50} was 212.70 µM (Hill coefficient 2.74, n=7).

3.3 Block by Strychnine

Cells were stimulated with 100 µM or 150 µM glycine for automated or manual patch-clamp recordings, respectively. Increasing concentrations of strychnine were co-applied and the mean current amplitudes were plotted versus the strychnine concentration. The dose response curve was generated, for manual patch-clamp recordings an IC_{50} of 51.87 nM was determined (Hill coefficient: 1.33). For automated patch-clamp recordings an IC_{50} of 12.55 nM was determined (Hill coefficient: 1.35).

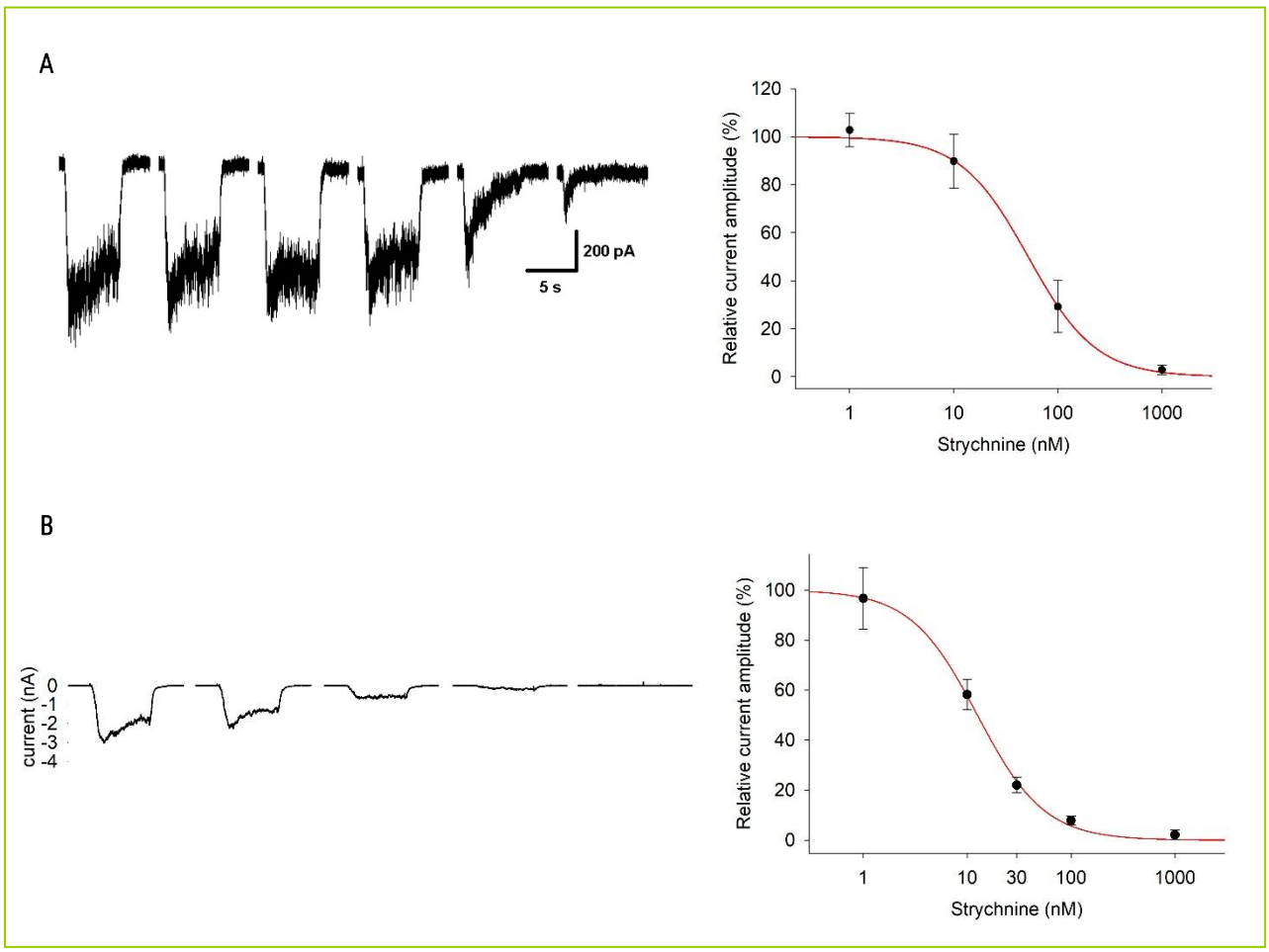

Fig.2: Strychnine block of GlyRα3 currents. **A)** Manual patch-clamp recordings: cells were stimulated with 150 µM Glycine. Glycine was applied twice followed by increasing concentrations of Strychnine (1.0, 10, 100 and 1000 nM) dissolved in bath solution, n=5). **B)** Automated patch-clamp recordings: cells were stimulated with 100 µM Glycine. Cumulative, increasing concentrations of Strychnine were applied (1.0, 10, 30, 100 and 1000 nM) dissolved in bath solution, n=7).

4 CELL CULTURE CONDITIONS

4.1 General

CHO GlyRα3 channel 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 Hygromycin 500 µg/mL. The CHO GlyRα3 cells are passaged at a confluence of about 50 to 80%.

- 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.

Table 1: Cell culture reagents

Product	Supplier	Order number
Nutrient mixture F-12 Ham (F12 Ham)	Sigma-Aldrich	N6658
Fetal Bovine Serine (FBS)	Gibco	10270-106
Penicillin / Streptomycin (100x)	Gibco	10378-016
Phosphate Buffered Saline (PBS, without Ca^{2+} and Mg^{2+})	Sigma-Aldrich	D8537
Hygromycin B (50 mg/mL)	Gibco	10687010
Detachin	Genlantis	T100T100
Trypsin EDTA (10x)	Sigma-Aldrich	T4174
DMSO	Sigma-Aldrich	D2438

For the preparation of 1X Trypsin/EDTA, the 10X solution is diluted in PBS (without Ca^{2+} and Mg^{2+}), aliquoted and stored in the freezer.

4.2 Recommended Complete Medium

- 500 mL F12 (HAM) with L-Glutamine
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Antibiotics

- CHO GlyRα3 clones were selected under Hygromycin 1000 µg/mL antibiotic pressure.
- To cultivate CHO GlyRα3 cells, a reduced antibiotic pressure (Hygromycin 500 µg/mL) should be used.

Remark: The permanent application of 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 5 mL complete medium
- Incubate cells at 37°C to allow the cells to attach to the bottom of the flask

- Once cells attach to the bottom of the flask, look healthy and proliferate, aspirate off the medium and replace with 5 mL complete medium containing selection antibiotics for cultivation (see 4.3)
- To check whether cells are attached properly, the flask can be gently moved while looking under the microscope
- If 48h after thawing the confluency is below 50%, replace the medium in the flask with fresh medium containing antibiotics
- Incubate cells at 37°C and check them daily until 50% to 80% confluency is reached.

4.5 Splitting Cells

- When cells are 50% to 80% confluent remove complete medium.
- Wash cells with 1xPBS to remove excess medium
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C.
- Detach cells by gently tapping the sides of the flask 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.
- Use remaining suspension for counting the cells.

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 and wash cells with 1xPBS.
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C
- Detach cells by gently tapping the sides of the flask, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells at 200 g using a centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 1.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.75 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.

4.8 Stability of CHO GlyRα3 cells

CHO GlyRα3 cells stably express functionally active GlyRα3 channels over 25 passages. Under recommended cell culture conditions, no variation in current density was observed over 25 cell splitting cycles.

5 SEQUENCE

5.1 GlyRα3, NP_006520.2

MAHVRHFRTLVSGFYFWEAALLLSLVATKETDSARSRSAPMSPSDFLDKLMGRTSGYDARIRPNFKGPPVNVTCNIFINS
FGSIAETTMDYRVNIFLRQKWNDPRLAYSEYPDDSLDLDPSMLDSIWKPDLFFANEKGANFHEVTTDNKLLRIFKNGNVL
YSIRLTLTLSCPMDLKNFPMDVQTCIMQLESFGYTMNDLIFEWQDEAPVQVAEGLTLPQFLLKEEKDLRYCTKHYNTGKF
TCIEVRFHLERQMGYYLIQMYIPSLLIVILSWVSFWINMDAAPARVALGITTVLTMTTQSSGSRASLPKVSYVKAIDIWM
AVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRKNKTEAFALEKFYRFSDMDDEVRESRFSFTAYGMGPCLQAKDGMTP
KGPNHPVQVMPKSPDEMRKVFIDRAKKIDTISRACFPLAFLIFNIFYWVIYKILRHEDIHQQQD*

5.2 Genetic Information

CHO GlyRα3 cells have been stably transfected with codon optimized cDNA encoding for the human GlyRα3 channel (NP_006520.2) by non-viral lipofection.

The expression of the GlyRα3 receptor is under the control of a CMV promoter, the Hygromycin resistance gene is controlled by a SV40 promoter. For amplification in e-coli, an Ampicillin resistance gene is located on the complementary strand under the control of a bla promoter.

6 CONTACT INFORMATION

6.1 Contact Address for Technical Support & Ordering Information

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