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).

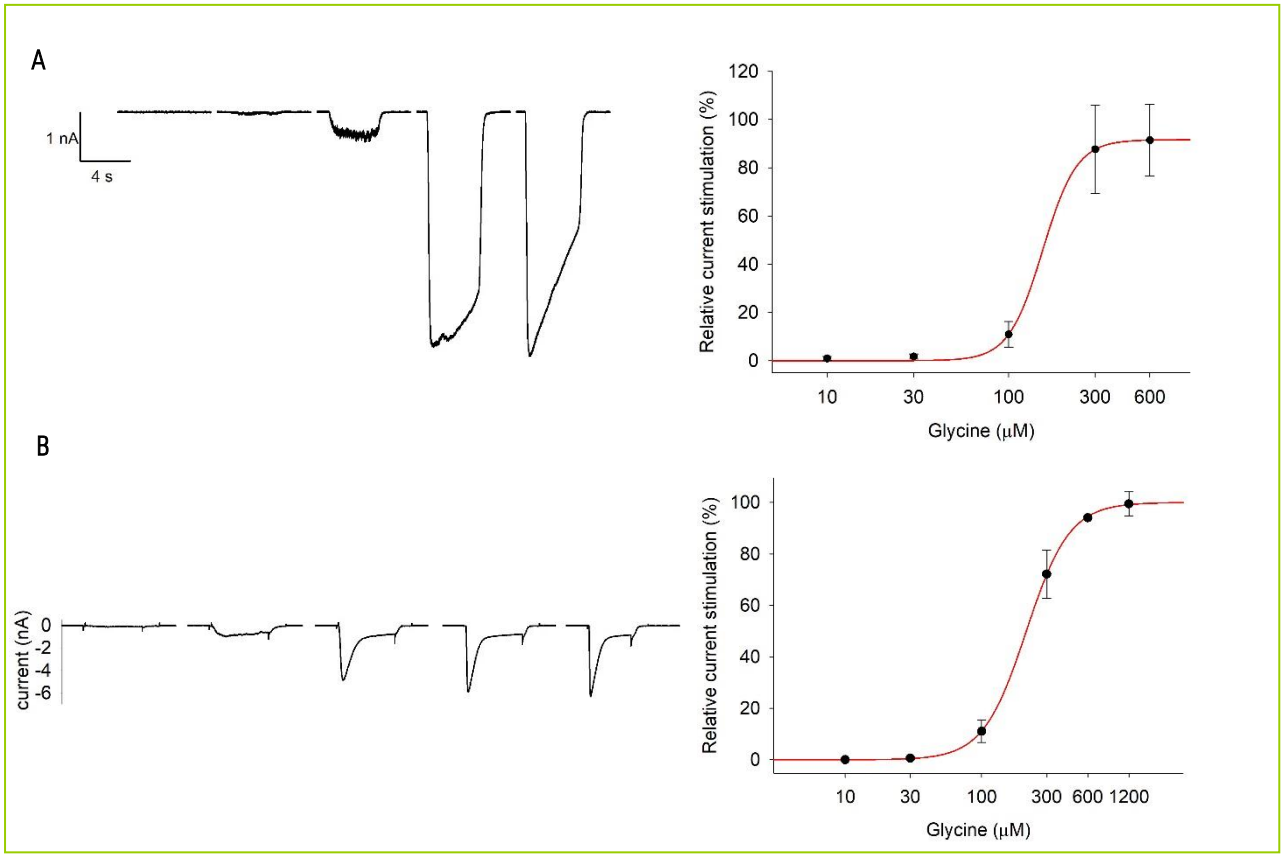

Fig.1: A) Dose-response curve to glycine using manual patch-clamp on GlyRα3. The calculated EC_{50} was 154.10 µM (Hill coefficient 4.62, n=4). **B)** Dose-response curve to glycine using automated patch-clamp on GlyRα3. The calculated EC_{50} was 212.70 µM (Hill coefficient 2.74, n=7).

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).

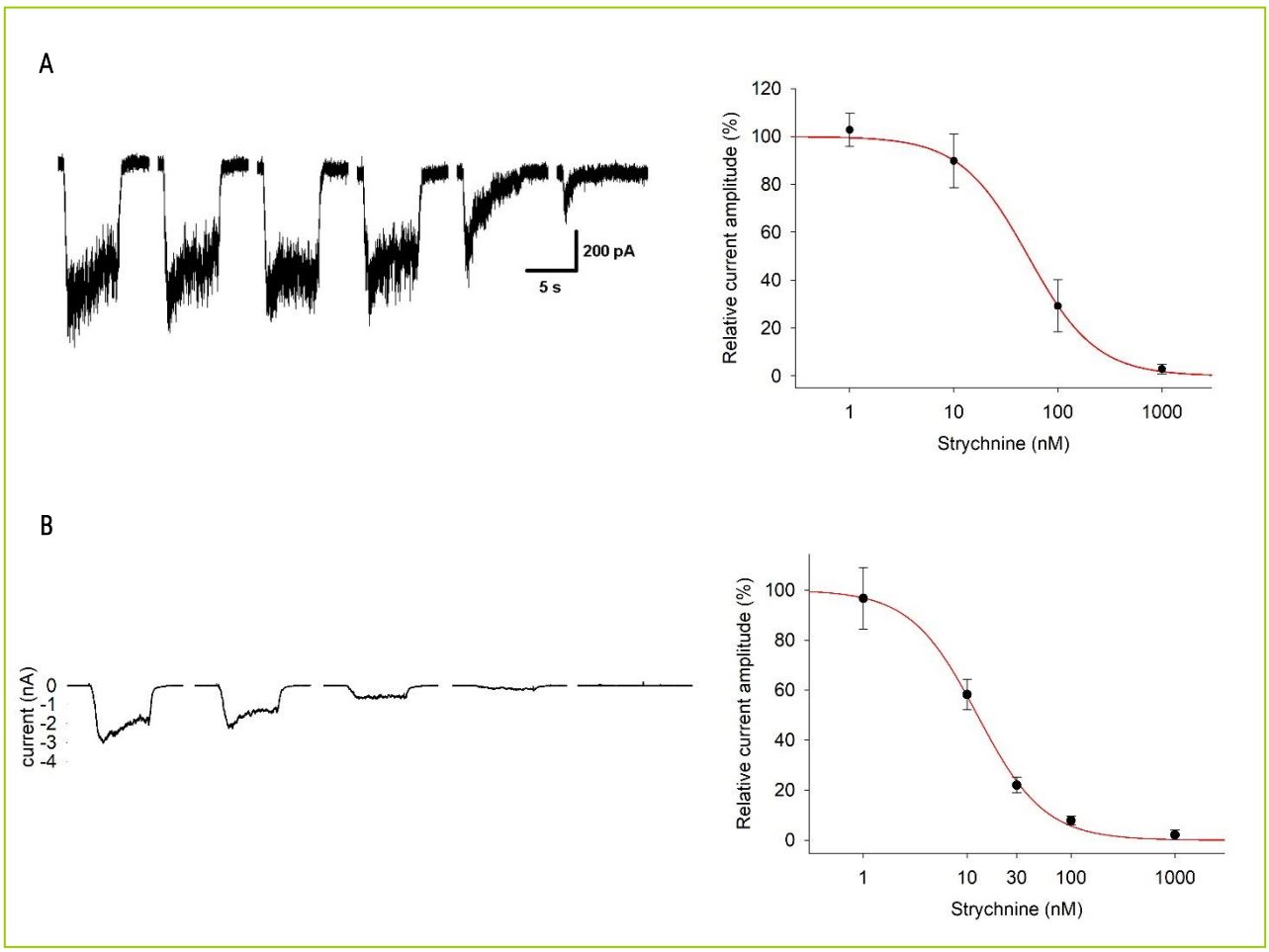

Fig.2: Strychnine block of GlyRα3 currents. **A)** Manual patch-clamp recordings: cells were stimulated with 150 µM Glycine. Glycine was applied twice followed by increasing concentrations of Strychnine (1.0, 10, 100 and 1000 nM) dissolved in bath solution, n=5). **B)** Automated patch-clamp recordings: 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 GlyRα_3 cells stably express functionally active GlyRα_3 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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