SHORT AND LONG GAMMA SUBUNIT SPLICE VARIANTS OF HUMAN GABA$_A$ RECEPTORS SHOW DIFFERENCES WHEN SUBJECTED TO ZINC

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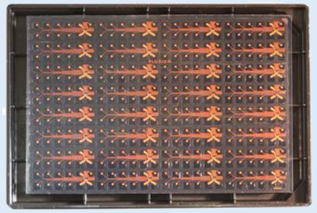
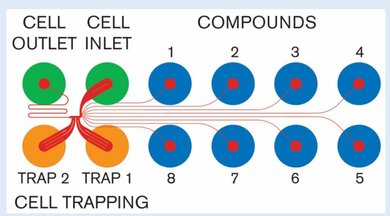
BACKGROUND

GABA$_A$ receptors, which are targeted by sleep aids, anti-anxiety medications, anesthetics, alcohol and neuro-steroids, regulate rapid inhibition in the mammalian brain. These receptors are made up of α, β and γ subunits and the γ subunit has two splice variants: S and L. These variants differ by 8 amino acids when integrated into the pentameric receptors. They have different patterns of distribution during development and in the adult brain, with L being more dominant in later stages of life. Although recorded ionic currents from these subunits tend to show little characterizing differences, this is not the case when zinc is present. A microfluidics-based automated patch clamp assay was used to investigate the differences in ionic current between these two splice variants under different conditions. Data will be presented from splice variants of human α1β2γ2, α2β2γ2 and α3β2γ2 GABA$_A$ receptors.

MATERALS & METHODS

Stable HEK-293 Cell lines expressing the different GABA$_A$ receptors were cultured at 37°C and 5% CO2. Culture media composition is listed below. Cells marked for experiments were kept in culture until they reached 65-75% confluence. Flasks were first washed with 10 ml of Ca^{2+} & Mg^{2+} PBS, followed by addition of Accutase® solution (Innovative Cell Technologies). After a brief incubation at 37°C for 3-5 minutes, the cell suspension was then spun down and re-suspended in 293 SFM II for at least 45 minutes (with gentle stirring). Plates were water rinsed and filled with intracellular solutions and compound doses. DMSO was added to solutions not containing drugs to achieve a concentration of less than or equal to 0.2%. Right before the start of the experiment, cells were re-suspended in extracellular solution at a final concentration of 6 million/ml and placed in the plate cell inlet wells. Cells were continuously held at -60 mV. GABA was added for 3 seconds with a wash period of 20 seconds.
All recordings were made with ensemble plates (20 cells) in the IonFlux system with whole cell patch clamp configuration. IonFlux plates, based on standard configurations of 96 or 384 wells can be preloaded with cells, compounds, and reagents using conventional liquid handlers or pipettors. Each pattern in an IonFlux plate has 8 compound wells, 2 trap recordings filled with intracellular fluid, 1 inlet for cells and extracellular solution, and 1 outlet for waste (below). Cells are patched in whole cell configuration using ensemble plate format.





Culture Media:
DMEM High Glucose w/pyruvate, 10% FBS, 1% Penicillin/Streptomycin, 10 µg/ml Blasticidin, 130 µg/ml Hygromycin, 2 µg/ml Puromycin
Intracellular solution (mM):
KF 70, KCl 60, NaCl 15, HEPES 5, EGTA 5, MgATP 4, (pH 7.3)
Extracellular solution (mM):
NaCl 137, KCl 4, $MgCl^2$ 1, $CaCl^2$ 1.8, HEPES 10, Glucose 10 (pH 7.45)
GABA dose response (µM):
0.3, 1, 3, 10, 30, 100, 300
Diazepam dose response (µM):
0.01, 0.03 ,0.1 ,0.3 ,1 , 3
Rc-Ome dose response (µM):
0.1, 0.3, 1, 3, 10, 30
Picrotoxin dose response (µM):
0.3, 1, 3, 10, 30, 100

Cell Lines	
α1β2γ2S	909-0068
α1β2γ2L	909-0069
α2β2γ2S	909-0070
α2β2γ2L	909-0071
α3β2γ2S	909-0072
α3β2γ2L	909-0073

CELL Microsystems

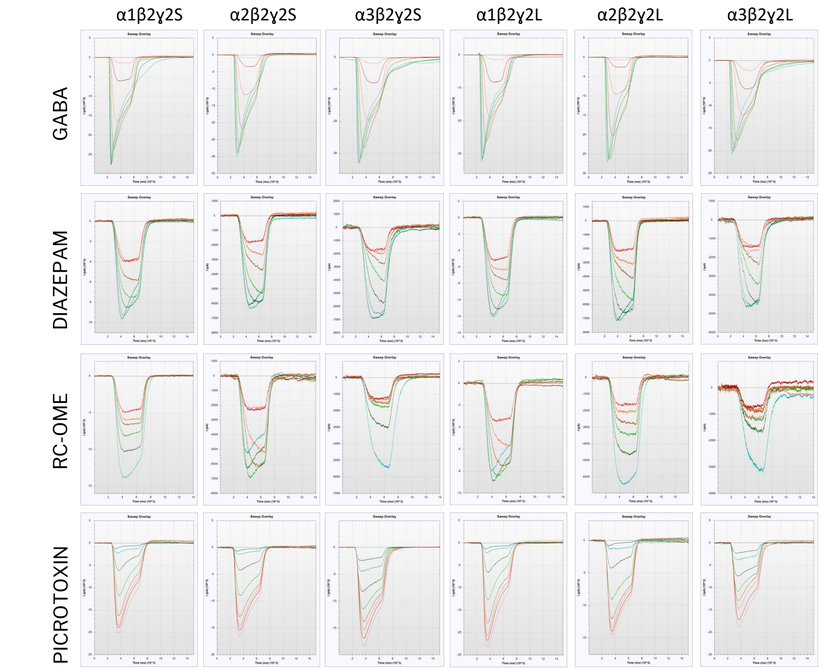
Validated 6 commercially available GABA $_A$ Receptors cell lines including L and S variants

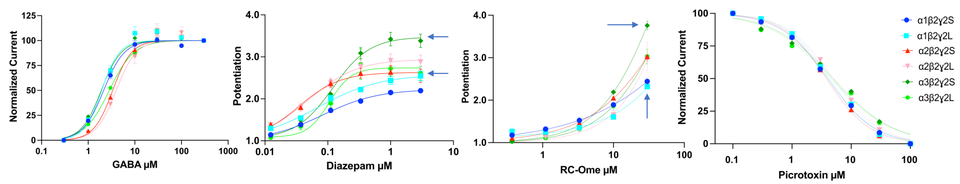
Demonstrated subtle selective modulation between L and S splices

Investigated off-target drug effects on GABA$_A$ Receptors

LEARN MORE

Response to GABA, Diazepam, RC-Ome and Picrotoxin

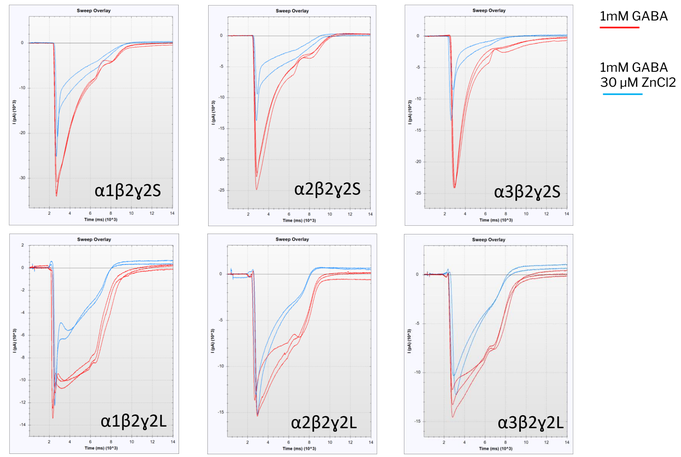


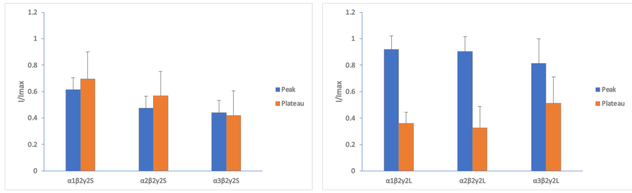


Representative superimposed sweeps from every cell line showing the GABA-induced current. The first row represent the effect of increasing doses of GABA. Increasing doses of Diazepam and Rc-Ome were sequentially co-applied with 1 µM GABA. Increasing doses of Picrotoxin were sequentially co-applied with 10 µM GABA. Dose response curves for every cell line (GABA and Picrotoxin n=8, Diazepam and Rc-Ome n=6). Error bars represent SEM. Arrows show the difference between S and L in potentiation.

µM	α1β2γ2S	α1β2γ2L	α2β2γ2S	α2β2γ2L	α3β2γ2S	α3β2γ2L
GABA	2.1	1.9	3.4	3.9	1.9	2.8
Diazepam	0.082	0.070	0.038	0.050	0.12	0.11
Picrotoxin	4.0	4.3	3.8	4.9	4.9	4.8

Effect of $ZnCl^2$ with 1mM GABA





Zn^{2+} effect was measured after an incubation of 30 seconds with 30 µM $ZnCl^2$ followed by 1 mM GABA + 30 µM $ZnCl^2$. Representative superimposed serial current sweeps showing alternating effect of GABA and GABA+$ZnCl^2$. Red traces are GABA alone (Before and after wash of $ZnCl^2$), blue traces are GABA+$ZnCl^2$. Below: Peak and plateau maximal current under zinc baseline adjusted to current without Zn2+.

Summary and References

With IonFlux technology, we were able to validate 6 different cell lines expressing α(1/2/3)β2γ2(L/S) GABA$_A$ receptors. We observed a major difference between α3β2γ2S α3β2γ2L with regard to diazepam and Rc-Ome potentiation (S showed higher response). We could not observe any significant differences between the L and S subunits when it came to GABA and picrotoxin responses. Due to solubility issues, we could not extrapolate the EC_{50} values for Rc-Ome. Zinc exhibited distinctive inhibition characteristics. S subtypes showed around 50% peak and plateau inhibition with zinc while L subtypes showed very little peak inhibition but with a significant plateau decrease. These results demonstrated selective activity in GABA$_A$ modulation between L and S variants offering potential application to drug development and off-target drug effects studies.

*Jiang, R. et al. Brit J Pharmacol **162**, 1326–1339 (2011).*
*Hosie, A. M., Dunne, E. L., Harvey, R. J. & Smart, T. G. Nat Neurosci **6**, 362–369 (2003).*