

Comparing Antagonistic Effects of Ginkgolide B and Picrotoxin on $\alpha 3$ Glycine Receptors

Sampurna Chakrabarti

University at Buffalo Department of Biological Sciences, University at Buffalo Department of Physiology and Biophysics

Mentor: Malcolm M. Slaughter

Introduction

GABA and glycine are the major fast inhibitory receptors in the CNS, the latter is less studied. The glycine receptors (GlyR) are ionotropic and conduct chloride ions into and out of the cell. These cys loop receptors are pentameric- made of two alpha and three beta subunits [1,6]. There is only one beta, but four alpha subtypes. Amongst the four isoforms of alpha subunits $\alpha 3$ and $\alpha 4$ are poorly understood. The major challenge is to functionally and pharmacologically differentiate between the subunits. In this study, we characterize the effects of glycine antagonists Ginkgolide B (GB) and Picrotoxin (PTX) on transfected HEK 293 cells expressing $\alpha 3$ GlyRs. Both GB and PTX act as antagonists to $\alpha 1$, $\alpha 2$ GlyRs and GABA receptors [2,3,4,5,7]. However, their role on $\alpha 3$ GlyR is not well understood.

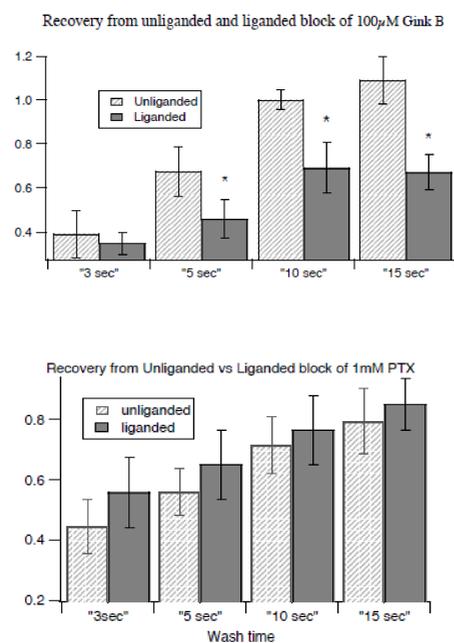
Different locations of the binding sites of antagonists can help in differentiating between the GlyR alpha subunits. Previous studies have shown that both GB and PTX can block homomeric $\alpha 1$ and $\alpha 2$ receptors only when the channel is open (liganded state) suggesting a pore blocking mechanism [3,7]. In this study, we analyze the blocking mechanism and recovery of $\alpha 3$ GlyR from GB and PTX application in both liganded and unliganded state.

Methods

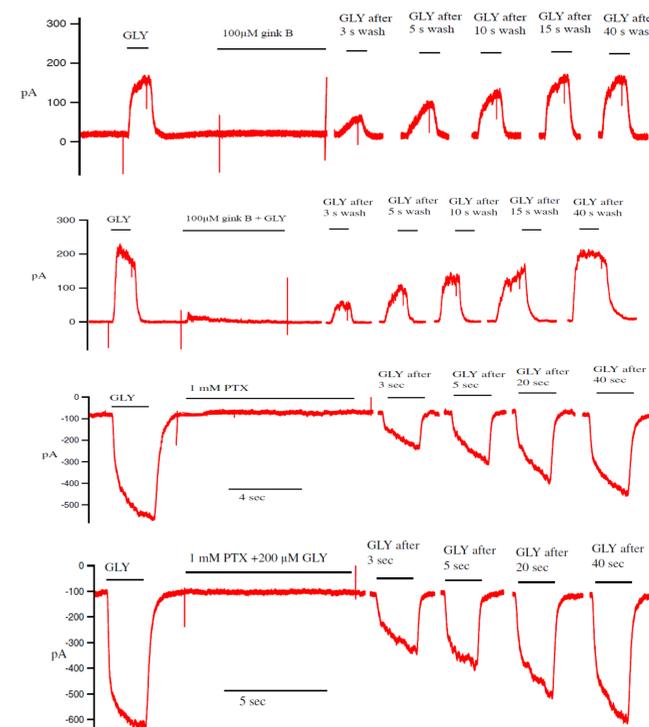
- Expressed alpha 3 receptors in HEK 293 cells by transfection
- Identified transfected cells by GFP using Fluorescence Microscopy
- Recorded via whole cell patch clamping



Results



- GB and PTX block $\alpha 3$ GlyRs in both the liganded and unliganded state
- Recovery from unliganded GB block is faster than liganded GB block ($p < 0.01$, $n = 5$)
- There is no significant difference between recovery rates of liganded and unliganded PTX block ($p > 0.05$, $n = 8$)



Conclusions

- The $\alpha 3$ GlyR, unlike the $\alpha 1$ or $\alpha 2$ GlyR, can bind GB when the channel is closed (unliganded block).
- GB also binds in the pore when the channel is open. This is evidenced by the voltage dependence of block and the difference in recovery time for liganded vs unliganded block.
- For PTX there is no voltage dependence of block and no significant difference in the recovery rates of liganded and unliganded block, indicating that PTX does not block in the pore.

References

- Duricic, Nela, et al. "Stoichiometry of the Human Glycine Receptor Revealed by Direct Subunit Counting." *The Journal of Neuroscience* 32.37 (2012): 12915-20. Print.
- Hawthorne, Rebecca, et al. "Molecular Determinants of Ginkgolide Binding in the Glycine Receptor Pore." *Journal of Neurochemistry* 98.2 (2006): 395-407. Print.
- Heads, Judith A., et al. "Structure-Activity Analysis of Ginkgolide Binding in the Glycine Receptor Pore." *Journal of Neurochemistry* 105.4 (2008): 1418-27. Print.
- Hill, R. G., M. A. Simmonds, and D. W. Straughan. "Antagonism of Gaba by Picrotoxin in the Feline Cerebral Cortex." *British journal of pharmacology* 44.4 (1972): 807-09. Print.
- Huang, Shelley H., et al. "Ginkgolides, Diterpene Trilactones of Ginkgo Biloba, as Antagonists at Recombinant A1 β 2 γ 21 Gabaa Receptors." *European Journal of Pharmacology* 494.2-3 (2004): 131-38. Print.
- Meyer, Guido, et al. "Identification of a Gephyrin Binding Motif on the Glycine Receptor B Subunit." *Neuron* 15.3 (1995): 563-72. Print.
- Wang, Dian-Shi, et al. "Mechanisms for Picrotoxin Block of A2 Homomeric Glycine Receptors." *Journal of Biological Chemistry* 281.7 (2006): 3841-55. Print.