Comparing Antagonistic Effects of Ginkgolide B and Picrotoxin on α3 Glycine Receptors

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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 α3 and α4 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 α3 GlyRs. Both GB and PTX act as antagonists to α1, α2 GlyRs and GABA receptors [2,3,4,5,7]. However, their role on α3 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 α1 and α2 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 α3 GlyR from GB and PTX application in both liganded and unliganded state.

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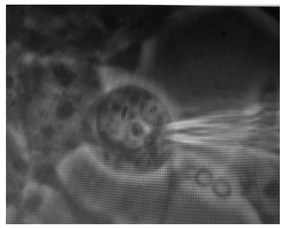
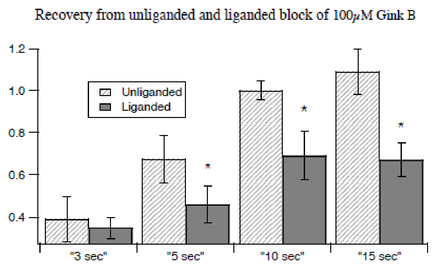
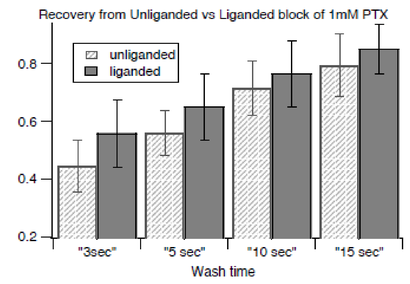
Methods

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

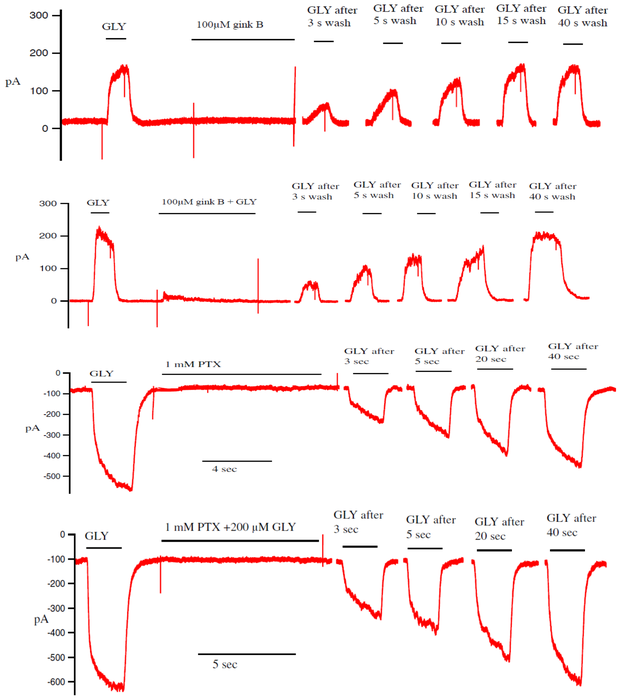






Results

- GB and PTX block α3 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 α3 GlyR, unlike the α1 or α2 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.

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