

## **Characterization of Ginkgolide B as an Antagonist of Alpha 3 Glycine Receptors**

Glycine receptors (GlyRs), a member of the “cys loop” receptor family, are one of the two major inhibitory receptors of the Central Nervous System. Each glycine receptor is composed of 2 alpha and 3 beta subunits. There are four different kinds of alpha subunits; the distinctions between which are not fully understood. The goal of the present study is to characterize the antagonistic activity of Ginkgolide B (GB) on alpha 3 glycine receptors and compare it to alpha 2 GlyRs. To this end, homomeric alpha 3 glycine receptors were expressed in HEK 293 cells and their chloride currents were recorded by means of whole cell patch clamping. It was seen that 100 $\mu$ M GB blocks both liganded and unliganded states of alpha 3 GlyRs which suggest the presence of more than one binding site. This property might be unique to alpha 3 GlyRs since previous studies indicate the unliganded state of alpha 1 and alpha 2 GlyRs are not blocked by GB. The GB block is voltage-dependent and is more effective when the cell is depolarized. Glycine receptors were found to recover faster from the unliganded (use-independent) block compared to the liganded (use-dependent) block at both depolarized and hyperpolarized voltages. There is very high amino acid homology in the structures of the alpha subunits, suggesting that the second blocking site might be due to only one to two amino acids in the extracellular domain of the receptor. In future, site directed mutagenesis will be used to test this hypothesis. Other antagonists will also be tested on the alpha 3 glycine receptor to decipher its physiology.

*Keywords: Glycine Receptors, alpha 3 subunit, Ginkgolide B, antagonist*