Pharmacological characterization of individual subtypes of α4β3δ $GABA_A$ receptor, include the novel description of β3δ $GABA_A$ receptor subtype

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γ-Aminobutric acid (GABA) is the main inhibitory neurotransmitters in the brain. It mediates its effect in part through activation of γ-aminobutyric acid type A receptors ($GABA_AR$), pentameric receptors that open a chloride-selective channel upon binding of GABA. Individual $GABA_ARs$ subunits are encoded by a number of individual genes, including α (1-6), β (1-4), γ, δ, π, ε and ρ. These subunits form a limited number of subtypes that differ in their pharmacology, sensitivity to GABA, distribution in the brain and physiological roles. Our focus is on the α4β3δ $GABA_AR$, which is located extrasynaptically and thought to contribute to tonic inhibition, activated by low concentrations of either ambient GABA, or spillover from synaptic transmission.

The two-electrode voltage clamp technique has long been used to study the function of $GABA_ARs$ and as a convenient method to identify putative pharmaceuticals. When expressing multimeric receptors *in vitro*, the analysis of electrophysiological recordings can be complicated by the presence of heterogeneous receptor populations with different pharmacological properties. In this study, we injected different combinations of α4, β3 and δ cRNA into *Xenopus laevis* oocytes and performed concentration-response curves to GABA through the two-electrode voltage clamp technique, with the cells held at −60mV. We also characterized the effects of several known pharmacological agents of extrasynaptic $GABA_ARs$, and characterized putative binding-site mutations previously identified.

We report that there are four possible receptor subtypes that we can identify to be expressed in *Xenopus* oocytes when α4, β3 and δ cRNA are injected: β3 homomeric; α4β3; β3δ and α4β3δ. The β3-homomeric receptor displays constitutive activity that is inhibited by zinc but exhibits no significant response to GABA (up to 1mM). GABA elicited chloride-currents at oocytes injected with α4 and β3, or β3 and δ alone, with an EC_{50} of 25μM and 26μM for the resultant α4β3 and β3δ subtypes respectively. Despite exhibiting similar pharmacological responses to GABA, the agent DS2 is an agonist of β3δ, but not α4β3 receptors. The pharmacology of β3δ receptor was further characterized in this study, and we identified that THIP and DS2 activate β3δ receptors, whereas zinc and gabazine inhibit GABA-elicited currents. When attempting to express the α4β3δ $GABA_AR$ *in vitro*, all these subtypes may contribute to the resultant GABA-elicited concentration response curve, complicating the analysis of the functional properties of α4β3δ. It has previously been demonstrated that GABA-binding site is located within the interface of α4 (complementary/−) and β3 (principle/+) subunits in the α4β3δ extrasynaptic $GABA_ARs$. However, GABA clearly activates β3δ receptors that lack an α4 subunit, and we hypothesized there is a novel GABA-binding site at the interface of β3 and δ subunits. Three critical residues, which were shown to be vital in α4β3δ receptor, were tested in β3δ receptors. $β3_{Y205C}$ shifts the GABA concentration-response curve by 1000-fold. $δ_{F72C}$ and $δ_{R218C}$ shifted only by 2-fold and 4-fold respectively indicating that $β3_{Y205C}$ may be directly involved in binding of GABA. In consideration of the β3-homomeric receptor and β3δ receptor above, δ subunit may be involved either in allosteric or gating mechanisms.

We have identified the potential for a mixed-population of receptor subtypes to be expressed in *Xenopus* oocytes injected with α4, β3 and δ. We manipulated various parameters of the expression system, including mRNA poly-adenylation and the relative subunit ratios to shift the resultant GABA-concentration response curve. The identification of a selective pharmacological agent for β3δ receptors would enable us to infer the relative subtype contributions to the resultant α4β3δ GABA-concentration response curve. This knowledge could be used to identify any role of β3δ receptors *in vivo*, enabling us to understand actions of pharmacological agents that act on α4β3δ receptors. This may lead to an increased understanding of the physiological role of these $GABA_AR$ subtypes.