Modulation of Cys-loop receptors to address CNS disorders

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The α4β2 nicotinic acetylcholine receptors are members of the Cys-loop family of ion channels. These receptor subtypes are widely expressed in the brain and are implicated in a wide variety of physiological processes. The α4β2 nicotinic acetylcholine receptors exist in two stoichiometries, (α4)2(β2)3 and (α4)3(β2)2, with different sensitivities to agonist, but their pharmacological profiles are not well understood. Methyllycaconitine is believed to be a competitive antagonist of nicotinic acetylcholine receptors. Using the two-electrode voltage clamp technique in the *Xenopus* oocyte expression system, we demonstrate that inhibition of α4β2 nicotinic acetylcholine receptors by methyllycaconitine is either surmountable or insurmountable depending on the different ratios of subunit mRNA injected. We propose that this is a result of the expression of different stoichiometries. Using an homology modelling approach, we identified D204 residue of the α4 subunit as interacting with the succinimide group of methyllycaconitine. After mutating this residue to a cysteine, we measured the rate of trapping of a methyllycaconitine analogue containing the reactive maleimide group. We demonstrate that this reaction results in reduced ACh-elicited currents in the (α4)3(β2)2 stoichiometry but not the (α4)2(β2)3 stoichiometry, indicating that MLA binds to the α4-α4 interface of the (α4)3(β2)2. Consistent with other studies, we propose that the α4-α4 interface is a structural target for potential therapeutics that modulate (α4)3(β2)2 nAChRs.