Are the putative N-terminal α-helix and preceding residues important in $GABA_{A/C}$ receptor function?

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The $GABA_{A/C}$ receptors are members of the pentameric Cys-loop superfamily of ligand-gated ion channels (pLGICs) and mediate inhibitory fast synaptic transmission in the nervous system. pLGICs comprise an N-terminal extracellular agonist-binding domain followed by a channel domain and intracellular domain. Available structural information shows the agonist binding domain comprises a β sandwich of ten β-strands, which form the agonist binding pocket, preceded by an N-terminal α-helix in eukaryotic structures, not present in prokaryotic structures. Sequence analysis of $GABA_{A/C}$ receptors predicts an α-helix in a similar position. This putative α-helix in $GABA_{A/C}$ receptors is, however, preceded by 10 to 46 additional residues not present in other pLGICs, which we term the N-terminal extension. The N-terminal α-helix has been shown to be functional essential in nicotinic acetylcholine receptors (Bar-Lev *et al.*, 2011; Castillo *et al.*, 2009). The role of the α-helix and N-terminal extension in $GABA_{A/C}$ receptors has not been tested and is the subject of this study. We found in both homomeric ρ1 $GABA_C$ and heteromeric α1β2γ2 $GABA_A$ receptors that the putative α-helix is essential for receptor function but not the N-terminal extension. Partial or complete removal of the N-terminal extension in ρ1 $GABA_C$ receptors did, however, affect cell surface expression and agonist sensitivity. Mutation of a putative furin protease cleavage motif within the N-terminal extension of ρ1 $GABA_C$ receptors likewise impaired cell surface expression. Our results support the essential role of the N-terminal α-helix in eukaryotic pLGICs and provide evidence that the N-terminal extension has a subtle modulatory role on receptor function.

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Bar-Lev DD, Degani-Katzav N, Perelman A, Paas Y. (2011) Molecular dissection of a Cl^--selective Cys-loop receptor points to components that are dispensable or essential for channel activity. *Journal of Biological Chemistry* **286:** 43830-41.