The Use of Concatemer Subunit Constructs to Study the Pharmacology of α4- and α6-Containing Neuronal Nicotinic Acetylcholine Receptor

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Purpose:

To define the molecular interactions between the positive allosteric modulator, CMPI and neuronal nicotinic acetylcholine receptors (nAChRs).

Methods:

We introduce specific amino acid mutations in plasmids encoding linked nAChR subunits (β2-α4-β2-α4-β2 and β2-α4-β2-α4-α4). Then we use these plasmids to prepare cRNA *in vitro* and inject into *Xenopus laevis* oocytes to express nAChR containing these mutations. Then we use two-electrode voltage-clamp electrophysiological recording *from Xenopus laevis* oocytes to measure effect of these mutations on CMPI potentiation.

Results:

We have identified several amino acids within the α4 subunit that are required for CMPI potentiation of (α4)3(β2)2 nAChR. We introduced these amino acid mutations in the last α4 of the pentameric construct (β2- α4- β2- α4- ***α4****) and currently testing the effect of these mutation on CMPI potentiation. Additionally, we individually replaced the α4 subunits within β2-α4-β2-α4-β2 and β2-α4-β2-α4-α4 pentameric constructs with a transcript for the human Alpha 6 subunits and created the following [(β2- **α6**- β2- α4- α4; β2- α4- β2- **α6**- α4; β2- **α6**- β2- α4- **α6**; β2- **α6**- β2- α4- β2; β2- α4- β2- **α6-**β2).study the effect of CMPI and other nAChR PAMs on α6-containing nAChR

Conclusions:

The α4:α4 subunit extracellular is a likely binding site for CMPI. Identifying binding site for CMPI and defining the structural feature of CMPI that are required for binding at the α4:α4 subunit extracellular interface will facilitate the structure-based design of novel nAChR PAMs. PAMs such as CMPI, represent a novel mechanism to pharmacological target (α4)3(β2)2 and α6(α4)2(β2)2 nAChRs and holds promise in the development of drug strategies to alleviate chronic pain, treat nicotine dependence, and slow cognitive decline associated with Alzheimer's disease.