Concatenated subunits reveal stoichiometry and subunit arrangement of five distinct α4β2 neuronal nicotinic acetylcholine receptors

Smulders CJGM, Van Kleef RGDM, Enter D, Raaben W, Den Dulk H*, Brouwer J*, *Vijverberg HPM*
Inst for Risk Assessment Sciences, Utrecht University, Utrecht, *Dept of Molecular Genetics, Leiden Inst of Chemistry, Leiden University, Leiden

By concatenation of cDNAs coding for rat α4 and β2 nAChR subunits tandem subunits have been prepared in which all possible pairs of the two subunits (αβ, βα, αα, and ββ) are coupled by a synthetic linker of glutamine residues. After injection of cDNAs coding for tandem subunits together with cDNAs coding for the α4 or β2 subunits into the nucleus of *Xenopus laevis* oocytes functional heteromeric nAChRs were expressed, which were investigated by two-microelectrode voltage clamp. The tandem constructs by themselves did not produce significant numbers of functional nAChRs. The αβ, βα, and ββ tandems combine with α4 to form nAChRs with low sensitivity to ACh. The βα tandem combines with β2 to produce nAChRs with a high sensitivity to ACh. The combination of αβ with β2 was little effective. The αα tandem with β2 produced a heterogeneous population of nAChRs with high and low sensitivity to ACh. The nAChRs with high sensitivity to ACh contain at least three β subunits, arranged as αβαββ or ααβββ. Inhibition of ACh-induced ion current by d-tubocurarine (d-TC) revealed additional populations of nAChRs with high and low sensitivity to d-TC. The nAChRs with high sensitivity to d-TC appear to contain at least three α subunits, arranged as αβαβα, αααββ, or αααβ. The effects of ACh and d-TC demonstrate the functionality of five types of α4β2 nAChR with different stoichiometries and arrangements of the α4 and β2 subunits. Further research with concatenated subunits may reveal the pharmacological and toxicological profiles of the distinct subunit assemblies of ligand-gated ion channels and may be essential for the understanding of the effects of existing and for the development of new therapeutic agents.

Henk PM Vijverberg, IRAS , Utrecht University, PO Box 80176, 3508 TD Utrecht, t 030 2535397, e-mail h.vijverberg@iras.uu.nl

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