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**Title:** Controlling the subunit order in the rat nicotinic acetylcholine receptor (nAChR) complex  
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**Authors:** \***R. P. YASUDA**, N. SAHIBZADA, K. J. KELLAR, B. B. WOLFE;  
Dept Pharmacol, Georgetown Univ., Washington, DC

nAChRs are generated when specific alpha and beta subunits assemble to form a pentameric receptor complex. Mammalian nervous system nAChRs may contain 3 or more different subunits (e.g., J.Neurosci. 22:8785, 2002; Mol.Pharmacol. 66:85, 2004, Mol.Pharmacol. 68:1656, 2005; J.Neurosci. 25:9258, 2005; Mol.Pharmacol. 70:1693, 2006). Using individual cDNAs, it is difficult, if not impossible, to generate a receptor of a specific subunit combination and order when two or more different subunits are present. We have utilized an approach demonstrated previously (Mol.Pharmacol. 69:558, 2006) to generate concatamerized rat nAChR subunits. The nAChR subunits are covalently attached to each other sequentially, which allows assembly of subunits in any desired stoichiometry and order. A plasmid, based on pcDNA3.1 was generated with 5 unique 8-cutter restriction sites that allow the insertion of 5 cassettes each encoding specific subunits of the nAChR and each covalently tethered to the next with a polyglutamine linker. The initial concatamer, beta4beta4alpha3beta4alpha3 appears to function identically to both native and recombinant alpha3beta4 nAChRs. Pharmacological experiments utilizing receptor binding and whole-cell patch-clamp recordings were done. Whole-cell patch-clamp recordings show that DHbetaE is a relatively weak inhibitor ( $IC_{50} > 40$  microM) of agonist-stimulated responses in the alpha3beta4 concatamer. This potency is similar to that observed at the native alpha3beta4 receptor in the pineal and the heteromeric receptor in cells transfected with alpha3 and beta4 subunit cDNA. In contrast, DHbetaE is potent at blocking responses in alpha3beta2 cells ( $IC_{50} \ll 40$  microM). Cytisine is an agonist at the concatameric alpha3beta4 receptor, as it is in cells transfected with individual alpha3 and beta4 subunits, whereas it is an antagonist in alpha3beta2 cells. Characterization of [3H]epibatidine binding in cells transfected with the concatamer demonstrate that A-85380 inhibits [3H]epibatidine binding with a  $K_i$  nearly identical to that in mammalian cells transfected with alpha3 and beta4 subunit cDNA but with considerably lower potency compared to cells transfected with alpha4 and beta2 subunit cDNA. Interestingly, the density of concatamer binding sites is also up-regulated about 6-fold following incubation for 40 h with 100 microM nicotine, suggesting receptor stabilization by nicotine. Future experiments will explore the outcome of generating receptors with one binding site being alpha3beta4 and one binding site being alpha3beta2, a receptor subtype that has been found in the cerebellum and superior cervical ganglion.

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