



Pharmacology and function of rat $\alpha3\beta2\beta4$ nicotinic acetylcholine receptor (nAChR) concatamers

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Abstract:

$\alpha3\beta2\beta4$ nAChRs are pentameric receptor complexes in which the subunits can be arranged in 8 different rational orders. To understand the pharmacology and function of each of these unique complexes, a strategy of expressing a fusion protein (i.e., concatamer) composed of the five receptor subunits covalently fixed in a known order and stoichiometry was employed. The mammalian expression plasmid, pcDNA3.1, was modified by the addition of 5 unique restriction sites in the polyclonal region. These restriction sites are not present in rat $\alpha3$, $\beta2$, or $\beta4$ cDNAs. This approach is similar to that demonstrated by Groot-Kormelink et al. (Mol.Pharmacol. 69:558-63, 2006) for a cDNA that expresses an $\alpha3\beta4$ concatamer (CAT). CAT cDNAs were expressed in mammalian cells and both [³H]epibatidine receptor binding and whole-cell patch recording were performed. Inhibition of [³H]epibatidine receptor binding by sazetidine-A (highly selective for nAChRs containing $\beta2$ subunits) was done to examine the contribution of both $\alpha3\beta2$ and $\alpha3\beta4$ agonist binding sites present in the CATs. Whole-cell patch recording was done along with γ -tube application of the non-selective agonist acetylcholine (ACh), as well as cytosine, which is a full agonist at $\alpha3\beta4$ but a very weak partial-agonist at $\alpha3\beta2$ sites. ACh-stimulated whole-cell currents were examined before and during the bath application of dihydro- β -erythroidine at 40 μ M, which almost completely blocks $\alpha3\beta2$ nAChRs but not $\alpha3\beta4$ nAChRs. Rat $\alpha3\beta2$ WT, $\alpha3\beta4$ WT, $\alpha3\beta2$ CAT and $\alpha3\beta4$ CAT were used as controls for the $\alpha3\beta2\beta4$ CATs. These experiments are designed to elucidate the differences and the similarities among different nAChR subunit orders and stoichiometries, and to determine the contributions of the two ACh binding sites ($\alpha3\beta2$ and $\alpha3\beta4$), as well as the accessory subunit (i.e., non-agonist binding subunit) towards agonist-activated whole-cell currents.

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