A heterogeneous stoichiometry of α9α10 nicotinic acetylcholine receptors is detected by the selective conotoxin Vc1.1

N.L. Absalom,[1] G. Liang,[1] E. Pera,[1] C. Chu,[1] H.-L. Kim,[1] J.M. McIntosh[2,3] and M. Chebib,[1] [1]Faculty of Pharmacy, University of Sydney, NSW 2006, Australia, [2]Department of Psychiatry, University of Utah, Salt Lake City, Utah 84112, USA and [3]Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA.

Nicotinic acetylcholine (nACh) receptors are ligand-gated ion channels involved in fast synaptic transmission. nAChRs are pentameric complexes formed from combination of alpha and beta subunits, with the α9α10 heteromeric complex found in inner hair cells, dorsal root ganglion neurons and lymphocytes. The α9α10 receptor has previously been reported to form a stoichiometry of $(\alpha9)_2(\alpha10)_3$. The conotoxins Vc1.1 and RgIA are potent and selective inhibitors of acetylcholine-evoked currents in α9α10 receptors. We have investigated the stoichiometry of α9α10 receptors by conotoxin inhibition of ACh-evoked currents recombinantly expressed in Xenopus oocytes. We show that Vc1.1 inhibit ACh-evoked currents in a biphasic inhibition curve. We show that the characteristics of this curve can be altered by varying the ratio of α9 and α10 RNA injected into the oocytes from 1:1 to 10:1 α9:α10 ($n \geq 3$ for each ratio). Furthermore, the biphasic nature of the curve is almost completely removed by "flooding" the injection ratio with α10 subunits at a ratio of 1:3 α9:α10. We interpret these results as demonstrating that the conotoxin Vc1.1 does not inhibit ACh-evoked currents when binding at the α9-α10 and α9-α9 interfaces in an equivalent manner and that the biphasic nature of the curve is a result of a mixed population of the receptors, in contrast to inferred stoichiometry using agonist-evoked concentration-response curves. We conclude that the receptor can form in either the $(\alpha9)_2(\alpha10)_3$ or the $(\alpha9)_3(\alpha10)_2$ stoichiometry *in vitro*.