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1.5

Cloning and functional expression of the nicotinic acetylcholine receptor chaperone RIC-3 from *Xenopus laevis*

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Xenopus laevis oocytes provide a heterologous expression system convenient for the functional analysis of recombinant ligand-gated ion channels such as nicotinic acetylcholine receptors (nAChRs) [1]. RIC-3, first identified in *Caenorhabditis elegans*, is a chaperone protein which enhances the expression of certain nAChR subtypes. A recent study examining *Drosophila* and human cell lines showed host-cell specific effects of RIC-3 [2]. To facilitate the study of nAChR function in Xenopus oocytes, we cloned the full length *Xenopus laevis ric-3* (*Xric-3*). As with the human *ric-3*, *Xric-3* possesses two membrane spanning regions and a coiled coil domain. We found that *Xric-3* increased the current amplitude of the human α7 nAChRs and its *C. elegans* homolog, ACR-16, when co-expressed with receptor RNA in Xenopus oocytes without affecting agonist potency. Our findings suggest that the host cell specificity of RIC-3 enhancement of α7-like nAChRs, although a feature of differentiated cells is less apparent in oocytes.

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1.6

Functional expression of an α5β2 nicotinic acetylcholine receptor

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The α5 subunit belongs to the family of nicotinic acetylcholine receptors (nAChRs), which are ligand-gated, cation selective channels. The α5 is predominantly expressed in limbic and autonomic regions [1–3]. Although α5 subunits are apparently associated with β2 or β4 subunits in neurons [1], no functional expression has been reported to date. It has, therefore, been suggested that these subunits merely act as accessory subunits [4]. Here. we report for the first time expression of functional nAChRs with only α5 combined with β2 or β4 subunits. Functionality of the human α5 was observed only when the cDNA encoding for this subunit contained its adjacent untranslated region (UTR). When expressed in Xenopus oocytes α5 containing receptors (α5β2 or α5β4) displayed robust currents in response to ACh and were typically in the μA range. The α5β2 nAChR displayed a high sensitivity to ACh with an EC_{50} of 1.63 ± 0.15 μM which is about 10-fold more sensitive than the major brain α4β2 receptors. These receptors also displayed a high sensitivity to nicotine with an EC_{50} of 0.58 ± 0.17 μM. Altogether these data demonstrate that functional receptors can be obtained with a binary combination of α5 with a β subunit and that these receptors may play an important physiological role both in the central and peripheral nervous system. The high degree of correlation between smoking and cancer in human with α5 and its non-synonymous single nucleotide polymorphism further calls for a better understanding of the functional role of this subunit. These data open new avenues for the screening of the physiological properties of α5 containing nAChRs.

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1.7

Gain of function mutants in human or mouse nAChR β3 subunits interchangeably activate either human or mouse α6β4*-nAChR, but not human or mouse α6β2*-nAChR

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It has been difficult to demonstrate function of heterologously expressed nAChR containing α6 and β3 subunits. Recently Dash et al. [1] confirmed that wild type, human nAChR β3 subunits have a dominant-negative effect on the function of α6β4*-nAChR subtypes but also found that β3 subunit gain of function mutant(s) potentiate function of human α6β4*-nAChR. Function of human α6β2*-nAChR was absent in the presence of either wild type or gain of function β3 subunits. This is puzzling, because there is function of α6β3*-nAChR containing wild type β3 subunits in rodents. In order to better understand α6β3*-nAChR, we interchangeably expressed mouse or human wild type or gain of function β3 subunits with human or mouse α6 and β2 or β4 subunits and determined functional features of expressed receptors. Gain of function mutants (M2 second transmembrane domain 9' or 13' positions) in either human or mouse β3 subunits potentiate function of either human or mouse α6β4*-nAChR but not of human or mouse α6β2*-nAChR. However, there was no function in oocytes expressing human α6 and either β2 or β4 subunits along with wild type, mouse β3 subunits. Similarly, wild type, human β3 subunits failed to produce functional receptors when