38 and UNC-29 co-localise on the muscle cell membrane. We have co-expressed *Ascaris* UNC-38 and UNC-29 in *Xenopus* oocytes to form functional ACh-, nicotine and levamisole-gated ion channels, the first successful heterologous expression of a parasite nAChR. Changing the subunit stoichiometry produces receptor populations with different pharmacological properties: injecting different RNA ratios to drive expression towards a 3:2 UNC-38:UNC-29 stoichiometry produces a receptor population more sensitive to nicotine and oxantel, whereas driving expression to favour a 2:3 UNC-38:UNC-29 stoichiometry produces receptors more sensitive to levamisole and pyrantel. The pharmacology of these receptors resembles the L- and N-subtypes previously observed in native *A. suum* muscle cell membranes. In addition, we describe a novel nAChR subunit gene, *acr-26*, that is conserved in several evolutionary distinct parasitic species but, to date, not in any free-living or plant parasitic species. Immunofluorescence using an antibody against *A. suum* ACR-26 designed and tested in-house demonstrated expression in the head region of the nematode. Sequence data similarities with other nAChR subunits, such as the nematode ACR-16 and the vertebrate α7 and α9 subunits, combined with a computer modelling approach predicted that ACR-26 could form a homomeric receptor. We injected cRNA encoding ACR-26 into *Xenopus* oocytes and observed that a novel homomeric receptor was expressed in these cells, forming cation channels sensitive to both acetylcholine and nicotine.

doi:10.1016/j.bcp.2009.06.031

1.3

Pharmacological chaperoning of nicotinic receptors begins in the endoplasmic reticulum: Compartments and stoichiometries

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Pentameric α4β2 neuronal nicotinic acetylcholine receptors (nAChRs) assemble in two possible stoichiometries, $(\alpha4)_2(\beta2)_3$ or $(\alpha4)_3(\beta2)_2$. The proportion of the total receptor population represented by each stoichiometry in a cell is influenced by the local environment. *Se*lective *pha*rmacological *ch*aperoning of nicotinic *a*cetylcholine receptor (nAChR) *n*umber and *s*toichiometry (SePhaChARNS) is an important aspect of nicotine addiction and can explain the inadvertent therapeutic effects of smoking in Parkinson's disease. We employed fluorescent protein (FP)-tagged nicotinic acetylcholine receptor (nAChR) subunits to study the effects of nicotine and cytisine on: (1) intracellular receptor stoichiometry using pixel-by-pixel Förster Resonance Energy Transfer (FRET) and (2) trafficking of assembled nAChRs to the plasma membrane (PM) by total internal reflection fluorescence microscopy (TIRFM). Neuroblastoma (N2a) cells were transiently co-transfected with α4mCherry and β2GFP nAChR subunits. Nicotine (1 μM for 4 h) incubation increased the assembly of the $(\alpha4mCherry)_2(\beta2GFP)_3$ nAChR stoichiometry. Subcellular stoichiometry analysis revealed that nicotine induced preferential $(\alpha4mCherry)_2(\beta2GFP)_3$ receptor assembly in the endoplasmic reticulum (ER). TIRFM showed that nicotine exposure restricted localization of the newly assembled $(\alpha4GFP)_2(\beta2)_3$ receptors to the ER. Conversely, cytisine treatment (1 μM for 4 h) of α4mCherry and β2GFP transfected N2a cells resulted in preferential assembly of the $(\alpha4mCherry)_3(\beta2GFP)_2$ stoichiometry in the ER and an increase in surface trafficking of assembled nAChRs relative to non-treated controls. To study the influence of β2 on α4β2 nAChR trafficking, N2a cells were transiently co-transfected with either α4GFPβ2 or α4GFPβ4 and imaged 48 h post-transfection by TIRFM. mCherry with a lyn kinase membrane localization signal was included in transfections to visualize the PM. The α4GFPβ2 receptors trafficked to the PM in ~10 % of the cells while ~90% of cells displayed α4GFPβ4 at the PM. Together, these data reveal a rate-limiting role for β2 subunits in ligand-induced α4β2 nAChR trafficking and stoichiometry-based differences in subcellular receptor localization.

Acknowledgments: R.P. and R.S. contributed equally to this work. Grants: NS11756, DA17279, Michael J. Fox Foundation (RS), Philip Morris, Targacept. Fellowships: Ford and APA-DPN (RP). AHA post-doctoral (FJM).

doi:10.1016/j.bcp.2009.06.032

1.4

Pharmacological chaperoning of nicotinic receptors begins in the endoplasmic reticulum: High-resolution imaging

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Nicotine addiction is the world's leading preventable cause of mortality. Smokers also have a much lower incidence of Parkinson's disease. Previous experiments show that ligand interactions with α4- and β2-nicotinic receptor subunits are necessary and sufficient for nicotine addiction. A plausible cellular/molecular mechanism for some responses to nicotine exposure is *se*lective *pha*rmacological *ch*aperoning of *a*cetylcholine *r*eceptor *n*umber and *s*toichiometry (SePhaChARNS). To investigate SePhaChARNS in a neuronlike environment, we used single-molecule resolution fluorescence microscopy to monitor localization and trafficking of α4GFPβ2 and α4GFPβ4 receptors expressed in mouse neuroblastoma (N2a) cells. As in previous investigations on native neurons and heterologous expression systems, we find large pools of endoplasmic reticulum (ER) localized α4GFPβ2 receptors. Strikingly, cells expressing α4GFPβ4 display plasma-membrane (PM) localized receptors. Pharmacological chaperoning was investigated by incubating N2a cells expressing α4GFPβ2 receptors in nicotine or the partial agonist cytisine. Furthermore, we simultaneously imaged α4GFPβ2 receptors and a mCherry-tagged ER exit site (ERES) marker to monitor the ER exit of α4GFPβ2 receptors and the associated changes in ERES. Nicotine induced an increase of ER localized α4GFPβ2 receptors, and ERES activity did not change markedly. In contrast, cytisine treatment increased the number of ERES fusion events, which may be an ERES adaptive response to increased cargo load. Consistent with an increase in cargo load we observed increased α4GFPβ2 receptor PM localization. Data from cytisine and nicotine treatments directly indicate that pharmacological chaperoning is initiated in the ER. Nicotine and cytisine induce assembly of differential α4β2 nicotinic receptor stoichiometries (see accompanying abstract by Srinivasan et al.), which leads to differential receptor localization, trafficking and ERES response. Thus, high-resolution imaging of SePhaChARNS is providing data required to understand, and manipulate nicotinic receptors with drugs.