Identifying Organelle Specific Intracellular Changes in Nicotinic Acetylcholine Receptors in Response to Ligand Exposure Using Fluorescence Microscopy

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Overcoming addiction to nicotine is often the greatest hurdle in keeping tobacco users from quitting, often leading to an untimely death related to tobacco consumption. Nicotine is known to alter a diverse set of properties in some subtypes of pentameric ligand-gated ion channels known as nicotinic acetylcholine receptors (nAChRs), leading to an incomplete link between nAChRs and nicotine addiction. Nicotine increases trafficking and expression of the α4β2 subtype, as well as alters the stoichiometric combination of subunits. The two stoichiometries of α4β2, (α4)2(β2)3 or (α4)3(β2)2, differ in terms of agonist sensitivity, calcium permeability, and rates of desensitization, making it crucial to determine distributions of each version to identify a drug target. We have found that the primary metabolite of nicotine, cotinine, also alters α4β2 in a similar way, supporting the hypothesis that nicotine interacts with and modifies receptors intracellularly. To evaluate changes in assembly within the endoplasmic reticulum, we developed a novel method to separate endoplasmic reticulum and plasma membrane derived nanoscale vesicles that encapsulate a single nAChR. Once organelle specific nanovesicles are isolated, we use single molecule fluorescence techniques to evaluate differences in nAChR stoichiometry when exposed to a ligand such as nicotine or a smoking cessation agent. The alpha subunits in these nAChRs each contain a green fluorescent protein (GFP) that can be detected using fluorescence microscopy. The GFP within the alpha subunit stochastically undergoes photobleaching, losing the ability to fluoresce, after excitation. This is measured by a stepwise decrease in fluorescence over time, with each single step corresponding to one single GFP-labeled alpha subunit. The number of measured bleaching steps then corresponds to the number of alpha subunits present in the nAChR pentamer, and thus the stoichiometry. We detect an increase in the high sensitivity (α4)2(β2)3 version within the endoplasmic reticulum when nicotine is present. Detecting intracellular changes in stoichiometry suggests nicotine acts on nascent, unassembled subunits within the endoplasmic reticulum.