Beta2 Nicotine Receptor Subunits: Biomarkers for Dependence
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3R01DA036061-03S1

Abstract:

The requested supplement produces and exploits a set of three live-cell assays, suitable for regulating tobacco product additives such as (a) menthol in tobacco and (b) many flavorings in the liquids of electronic nicotine delivery systems (ENDSs). In more detail, existing data show that chronic exposure to menthol (> one day) changes the properties of nAChRs: subunit stoichiometry, trafficking from endoplasmic reticulum (ER) to plasma membrane (PM), and response to agonists.

Supplemental Aim 1: Selecting human neuroblastoma cell lines transfected with fluorescent human nicotinic acetylcholine receptors (nAChRs). The combinations to be included are α4β2, α6β2, α6β2β3, and α3β4. These represent the majority of the nicotine-sensitive nAChR types in the brain and periphery.

Supplemental Aim 2: Runs in parallel to Aim 1, beginning with the mouse material we have used till now. We will develop and standardize three modest-throughput assays for assessing the newly discovered effects of menthol on the properties of nAChRs. The three assays will be chosen from ten modalities already known to measure these effects of menthol (Henderson et al., 2016). These tedious, manual measurements are presently unsuitable for regulatory use. One group of measurements includes patch-clamp electrophysiological assays; these will be converted for use with a planar patch-clamp system. These measurements correlate acetylcholine-induced amplitudes, dose-response relations, and desensitization with nAChR numbers and compositions.

A second group comprises biophotonic measures for nicotine- or flavoring-induced changes in nAChR properties. These measurements include several modalities that measure the movements of nAChRs from the endoplasmic reticulum (ER) to the plasma membrane (PM), the subunit stoichiometry in ER and PM, and the number of ER exit sites. A fallback assay for the biophotonic group will exploit biosensor-based measurements for the influx of Ca^{2+} through nAChR channels.

Supplemental Aim 3: Uses the modest-throughout assays to generate proof-of-principle for regulatory purposes. We will confirm and extend a preliminary data set which suggests that chronic exposure to (-) menthol to or (+) menthol has distinguishable effects on the measured parameters. We extend the existing dataset to distinguish among the effects of the two menthol isomers, both of which are now added indiscriminately to tobacco cigarettes and to ENDSs.