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Title: Genetically Encoded Biosensors for Nicotinic Ligands

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Abstract: Nicotinic agonists play an important role in addiction, withdrawal, neuroprotection, cognitive behavior, and neural pharmacology. Previous work with nicotine has shown that, in addition to its canonical actions on the plasma membrane (PM) (such as receptor activation and desensitization), pharmacological chaperoning of receptors by nicotine begins intracellularly in organelles such as the endoplasmic reticulum (ER). Using our array of drug-specific biosensors, we have examined the pharmacokinetic properties of both nicotine and varenicline (Chantix) as these drugs cross into and out of the PM and ER. In this study, we report further findings for several additional candidate smoking cessation drugs – cytisine (Tabex) and dianicline – to compare with the aforementioned nicotinic agonists, as well as demonstrate the ability of the biosensor paradigm to potentially improve the pharmacokinetic profile of medicinal compounds. Our sensors utilize OpuBC, a monomeric bacterial periplasmic binding protein (PBP) which contains (a) a binding site for amines including a cation-π box, and (b) a ligand-induced “Venus flytrap” conformational change invoked by binding of target ligand. We inserted circularly permuted “superfolder” GFP (cpGFP), flanked by several-residue linkers, within inter-domain hinge regions and applied directed evolution, including X-ray crystallography, to optimize sensing for each drug of interest. Our iDrugSnFRs (“intensity-based Drug-Sensing Fluorescent Reporters”) can detect their drug partner with responses of $\Delta F/F0 > 1$ at the half-maximal effective concentration. Using targeting and retention sequences we direct the constructs to the ER or to the PM of clonal mammalian lines and cultured neurons. Live-cell video imaging shows that the kinetics of ER entry/exit differ among these nicotinic drugs by > 10-fold. These differences provide additional insights into aspects of nicotinic agonists, pharmacokinetics, organellar sequestration of drugs by acid trapping, protein trafficking and upregulation—all crucial facets in the expanded understanding of "inside-out" neuropharmacology of neural drugs.