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Effect of partial nicotinic agonists on real-time dopamine responses in rat nucleus accumbens: In vivo voltammetric study

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There is strong evidence that neuronal nicotinic receptors (NNRs) play a crucial role in the regulation of mesolimbic dopamine (DA) neurons but the exact receptor subtypes and mechanisms underlying their involvement are not understood. Elucidation of the role of receptor subtypes (e.g., α4-, α6- or α7-containing) in the ventral tegmental area (VTA) or nucleus accumbens (NAc) and their pharmacological properties (e.g., partial or full activation, inhibition, or desensitization) offers new insights for development of NNR-targeted therapies for smoking cessation and other conditions. Recent voltammetric studies on nicotinic activity at DA terminals in the striatum significantly contributed to the field but were limited by the properties of slice preparations or the use of nicotine ± antagonist approaches. The present work focused on the effects of nicotine and nicotinic partial agonists on accumbal DA release using fast-scan cyclic voltammetry *in vivo*. We used partial agonists (PAs) which in our previous studies exhibited medium efficacy and high potency (PA-A) or low efficacy and low potency (PA-B) relative to nicotine when measuring NNR-mediated [^{3}H]DA release from rat striatal synaptosomes. In the present study, nicotine (0.3 mg/kg, i.v.) induced marked DA efflux in the NAc of freely-moving and anesthetized rats. A 30-min pre-administration of PA-A (0.1 mg/kg and 0.3 mg/kg, i.p.) significantly diminished nicotine-evoked DA release in rat NAc. In addition, PA-A and PA-B (3 mg/kg, i.p.) and nicotine (0.3 mg/kg, i.v.) decreased the DA efflux elicited by electrical stimulation of the VTA (24 rectangular pulses, 60 Hz, 300 μA, 2 ms/phase) without affecting DA uptake. Importantly, both PA-A and PA-B attenuated nicotine-evoked DA responses in the NAc at doses that decreased nicotine self-administration in rats. Our study suggests the utility of *in vivo* voltammetry for translational studies of NNR-targeted drug candidates.

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An mGlu2/3 receptor agonist blocks increases in nucleus accumbens shell dopamine induced by self-administered, but not experimenter-administered, nicotine in rats

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Metabotropic glutamate (mGlu) 2/3 receptors that negatively regulate glutamatergic transmission are critically involved in the reinforcing effects of drugs of abuse, including nicotine. mGlu2/3 receptor agonist, LY379268 (1 mg/kg), has previously been shown to decrease nicotine self-administration and cue-induced nicotine-seeking behavior in rats. In addition, chronic nicotine self-administration resulted in downregulation of mGlu2/3 receptor function in mesocorticolimbic brain areas. We hypothesize here that LY379268 decreased the reinforcing effects of nicotine by attenuating nicotine-induced increase in NAcc dopamine. Using *in vivo* microdialysis, the present study examined the effect of systemic LY379268 (1 mg/kg, s.c.) pretreatment on nicotine-induced increase in NAcc shell dopamine and its metabolites (DOPAC, HVA and 5HIAA) in rats with a history of nicotine self-administration. Nicotine was administered either through an experimenter-administered subcutaneous injection (0.4 mg/kg, base) or through a single self-infusion of nicotine (0.06 mg/kg, base). Systemic LY379268 (1 mg/kg) pretreatment abolished NAcc shell dopamine increase after nicotine self-administration in nicotine-experienced rats. However, pretreatment with LY379268 (1 mg/kg) had no effect on the experimenter-administered nicotine-induced increase in dopamine in the NAcc shell in nicotine-experienced rats. Furthermore, LY379268 pretreatment did not influence the increase in dopamine metabolites after either self-administration of nicotine or experimenter-administered nicotine. These data indicate that the mGlu2/3 receptor agonist LY379268 plays an important role in blocking the combined effect of both nicotine and stimuli associated with nicotine self-administration. Further, based on these data we hypothesize that mGlu2/3 receptors play a more critical role in regulating the dopamine response to nicotine in the presence of stimuli associated with nicotine as compared to nicotine alone.

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3.8

$GABA_B$ receptor positive modulators: Effects on nicotine self-administration and cue-induced reinstatement of nicotine-seeking behavior in rats

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γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and is implicated in the modulation of central reward processes. Acute administration of γ-aminobutyric acid B ($GABA_B$) receptor agonists or positive modulators decreased self-administration of various drugs of abuse, such as nicotine, cocaine, ethanol and heroin, and inhibited cue-induced reinstatement of nicotine- and cocaine-seeking behavior. $GABA_B$ receptor positive modulators may be potentially improved therapeutic compounds for the treatment of drug dependence than $GABA_B$ receptor agonists due to fewer adverse side-effects. BHF177, a newly synthesized $GABA_B$ receptor positive modulator, decreased nicotine self-administration under a fixed-ratio 5 (FR5) and a progressive-ratio (PR) schedule of reinforcement in Wistar rats, while it did not affect food-maintained responding [1]. The present study investigated the effects of administration of another newly synthesized $GABA_B$ receptor positive modulator, BIK998, on nicotine- and food-maintained responding under a FR5 and a PR schedule of reinforcement. It also investigated the effects of BHF177 on cue-induced reinstatement of nicotine- and food-seeking behavior. Administration of BIK998 (0, 20, 40, 80 mg/kg, PO) did not affect nicotine self-administration, neither in the FR5 nor the PR schedule of reinforcement in either nicotine- or food-responding groups. The lack of effects seen with BIK998 may be attributed to sub-

optimal bioavailability. Pharmacokinetic data have indicated that BIK998 concentrations in the brain and plasma are approximately 50% in comparison to BHF177 concentrations after oral administration of the same dose. Administration of BHF177 (0, 2.5, 5, 10, 20, 40 mg/kg, PO), after a 10-day extinction phase, selectively and dose-dependently blocked cue-induced reinstatement of nicotine-, but not food-seeking behavior, reflecting a selective prevention of cue-induced reinstatement of nicotine-seeking behavior and not that of a natural reinforcer, such as food. These findings add to previously published data on the effects of BHF177 on nicotine self-administration and suggest that the $GABA_B$ receptor positive modulator BHF177, or other similar $GABA_B$ receptor positive modulators, could be useful therapeutics for the treatment of different aspects of nicotine dependence, by assisting both in smoking cessation by decreasing the reinforcing effects of nicotine (as shown previously), as well as in preventing relapse to smoking in humans, as suggested by the blockade of cue-induced reinstatement of nicotine-seeking in rats (present studies).

Reference

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3.9

The localization of neuronal nicotinic receptors (nAChRs) in the zebra finch brain tested under naïve, nicotine-on board and nicotine withdrawal conditions

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Nicotine improves cognitive function, but its adverse effects make it problematic as a treatment for diseases of cognitive dysfunction. The expression of the neuronal nicotinic acetylcholine receptors (nAChRs) alpha7 and alpha4beta2 is altered in diseases such as autism, depression, schizophrenia, Alzheimer's and Parkinson's disease. Agents that target these specific subtypes of nAChRs show great promise for cognitive enhancement. Over the years the precise mapping of subcellular and neuroanatomical localization of nAChRs, among which the alpha7 and alpha4beta2, is studied in a plethora of animal models, including humans. However, the expression of the nAChRs in the zebra finch brain has never been examined. This is a striking fact, as the zebra finch is a well-recognized animal model to study cognitive functioning. Therefore, we argue that the zebra finch can be used as an innovative test model in the search of neuroprotective ligands, which can potentially lead to the development of new therapies for (age-related) neurodegenerative diseases. Over the last 3 years our laboratory developed a behavioral model to test *in vivo* nicotine administration in zebra finches. We gained information on the pharmacokinetic and pharmacodynamics of nicotine in the zebra finch. As no information was available on the localization and expression levels of neuronal nAChRs, we performed an in situ hybridization using iodine-125 labeled epibatidine, in competition with iodine-125 labeled and unlabeled cytisine and alpha-conotoxin MII. In addition we labeled sections with iodine-125 alpha-bungarotoxin. Brain tissue from a naïve bird showed a pronounced alpha-bungarotoxin labeling in the cortex, hippocampal area, and the lateral forebrain bundle, pointing towards alpha7 sensitive sites. Labeling of the sections with cytisine showed the presence of alpha4beta2 sensitive sites in the cortex, hypothalamic area and some layers of the tectum opticum. Alpha-conotoxin MII showed the most pronounced labeling in the cortex, while in the striatum the labeling was less intense, pointing towards alpha6beta2 and potential alpha3beta2 sensitive sites. Currently, we are evaluating adult male zebra finch sections tested under the following conditions: nicotine-on board, nicotine-withdrawal (24 hr, 3 months and 16 months following the last nicotine administration). Based on this initial study, we provide evidence that the zebra finch can be used as an animal model in nicotine research with unlimited potential, not only in respect to cognition, but also in studies related to nicotine's addictive and dependence properties.

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3.10

Preclinical properties of the α4β2 nAChR partial agonists varenicline, cytisine and dianicline translate to clinical efficacy for nicotine dependence

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Varenicline, cytisine and dianicline are α4β2 nAChR partial agonists that have been in clinical smoking cessation trials [1–3], in which varenicline was found to have a significantly higher end-of-treatment odds ratio (3.7) than cytisine or dianicline (≤1.9). We investigated which preclinical pharmacodynamic and pharmacokinetic properties would have predictive validity for clinical efficacy by measuring binding affinities, functional efficacies, as well as activation and desensitization potencies at α4β2 and α7 nAChRs *in vitro*. In addition, rat plasma and brain pharmacokinetics were determined to estimate steady state human unbound brain concentrations at the recommended doses of the three agents, for a comparison of therapeutic brain concentrations with desensitization and activation potencies. With a brain to plasma ratio (B/P) ≥ 1 and very high affinity for α4β2 nAChRs (K_i = 0.4 nM), varenicline reaches sufficient free brain concentrations (30–130 nM) to significantly desensitize and slightly activate α4β2 nAChRs. At therapeutic levels, varenicline partially desensitizes but does not activate α7 nAChRs. By comparison, peak nicotine brain concentrations in smokers, estimated to be ~500 nM, will also desensitize and activate α4β2 nAChRs (K_i = 6 nM) but will have no activity at α7 nAChRs. In contrast, predicted human brain concentrations of dianicline (40–85 nM) and cytisine (2–10 nM) are orders of magnitude below the concentrations required for receptor desensitization and activation. In the case of dianicline, this is due to a combination of limited brain penetration (B/P = 0.3) and weak *in vitro* binding (K_i = 105 nM) and functional potencies. Cytisine has high binding affinity (K_i = 2 nM) and functional potencies, but human brain concentrations are insufficient because of minimal brain penetration (B/P = 0.1). These data suggest a plausible explanation for the lower clinical efficacy of cytisine and dianicline compared to varenicline. This translational study based on PK-PD data suggests that an α4β2 nAChR partial agonist will be most efficacious as a nicotine dependence treatment if the compound has (a) potent binding affinity to α4β2 nAChRs, (b) adequate brain entry for interaction with central α4β2 nAChRs, (c) high enough brain concentrations for both inactivation and at least minimal activation of α4β2 nAChRs, and (d)