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)