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)

sufficient affinity to block nicotine's reinforcing effect by preventing binding of nicotine at α4β2 nAChRs when smoking.

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3.11

Low efficacy partial agonists of the α4β2 nicotinic acetylcholine receptor (nAChR). Does functional efficacy govern in vivo response?

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Nicotinic acetylcholine receptor (nAChR) partial agonists are promising medicinal targets as treatments for cognition, pain, schizophrenia, addiction and depression. As mediators of cholinergic signaling, partial agonists with differing functional efficacies are of interest as this property could be a critical variable in determining treatment effectiveness. Here, we describe an enantiomeric pair of molecules closely related to varenicline, the first approved nAChR partial agonist, and their evaluation in in vitro and in vivo preclinical models relevant to nicotine addiction and other indications.

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3.12

The nAChR agonist AMOP-H-OH ('sazetidine-A') exhibits reinforcing, but not withdrawal-alleviating, properties in rats

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The novel nAChR ligand AMOP-H-OH ('sazetidine-A') has been reported as either a full or partial agonist at high-affinity nicotinic acetylcholine receptors (nAChRs). The present studies aimed to test the hypothesis that if AMOP-H-OH is an agonist at high-affinity nAChRs, it will exhibit reinforcing and perhaps withdrawal-alleviating properties in rats trained to self-administer nicotine or chronically exposed to nicotine via subcutaneous osmotic minipumps, respectively. Rats were trained to self-administer nicotine under a fixed-ratio 3 schedule of reinforcement, and a nicotine dose–response function (0, 0.01, 0.03, 0.06, 0.1 mg/kg/inf) was determined. Nicotine-trained rats were then allowed to self-administer a range of doses of AMOP-H-OH (0.01, 0.03, 0.06, 0.1, 0.3 mg/kg/inf). The effects of AMOP-H-OH, the non-competitive neuronal nAChR antagonist mecamylamine or the high-affinity nAChR partial agonist varenicline on the reinforcing effects of nicotine were determined. Finally, naive rats were prepared with subcutaneous osmotic minipumps containing either nicotine (3.16 mg/kg/day, free base) or saline. Six days later, the minipumps were removed and the effects of acute pre-treatment with AMOP-H-OH, varenicline and nicotine on the somatic signs of nicotine withdrawal were assessed. AMOP-H-OH exhibited a dose-response function that was shifted to the right compared to nicotine. The reinforcing effects of nicotine were attenuated by AMOP-H-OH, mecamylamine and varenicline. Varenicline and nicotine, but not AMOP-H-OH, attenuated somatic signs of nicotine withdrawal in rats. The present studies observed dose-sensitive changes in AMOP-H-OH self-administration similar to nicotine, thereby indicating that AMOP-H-OH is an agonist at high-affinity nAChRs *in vivo*. Interestingly, AMOP-H-OH failed to attenuate the somatic signs of nicotine withdrawal, most likely due to a lack of efficacy at β4-containing nAChRs. The present studies confirmed previously reported effects of varenicline on nicotine self-administration, and extended the varenicline literature by demonstrating varenicline-induced attenuation of somatic signs of nicotine withdrawal. Future studies should further characterize the reinforcing properties of AMOP-H-OH, assess the effects of AMOP-H-OH on nicotine withdrawal-associated changes in brain reward function and neurochemistry, and assess the effects of AMOP-H-OH in preclinical models of relapse.

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Section 4. Pain and other indications

4.1

In vitro pharmacological profile of a novel α4β2 positive allosteric modulator NS9283 (A-969933)

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Nicotinic agonists of the α4β2 nAChR subtype are considered as potential therapeutic agents for treating pain with supporting evidence provided by compounds such as ABT-594 and ABT-894. An approach to enhance the function of α4β2 nAChRs is by positive allosteric modulation. In this study, we describe the in vitro pharmacological profile of a novel positive allosteric modulator (PAM) of α4β2 nAChRs, NS9283 (A-969933), based on radioligand binding, Ca^{2+} imaging, and electrophysiology. NS9283 (at ≤10 μM) did not displace the binding of orthosteric ligands including [^{3}H]cytisine at rat α4β2* (cortex), [^{3}H]A-585539 at rat α7* (cortex), or [^{3}H]epibatidine at human α3* (IMR-32). NS9283 did not directly evoke Ca^{2+} responses in HEK-293 cells expressing hα4β2 nAChRs but potentiated the submaximum agonist evoked (nicotine or ABT-594) responses (EC_{50} ~ 0.4 μM). In the presence NS9283 (3 or 10 μM), the agonist concentration-responses, in HEK-293 α4β2 cells, were also potentiated by increases in potency, maximum efficacy, and Hill slope. Interestingly, the agonist responses to ACh and nicotine were affected more robustly than for ABT-594 and ABT-894. Effects of NS9283 were also examined at human and rat α4β2 nAChRs expressed in oocytes by two electrode voltage clamp (POETs) where the submaximum agonist evoked responses were enhanced concentration-dependently (EC_{50} ~ 0.3 μM) as well as were the agonist evoked concentration-responses in the presence of NS9283 (10 μM). NS9283 did not potentiate the responses at human α3β4 (Ca^{2+} imaging in HEK-293/α3β4 cells or IMR-32 cells, IC_{50} ~ 10 μM, and expressed in oocytes, TEVC, IC_{50} ~ 70 μM),