4.4

Positive allosteric modulation of α4β2 nicotinic receptors potentiates some CNS effects of the α4β2 agonist, ABT-594

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Combination of an α4β2 positive allosteric modulator (PAM), NS9283 (A-969933), with inactive doses of a full α4β2 nicotinic receptor agonist, ABT-594, results in a marked, dose-related increase in pain relief in several animal pain models (see Lee et al., accompanying abstract) leading to an increased therapeutic window vs. gastrointestinal side effects. To evaluate the effect of this combination on CNS side effects, we examined A-969933 and ABT-594 alone or in combination in 3 assays: cortical activation measured by slow-wave electroencephalogram (EEG), nicotine (NIC) drug discrimination, and NIC- or ABT-594-sensitized locomotor activity. Sprague-Dawley rats implanted with cortical electrodes were injected with ABT-594 (0, 0.01 μmol/kg) and/or A-969933 (3 μmol/kg), prior to EEG testing. A-969933 significantly potentiated ABT-594-induced lowering of EEG amplitude, but neither had significant effect when administered alone. Other rats were trained to discriminate a 2.5 μmol/kg dose of NIC from saline in a 2-lever drug discrimination task. A dose-response test with ABT-594 (0, 0.0062, 0.019 and 0.062 μmol/kg) showed full generalization to the NIC cue at 0.062-μmol/kg (ED_{50} = 0.03 μmol/kg). A-969933 (0.3, 3, 30 μmol/kg) given alone produced only saline lever selection, while combined injection of A-969933 (3 μmol/kg) and a non-effective dose of ABT-594 (0.01 μmol/kg) resulted in a non-significant 2.6-fold increase in NIC-lever selection. Locomotor activity (LMA) was recorded 4 d/week in 60-min sessions following injections of vehicle, 0.01 or 0.3 μmol/kg ABT-594, 3.5 μmol/kg A-969933, or combination of the low dose of ABT-594 and A-969933. LMA rapidly sensitized in rats receiving 0.3 μmol/kg ABT-594, resulting in ∼4-fold increases in activity, but LMA failed to increase above that of vehicle in the other dose groups. In a separate LMA experiment, rats injected with vehicle, 0.1 μmol/kg ABT-594, or 0.4 mg/kg NIC 4 d/week displayed a multi-fold increase in LMA for the ABT-594 and NIC groups. Following 3½ weeks of sensitization, challenge injections of 3.5 or 10 μmol/kg A-969933 alone produced only vehicle levels of LMA, as did challenge injections of the high dose of A-969933 plus 0.01 μmol/kg ABT-594. Overall, the combination of this α4β2 PAM and α4β2 agonist at doses producing marked alleviation of pain in various pain assays results in (1) cortical activation similar to higher doses of the agonist alone, (2) incomplete generalization to the NIC stimulus, and (3) no apparent increase in LMA activity in sensitized rats. These data suggest that activation of nicotinic α4β2 receptors contribute to some, but not all, behavioral properties of nAChR ligands such as ABT-594.

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4.5

GZ556A and ZZ204G are novel small molecule antagonists of α9α10 nAChRs and are analgesic in rats

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Chronic pain afflicts tens of millions of individuals worldwide. Although there are numerous analgesics, these work through a limited number of mechanisms of action. Often, even complex combinations of these medications are insufficient to adequately treat disabling pain. Neuropathic pain is particularly treatment resistant. Recent studies indicate that small peptides from cone snails (α-conotoxins) are analgesic. These peptides potently block α9α10 nAChRs. In an effort to obtain small molecule analgesics that work by the same mechanism we evaluated a series of *tris*- and *tetrakis*-quaternary ammonium salts. Two compounds, GZ556A and ZZ204G potently blocked rat α9α10 nAChRs expressed in *Xenopus* oocytes, with IC_{50} values of 4.3 (2.2–8.0) nM and 0.65 (0.46–0.92) nM respectively (numbers in parenthesis are 95% confidence intervals). In addition, these compounds were 10- to 5000-fold less potent on other tested nAChR including α1β1δε, α2β2, α2β4, α3β2, α3β4, α4β2, α4β4, α6/α3β2β3, α6/α3β4 and α7 subtypes. GZ556A and ZZ204G were tested in the rat chronic constriction injury and formalin models of neuropathic and persistent pain. Both compounds produced analgesia at doses lower than those that caused motor impairment as assessed by rotorod. Tolerance to analgesic effects is a major limiting factor in opiate-based medications. Tolerance was not observed with either GZ556A or ZZ204G over seven days of administration. GZ556A and ZZ204G represent novel α9α10 nAChR antagonists and lead compounds for analgesics.

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4.6

Antidepressant-like activity of AMOP-H-OH ('sazetidine-A') in the forced swim test is mediated by high affinity nicotinic acetylcholine receptors

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Both preclinical and clinical data suggest a role for nicotinic acetylcholine receptors (nAChRs) in depression. Both nAChR agonists and antagonists reduce depressive symptoms in humans and have antidepressant-like effects in rodent models. This study evaluated the action of the selective α4β2 partial agonist AMOP-H-OH (6-[5-(Azetidin-2-ylmethoxy) pyridin-3-yl]hex-5-yn-1-ol (aka Sazetidine-A)) in the forced swim test in mice. AMOP-H-OH produced a robust reduction in immobility in the forced swim test in comparison to weaker effects seen with the α4β2 partial agonist varenicline and the non-competitive nAChR antagonist mecamy-