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-

lamine. The antidepressant-like effect of AMOP-H-OH in forced swim was completely reversed by mecamylamine and the high affinity nAChR antagonist dihydro-β-erythroidine (DHβE), but not by the α7 nAChR antagonist methyllycaconitine (MLA). AMOP-H-OH was long lasting in the forced swim test with efficacy observed up to 4 h after treatment, an effect that was also completely reversed by mecamylamine. A pharmacokinetic study was carried out to determine if the duration of action of AMOP-H-OH could be correlated with plasma and brain levels. Mice were treated with 1 or 3 mg/kg of AMOP-H-OH and plasma and brains were collected 0.25, 0.5, 1, 2, and 4 h after i.p. injection. Although plasma levels showed a dose response relationship 15 min after administration, levels were nearly non-detectable by 30. Brain levels of AMOP-H-OH reached only low levels 15 min after administration and were at or below detection level at the later time points. Similar dissociations between pharmacokinetic and pharmacodynamic (PK/PD) profiles have been noted for other nicotinic compounds. The superior efficacy of AMOP-H-OH in forced swim compared to varenicline and mecamylamine suggests that this class of compounds may provide novel opportunities for the development of drugs to treat depression.

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4.7

nAChR agonists reduce L-dopa-induced dyskinesias in parkinsonian rats

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Dyskinesias are debilitating movement abnormalities that arise with L-dopa therapy in Parkinson's disease. Currently treatment options to decrease their occurrence include a reduction in L-dopa dosage or the use of drugs such as amantadine that have only limited efficacy. Our recent data show that nicotine treatment effectively attenuates L-dopa-induced dyskinesias in parkinsonian monkeys and rats. Since nicotine activates multiple nicotinic receptors (nAChRs), we initiated a series of experiments with receptor agonists to identify whether more selective receptor drugs may reduce L-dopa-induced dyskinesias. For these studies, we used a well-characterized parkinsonian animal model of dyskinesias, L-dopa-treated unilateral 6-OHDA-lesioned rats. Several nAChR agonists were tested including (1) RJR-2403 that is selective for α4β2* nAChRs, (2) A-85380 that interacts with both α4β2* and α6β2* nAChRs and (3) varenicline that targets α4β2*, α6β2*, α3β4* and α7 nAChRs. For the experiments with RJR-2403, L-dopa-treated dyskinetic lesioned rats L-dopa were given varying doses of RJR-2403 (0.1, 0.2 and 1.4 mg/kg/day) via minipump for a several week period. No significant declines were obtained in L-dopa-induced abnormal involuntary movements (AIMs) with RJR-2403, although there was a small trend for improvement. The lack of efficacy of this agonist may be because it only targets α4β2* nAChRs, or possibly due to a sub-optimal pharmacokinetic profile. After a washout period, the rats were next treated with a drug with a somewhat wider nAChR profile, the β2-directed nAChR agonist A-85380. It was injected twice daily for 4 days at doses ranging from 0.10 to 0.75 μmol/kg, as previously described. L-dopa-induced AIMs were rated on the 4th day of injection. A small (15%) but significant decline was observed in L-dopa-induced AIMs with the 0.18 μmol/kg dose. There was no decline in the antiparkinsonian effect of L-dopa, as assessed using the asymmetric forelimb or cylinder test. We then did a series of experiment with varenicline, an agonist that interacts with multiple nAChR subtypes. Varenicline (1 mg/kg) was administered to L-dopa-treated lesioned rats twice daily via sc injection for 4 days. This drug also significantly reduced L-dopa-induced AIMs (25%) without affecting parkinsonian behavior. Overall, these data show that agonists that target multiple CNS nAChR subtypes reduce L-dopa-induced AIMs with no appreciable effects on parkinsonism. The results should aid in the development of selective therapies using nAChR subtype agonists to reduce L-dopa-induced dyskinesias in Parkinson's disease.

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Section 5. Peripheral nAChRs

5.1

Importance of nicotinic acetylcholine receptors in the visual adverse effects associated with telithromycin

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Adverse effects associated with use of the macrolide antibiotic telithromycin include "blurred vision" associated with impairment of accommodation and, in myasthenia patients, exacerbated muscle weakness. A first clue about blurred vision came from the relatively high prevalence of such symptoms reported by subjects exposed to telithromycin compared to structurally related macrolides (clarithromycin or azithromycin). As macrolides do not readily cross the blood brain barrier, blurred vision must arise from disruption of function in the peripheral nervous system. Since cholinergic neurotransmission plays a determinant role in peripheral nervous transmission we evaluated the possible interaction of macrolides with neuronal nicotinic acetylcholine receptors (nAChR) and compared the effects of telithromycin, clarithromycin, azithromycin and CEM-101, a novel fluoroketolide. Effects of macrolides on the functional properties of nAChRs were evaluated using human receptors expressed in *Xenopus* oocytes. Exposure to a low concentration of telithromycin (2 μM) inhibited by 85% or more the ACh-evoked currents at α3β4 and α7 nAChR while much less inhibition was observed with azithromycin, clarithromycin or CEM-101. As the α3β4 and α7 receptors are the major constituents of ganglionic transmission, inhibition of their activity will impair or even suppress neurotransmission in peripheral ganglia. Dysfunction of the ciliary ganglion is expected to cause a loss of control of pupillary constriction and ciliary muscle contraction. Both effects thereby may combine to produce a reduction in the depth of field and of accommodation and cause a loss of focusing. This should result in profound vision disturbance and "blurred vision" for objects in the near and intermediate vision.

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