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

Acknowledgements: This work was supported by the Swiss National Science Foundation to D.B.

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doi:10.1016/j.bcp.2009.06.077

3.11

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

Jotham W. Coe*, Paige R. Brooks, Michael C. Wirtz, Michael G. Vetelino, Eric P. Arnold, Steven B. Sands, Thomas I. Davis, Lorraine A. Lebel, Carol B. Fox, Alka Shrikhande, Robert S. Mansbach, Leslie K. Chambers, Charles C. Rovetti, David W. Schulz, F. David Tingley III, Brian T. O'Neill, Hans Rollema

Neurosciences Chemistry, Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, United States

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.

doi:10.1016/j.bcp.2009.06.078

3.12

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

Adrian Hackett*, Barbara Caldarone, Alan P. Kozikowski, Afshin Ghavami, Berend Olivier, Taleen Hanania, Neil E. Paterson

PsychoGenics, Inc., 765 Old Saw Mill River Rd., Tarrytown, NY 10591, United States

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.

doi:10.1016/j.bcp.2009.06.079

Section 4. Pain and other indications

4.1

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

J. Malysz*, T. Dyhring, P.K. Ahring, G.M. Olsen, D. Peters, J.H. Gronlien, C. Wetterstrand, H. Ween, M. Haakerud, K. Thorin-Hagene, E. Andersen, D.J. Anderson, M. Hu, P.E. Kroeger, C.-H.L. Lee, M. Gopalakrishnan, D.B. Timmermann

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott, Abbott Park, IL, USA and Drug Discovery, NeuroSearch, Ballerup, Denmark

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),

human α7 nAChRs (expressed in oocytes, TEVC, $IC_{50} \sim 15\ \mu M$), human 5-HT_{3A} (expressed in oocytes, TEVC, $IC_{50} > 30\ \mu M$). In contrast, NS9283 was able to potentiate submaximum nicotine evoked responses at human α4β4 receptors ($EC_{50} \sim 0.3\ \mu M$), expressed in HEK-293 cells, and to enhance agonist concentration responses suggesting that this compound interacts with the α4 subtype. In summary, this study identifies NS9283 as a novel and selective positive allosteric modulator of the α4 containing nAChRs, including the α4β2 subtype that will be useful in further defining physiological roles of these nAChRs both in *in vitro* and *in vivo* studies.

doi:10.1016/j.bcp.2009.06.080

4.2

Improving the efficacy-tolerability profile of nAChR agonists for the treatment of neuropathic pain in combination with positive allosteric modulators

Chih-Hung Lee*, Chang Zhu, Thomas Campbell, Thomas Shaughnessy, Prisca Honore, James Polakowski, Murali Gopalakrishnan

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500, United States

Various neuronal nAChR subunits (α2-α10 and β2-β4) are differentially expressed throughout the nervous system and combine to form diverse subtypes with a wide range of physiological and pharmacological profiles. Gene knockout and antisense studies coupled with pharmacological studies with nAChR agonists have documented a clear role of α4β2 activation in analgesia. Studies conducted at Abbott and elsewhere have demonstrated that α4β2 nAChR agonists possess potential as broad-spectrum analgesics based on preclinical studies demonstrating their efficacy in diverse pain states including multiple forms of acute, chronic, inflammatory and neuropathic pain. ABT-594, for the first time, provided clinical validation to the nAChR agonist pharmacology as a novel mechanism for treatment of pain. However, ABT-594 was poorly tolerated at these doses, particularly with respect to the side effects of nausea and emesis, thought to be mediated by activation of the ganglionic-type (α3-containing) receptors. An alternate approach is to selectively modulate the α4β2 nAChR via positive allosteric modulation. Positive allosteric modulators (PAMs) are compounds that do not interact with the agonist binding sites or possess intrinsic activity at the receptor *per se*, but potentiate the effects of the agonist. Here, we report that A-969933 (NS-9283) was found to selectively enhance the potency of a range of nAChR agonists at α4β2 but not α3β4 nAChRs. Studies were conducted in the Chung model of neuropathic pain to establish the efficacy of nAChR agonists such as ABT-894 could be enhanced in combination with PAMs without affecting the tolerability profile (gastrointestinal, cardiovascular, etc.). These preclinical studies collectively demonstrate that the pain efficacy of clinically well-tolerated doses of ABT-594 in humans can be significantly enhanced by co-administration with the α4β2 PAM.

doi:10.1016/j.bcp.2009.06.081

4.3

In vivo characterization of the co-administration of α4β2 neuronal nicotinic receptor agonist and positive allosteric modulator in experimental pain in rats

Chang Z. Zhu*, Chih-liang Chin, Chengmin Zhong, Joe Mikusa, Prasant Chandran, Anita Salyers, Erica Wensink, Gricelda Silmer, La Geisha Lewis, Donna Gauvin, Scott Baker, Ann Tovcimak, Jordan Brown, Nathan Rustay, Gerard B. Fox, Michael W. Decker, Chih-Hung Lee, Murali Gopalakrishnan, Prisca Honore

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500, United States

Positive modulation of the neuronal nicotinic acetylcholine receptor (nAChR) α4β2 subtype by selective positive allosteric modulator (PAM) NS-9283 (A-969933) has been demonstrated to potentiate the nAChR agonist ABT-594-induced analgesic activity in preclinical neuropathic pain, without worsening ABT-594-assocated adverse effects (see Lee et al., accompanying abstract). To determine whether this beneficiary is neuropathic pain limited, the present study examined the analgesic activity and adverse efficacy profile across variety of animal models, utilizing the combined administration of ABT-594 and NS-9283 (A-969933) in rats. The effects of the combined therapy on drug-induced brain activities were also determined using pharmacological magnetic resonance imaging (phMRI). In carrageenan-induced thermal hyperalgesia, co-administration of NS-9283 (A-969933) (3.5 μmol/kg ip) induced leftward shift of the dose–response of ABT-594 by 5 fold (EC_{50} = 30 nmol/kg vs 150 nmol/kg). In rat paw skin incision model of post-operative pain, co-administration of NS-9283 (A-969933) similarly induced leftward shift of ABT-594 by 4 fold (EC_{50} = 30 nmol/kg vs 120 nmol/kg). In monoiodo-acetate (MIA) induced knee joint pain, co-administration of 969933 enhances the capacity of ABT-594 returning to the normalcy by 3 fold (EC_{50} = 30 nmol/kg vs 120 nmol/kg). In phMRI, our data also show that, compared to the brain activity patterns obtained by infusing ABT-594 alone at various doses, co-administration of ABT-594 (0.03 μmol/kg iv) with NS-9283 (A-969933) (3 mg/kg po) leads to a leftward shift of dose–response in cortical activation, without activating the emetic center in brainstem. Interestingly, CNS effects of ABT-594 observed at supra-therapeutic doses (effects on balance and co-ordination, temperature, locomotor activity) were not exacerbated in presence of the efficacious dose of NS-9283 (A-969933) (3.5 μmol/kg). These results demonstrate that selective positive allosteric modulation at the α4β2 nAChR potentiates nAChR agonist-induced analgesic activity across rat pain models without altering adverse effects, suggesting that selective positive modulation of α4β2 nAChR subtype by PAM may represent a novel analgesic approach.

doi:10.1016/j.bcp.2009.06.082