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.

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4.2

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

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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.

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4.3

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

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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.

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