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Effects of nicotine in combination with drugs described as positive allosteric nicotinic acetylcholine receptor modulators in vitro: discriminative stimulus and hypothermic effects in mice

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ABSTRACT

Some drugs that are positive allosteric nAChR modulators in vitro, desformylflustrabromine (dFBr), PNU-120596 and LY 2087101, have not been fully characterized in vivo. These drugs were examined for their capacity to share or modify the hypothermic and discriminative stimulus effects of nicotine (1 mg/kg s.c.) in male C57Bl/6J mice. Nicotine, dFBr, and PNU-120596 produced significant hypothermia, whereas LY 2087101 (up to 100 mg/kg) did not. Nicotine dose-dependently increased nicotine-appropriate responding and decreased response rate; the respective ED_{50} values were 0.56 mg/kg and 0.91 mg/kg. The modulators produced no more than 38% nicotine-appropriate responding up to doses that disrupted operant responding. Rank order potency was the same for hypothermia and rate-decreasing effects: nicotine > dFBr > PNU-120596 = LY 2087101. Mecamylamine and the α4β2 nAChR antagonist dihydro-β-erythroidine, but not the α7 antagonist methyllycaconitine, antagonized the hypothermic effects of nicotine. In contrast, mecamylamine did not antagonize the hypothermic effects of the modulators. The combined discriminative stimulus effects of DFBr and nicotine were synergistic, whereas the combined hypothermic effects of nicotine with either dFBr or PNU-120596 were infra-additive. PNU-120596 did not modify the nicotine discriminative stimulus, and LY 2087101 did not significantly modify either effect of nicotine. Positive modulation of nicotine at nAChRs by PNU-120596 and LY 2087101 in vitro does not appear to confer enhancement of the nAChR-mediated hypothermic or discriminative stimulus effects of nicotine. However, dFBr appears to be a positive allosteric modulator of some behavioral effects of nicotine at doses of dFBr smaller than the doses producing unwanted effects (e.g. hypothermia) through non-nAChR mechanisms.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels activated by acetylcholine as well as various exogenous compounds, including nicotine. Disruption and dysregulation of nAChR signaling has been implicated in multiple pathologies, including addiction (Henningfield et al., 1985; Stolerman and Jarvis, 1995), Alzheimer's disease (Court et al., 2001), schizophrenia (Young and Geyer, 2013), and Parkinson's disease (Quik and Wonnacott, 2011). Currently approved therapeutic strategies that target nAChRs include nAChR agonism and acetylcholinesterase inhibition (Buccafusco, 2004; Newhouse et al., 2004).

There are limitations to using these therapeutic strategies, however. Acetylcholine is also the endogenous ligand for muscarinic acetylcholine receptors (mAChRs), and unintended activation of mAChRs by therapies targeting nAChRs can cause undesired effects, most commonly nausea and vomiting (Inglis, 2002). Although many nAChR agonists have some degree of selectivity for nicotinic versus muscarinic receptors, acetylcholinesterase inhibitors act indiscriminately at both types of receptor. Furthermore, orthosteric activation by acetylcholine and other agonists triggers rapid desensitization of nAChRs, reducing the effectiveness of continuous or repeated dosing regimens (James et al., 1994).

Allosteric modulators differ from orthosteric agonists in that they bind to a receptor site that is distinct from the orthosteric site. Allosteric modulators often do not have effects in the absence

Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; dFBr, desformylflustrabromine; DHβE, dihydro-β-erythroidine; FR, fixed ratio; LY 2087101, [2-[(4-fluorophenyl)amino]-4-methyl-5-thiazolyl]-3-thienylmethanone; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; PNU-120596, N-(5-chloro-2,4-dimethoxyphenyl)-N′-(5-methyl-3-isoxazolyl)-urea

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of an orthosteric ligand; instead allosteric modulators change the affinity and/or efficacy of the orthosteric ligand (Uteshev, 2014). Positive allosteric nAChR modulation of an orthosteric ligand (e.g. nicotine) decreases the amount of orthosteric ligand required for an effect, thereby resulting in less unwanted effects such as receptor desensitization produced by the orthosteric ligand (Williams et al., 2011). Thus, allosteric modulation of nAChRs is an alternative therapeutic strategy than can potentially circumvent the limitations inherent in targeting the orthosteric site.

Two nAChR subtypes predominate in the central nervous system: the heteromeric α4β2 subtype and the homomeric α7 subtype. There is evidence to suggest that the α4β2 nAChR subtype is of primary importance in the abuse- and dependence-producing properties of nicotine (Besson et al., 2006; Picciotto et al., 1998; Tapper et al., 2004), while the α7 nAChR subtype is responsible for the cognitive-enhancing effects of nicotine (Pichat et al., 2007; Roncarati et al., 2009; Wallace et al., 2011). Drugs that selectively target a particular nAChR subtype have the advantage of producing only those effects mediated by that nAChR subtype. Although allosteric modulators selective for specific nAChR subtypes in vitro have been developed, there is limited in vivo data to support the effectiveness of these compounds. Therefore, three positive nAChR allosteric modulators reportedly differing in nAChR subtype selectivity in vitro were chosen for examination in vivo: desformylflustrabromine (dFBr), PNU-120596 and LY 2087101.

In cells transfected with one subtype of nAChRs, dFBr, PNU-120596 and LY 2087101 alone do not mimic the effects of an orthosteric nAChR agonist and they vary in selectivity for nAChR subtypes: dFBr is selective for the α4β2 nAChR subtype (Kim et al., 2007; Sala et al., 2005), PNU-120596 is selective for the α7 nAChR subtype (Hurst et al., 2005) and LY 2087101 is non-selective for α4β2 and α7 nAChRs (Broad et al., 2006). When tested in combination with acetylcholine, dFBr was initially found to increase the maximum current for cells transfected with α4β2 nAChRs, but not for α7 nAChRs (Sala et al., 2005). Additional studies replicated this finding at α4β2 nAChRs, but found that the concentration-response function was biphasic, revealing subsequent inhibition at higher concentrations of dFBr (Kim et al., 2007). In tests with other nAChR agonists, combination with dFBr increased the maximum effect of low efficacy agonists (e.g. cytisine) more than it increased the maximum effect of high efficacy agonists (e.g. nicotine); dFBr had no effect on the potency of the nAChR agonists studied (Kim et al., 2007; Sala et al., 2005; Weltzin and Schulte, 2010). PNU-120596, on the other hand, was shown to increase the maximum effect of acetylcholine-induced current by 7-fold at the α7 nAChR, with no effect at other nAChR subtypes (Gronlien et al., 2007; Hurst et al., 2005). Furthermore, the selectivity of the effects of PNU-120596 in vivo was demonstrated by the ability to increase the antinociceptive effects of nicotine but not the antinociceptive effects of morphine (Freitas et al., 2013a). Unlike both dFBr and PNU-120596, LY 2087101 did not show selectivity for either the α4β2 or the α7 nAChR subtype in vitro; it did, however, produce increases in both the potency and the maximum amount of nicotine-induced currents (Broad et al., 2006).

There is evidence in vitro that dFBr, PNU-120596 and LY 2087101 are positive allosteric modulators with differing selectivity for the α4β2 and α7 nAChR subtypes, because they do not appear to activate these receptors on their own, but do increase the effects of both acetylcholine and nicotine. The aims of the current study were to examine the extent to which positive allosteric nAChR modulators: (1) exert nicotine-like effects in vivo; (2) enhance or otherwise modify the in vivo effects of nicotine; and (3) modulate nicotine differently as a function of the effect being modulated. Nicotine was chosen because it is used widely from tobacco products and in the form of nicotine replacement therapy, and the effects of the novel compounds were studied both alone and in combination with nicotine. The discriminative stimulus effects and hypothermic effects of nicotine were both studied because there is evidence that nAChR subtypes differentially mediate these effects (Rodriguez et al., 2014), which was expected to confer drug- and effect-dependent differences in their modulation. Drug discrimination appears to be mediated by the α4β2 nAChR subtype (Gommans et al., 2000; Shoaib et al., 2002). However, published data suggest that the hypothermic effects of nicotine are mediated by not only the α4β2 nAChR subtype (Rodriguez et al., 2014), but also perhaps the α7 nAChR subtype (Freitas et al., 2013a). Therefore, the nicotinic antagonists mecamylamine, dihydro-β-erythroidine (DHβE), and methyllycaconitine (MLA) were used to further characterize the nAChR mechanism underlying the hypothermic effects of drugs.

2. Materials and methods

2.1. Subjects

Male C57BL/6J mice were purchased at eight weeks of age from The Jackson Laboratory (Bar Harbor, ME). Forty mice were used for the hypothermia experiments and six mice were used for drug discrimination. Mice were habituated in the colony room for at least seven days before experiments began and housed in groups of four for hypothermia experiments or singly for drug discrimination experiments in cages (28 × 18 × 13 cm) with water continuously available. The mice used in the hypothermia experiment had continuous access to food (Harlan, Teklad 7912, Houston, TX) in the home cage. The mice used in the drug discrimination experiment were maintained at 85% free-feeding weight; these mice had access during experimental sessions to 0.6 cm^3 of 50% condensed milk (Borden Milk Products, Dallas, TX) and after experimental sessions to 2.5 g of Dustless Precision Pellets (500 mg, Rodent Grain-Based Diet, Bio-Serv, Frenchtown, NJ). All experiments were conducted during the light period of a 14/10 h light-dark cycle (lights on at 0600 h). The maintenance and experimental use of animals was carried out in accordance with the National Institute of Health's Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 2011). Protocols were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center, San Antonio.

2.2. Apparatus

Temperature was measured by inserting a probe (RET-3) (Physitemp Instruments, Inc., Clifton, NJ) attached to a microcomputer thermometer (7001H) (Physitemp Instruments, Inc.) 2 cm into the rectum. For drug discrimination, mice were placed in ventilated, sound-attenuating mouse operant chambers (MedAssociates, St. Albans, VT) with a house light located in the ceiling. On one wall were three holes spaced 5.5 cm apart (2.2 cm diameter each); each hole contained a photo beam and a light, and the center of each hole was 1.6 cm from the floor. In the center of the opposite wall was a fourth hole (also 2.2 cm diameter, center 1.6 cm from the floor) containing a dipper to which could be delivered 0.01 cm^3 condensed milk. An interface (MED-SYST-8) (MedAssociates) connected the operant chambers to a computer running Med-PC software (MedAssociates), which controlled and recorded all experimental events.

2.3. Drugs

Doses are expressed as the weight, in mg/kg, of the forms listed below, except in the case of nicotine, which is expressed as the