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Enhanced attenuation of nicotine discrimination in rats by combining nicotine-specific antibodies with a nicotinic receptor antagonist

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ABSTRACT

Tobacco addiction requires activation by nicotine of a variety of central nicotinic acetylcholine receptors (nAChRs). In animals, both nAChR antagonists and immunization against nicotine can reduce nAChR activation by nicotine and block a variety of addiction-relevant behaviors. However, clinical use of nAChR antagonists for smoking cessation is limited by dose-related side effects, and immunization does not reliably produce sufficient antibody levels in smokers to enhance smoking cessation rates. Combining these approaches may be one way of addressing the limitations of each while enhancing overall efficacy. This study examined the individual and combined effects of passive immunization with the monoclonal nicotine-specific antibody Nic311 and the nicotinic receptor antagonist mecamylamine (MEC) on nicotine's discriminative stimulus effects. Rats were trained to discriminate 0.4 mg/kg of nicotine from saline using a two-lever operant discrimination procedure. Antagonism of nicotine discrimination by Nic311 (160 mg/kg i.v.) and ascending doses of MEC (0.03, 0.1, 0.3, and 1.0 mg/kg s.c.) was assessed across four consecutive daily 2-min extinction test sessions using a 2×2 design. Nic311 alone produced a 24–48% reduction in % nicotine-lever responding (%NLR) across all four test sessions. MEC produced a dose-dependent decrease in %NLR, with no effect at the two lowest doses and 80–93% attenuation at the two highest doses. Nic311 combined with MEC significantly suppressed %NLR at every MEC dose (85–92% reduction across all four test sessions). Very low doses of MEC that were ineffective alone completely blocked nicotine discrimination when combined with Nic311. These data demonstrate that nicotine-specific antibodies and MEC can work synergistically to suppress the subjective effects of nicotine and suggest that low doses of MEC may significantly enhance the efficacy of immunotherapy.

1. Introduction

Nicotine is considered the principal constituent in tobacco responsible for initiating and maintaining tobacco addiction. It produces a constellation of neuropharmacological and behavioral effects that are similar to those produced by other drugs of abuse (Le Foll and Goldberg, 2006). These effects are mediated through nicotine's activation and desensitization of a variety of nicotinic acetylcholine receptors (nAChR) in brain (Changeux, 2010; Picciotto et al., 2008). Several medications currently used or under development for treatment of tobacco addiction act by altering nAChR activation by nicotine (Lerman et al., 2007).

Administration of a nAChR antagonist disrupts nAChR activation and can reduce addiction-relevant CNS and behavioral effects of nicotine. Mecamylamine, a noncompetitive and largely nonselective nAChR antagonist, reduces the reinforcing and discriminative stimulus effects of nicotine or tobacco in animals and humans (Lerman et al., 2007; Smith and Stolerman, 2009). It is currently the only nAChR antagonist approved for use in humans, albeit as an antihypertension medication. It has facilitated smoking cessation in clinical trials when combined with nicotine replacement therapy (Rose et al., 1998; Rose et al., 1994). However, its clinical development has been hampered because of its peripheral side effects at effective doses (e.g., constipation, abdominal cramps, dizziness (Rose et al., 1998; Tennant et al., 1984)). Preclinical development of other nAChR antagonists with efficacy similar to or better than mecamylamine, but with reduced peripheral side effects, has been an important focus in medication development for tobacco addiction (Dwoskin et al., 2009; Papke et al., 2008; Wilkins et al., 2002).

Immunotherapy presents an alternative means of reducing activation of nAChRs by nicotine that is mechanistically distinct from the use of a receptor antagonist. Vaccination with a nicotine immunogen elicits production of nicotine-specific antibodies that selectively bind and sequester nicotine in blood and thereby reduce the level of free or unbound nicotine that can distribute into brain and activate nAChRs. There are several potential advantages of immunotherapy over other approved or experimental pharmacotherapies for nicotine addiction

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(LeSage et al., 2006b). First, immunotherapies target nicotine itself rather than the brain receptors mediating nicotine's reinforcing effects and so do not block effects of endogenous acetylcholine. As such, nicotine vaccines do not have the central nervous system side effects associated with other types of medications. For this same reason, nicotine vaccines do not block peripheral nAChRs or produce the side effects that limit use of MEC. Second, reducing nicotine distribution to brain presumably decreases nicotine activation of all types of nAChRs, and therefore all of nicotine's neuropharmacological effects in brain that are vital to maintaining tobacco addiction. This is difficult to accomplish with any one or combination of nAChR-targeted medications other than nicotine itself. Immunization has been proven effective in reducing a variety of nicotine's CNS and behavioral effects in preclinical studies (e.g., DA release, locomotor activity, nicotine self-administration (Cornish et al., 2011; LeSage et al., 2006b; Moreno et al., 2010; Moreno and Janda, 2009; Roiko et al., 2009)) and increasing abstinence in Phase II clinical trials (Escobar-Chávez et al., 2011; Hatsukami et al., 2011). However, efficacy in Phase II trials has been limited to individuals with the highest serum antibody concentrations (e.g. top 30%), and preliminary results from Phase III trials suggest no effect of vaccine on 16-week continuous abstinence rates at 52 weeks from the quit date (although post hoc analysis indicated that antibody levels were positively correlated with abstinence rates, Fahim et al., 2011). The primary limitation of immunotherapy has been the modest and variable serum levels of antibody elicited by current vaccines.

Strategies are needed to address the limitations of nAChR antagonists and immunotherapy to improve their clinical potential. Although identifying improved nAChR antagonists and vaccines should be helpful for this purpose (e.g., Keyler et al., 2008; Moreno et al., 2010; Papke et al., 2008; Pravetoni et al., 2012; Wooters et al., 2011), new "second-generation" medications have not yet entered clinical trials. Alternatively, combining current nAChR antagonist medications and vaccines might enhance their efficacy. Receptor-based and immunologic treatments are attractive complements because their mechanisms (pharmacodynamic versus pharmacokinetic, respectively) are distinct, yet they target the same process. Each interrupts nAChR activation at one of two critical and sequential steps toward receptor activation; nicotine distribution to the receptor (immunotherapy), and the extent of receptor binding (competitive antagonist) or activation once bound (noncompetitive antagonist). The goal of this approach would be to achieve a high degree of blockade and efficacy using sub-toxic doses of MEC and achievable antibody concentrations via immunization. As a result, vaccine efficacy might be enhanced, while side effects of nAChR antagonism are minimized.

The purpose of the present study was to examine the separate and combined effects of immunization with the monoclonal nicotine-specific antibody Nic311 and MEC on nicotine's discriminative stimulus effects in rats. Immunization against nicotine can be achieved via vaccination or direct administration of antibodies (passive immunization). We chose the latter for this initial proof-of-principle study because, in contrast to vaccination, serum antibody concentrations can be precisely controlled and immediately achieved.

2. Materials and methods

2.1. Animals

Twenty-three male Holtzman rats (Harlan, Indianapolis) weighing 300–350 g at the start of the experiment were maintained with limited access to food (18 g/day rat chow) and unlimited access to water. Each rat was individually housed in a temperature- and humidity-controlled colony room under a reversed 12 h light/dark cycle (lights off at 10:00 am). Animal husbandry and experimental protocols were approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation, and were in accordance with the 2011 National Research Council Guide for the Care and Use of Laboratory Animals (8th edition), and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003).

2.2. Apparatus

Experimental sessions occurred in sixteen identical operant-conditioning chambers (ENV-008, Med Associates Inc., St. Albans, VT). The front panel contained two response levers, a stimulus light over each response lever, and an aperture between the levers for the delivery of 45-mg food pellets (PJAI-0045, Research Diets, New Brunswick, NJ). A house light was located on the back panel near the chamber ceiling to provide ambient illumination. Each chamber was enclosed in a sound-attenuating box equipped with an exhaust fan that provided masking noise.

2.3. Drugs

Nicotine bitartrate and mecamylamine (Sigma Chemical Co., St. Louis, MO) were dissolved in sterile saline. The pH of the nicotine solution was adjusted to 7.4 with dilute NaOH. All nicotine doses and concentrations are expressed as that of the base. MEC doses are expressed as that of the salt. The nicotine-specific monoclonal antibody Nic311 is an $IgG1_{\kappa}$ derived from mice immunized with the immunogen 3′-aminomethylnicotine conjugated to recombinant Pseudomonas exoprotein A, and has a Kd for nicotine of 60 nM and <1% cross-reactivity with mecamylamine, nicotine metabolites or a variety of neurotransmitters including acetylcholine (Keyler et al., 2005). Nic311 was purified by protein G chromatography to ≥95% of the total protein content with endotoxin levels of <0.2 EU/mg. Nic311 was diluted in 2 ml phosphate-buffered saline (concentration of approximately 30 mg/ml). The Nic311 dose of 160 mg/kg was selected based on pilot data indicating that it produces a partial attenuation of nicotine discrimination, allowing for detection of added effects by mecamylamine. Control IgG was human polyclonal IgG (Gammagard; Baxter Healthcare Corp., Westlake Village, CA) that does not bind nicotine or alter nicotine pharmacokinetics or behavior in rats (Cornish et al., 2011).

2.4. Nicotine discrimination training

The training procedures that were used have been described in detail elsewhere (LeSage et al., 2009). Briefly, rats were trained to discriminate nicotine alone (0.4 mg/kg, s.c.) from saline using a 2-lever discrimination procedure. Lever pressing was reinforced under a terminal variable-interval of 15 s schedule using 45-mg food pellets. Discrimination was assessed twice weekly (Tues and Fri) during 2-min extinction test sessions. Discrimination was considered stable when a) >80% responding occurred on the injection-appropriate lever during two consecutive saline and nicotine test sessions, b) >95% injection-appropriate responding occurred on six consecutive training sessions, and c) response rates (total responses/session) were stable (no trend across these four test sessions and six training sessions).

After a stable discrimination performance was achieved, a nicotine generalization dose–effect function was determined, involving substitution of a range of nicotine doses (0.0, 0.05, 0.1, 0.2 and 0.4 mg/kg) during Tues and Fri test sessions. Doses were administered in a mixed sequence that was counterbalanced across subjects. At least 2 weeks after the acute dose–response determination and when performance was stable, four consecutive daily test sessions with the training dose were conducted Tues–Fri to assess the stability of discrimination of the training dose across repeated test sessions. This repeated-testing procedure allowed studying the initial time course of Nic311 effects (see below). Rats that failed to meet the discrimination criteria (at least 80% responding on the nicotine lever) on any of these 4 consecutive tests were excluded from the study. Those that met the criteria on every