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New insights on the effects of varenicline on nicotine reward, withdrawal and hyperalgesia in mice

Deniz Bagdas [a, b, *], Yasmin Alkhlaif [a], Asti Jackson [a], F. Ivy Carroll [c], Joseph W. Ditre [d], M. Imad Damaj [a, b]

[a] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, United States
[b] The Center for the Study for Tobacco Products, Virginia Commonwealth University, Richmond, VA, United States
[c] Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC, United States
[d] Department of Psychology, Syracuse University, Syracuse, NY, United States

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ABSTRACT

Varenicline, a partial agonist for α4β2* nicotinic acetylcholine receptors (nAChRs) and a full agonist for α3β4 and α7 nAChRs, is approved for smoking cessation treatment. Although, partial agonism at α4β2* nAChRs is believed to be the mechanism underlying the effects of varenicline on nicotine reward, the contribution of other nicotinic subtypes to varenicline's effects on nicotine reward is currently unknown. Therefore, we examined the role of α5 and α7 nAChR subunits in the effects of varenicline on nicotine reward using the conditioned place preference (CPP) test in mice. Moreover, the effects of varenicline on nicotine withdrawal-induced hyperalgesia and aversion are unknown. We also examined the reversal of nicotine withdrawal in mouse models of dependence by varenicline.

Varenicline dose-dependently blocked the development and expression of nicotine reward in the CPP test. The blockade of nicotine reward by varenicline (0.1 mg/kg) was preserved in α7 knockout mice but reduced in α5 knockout mice. Administration of varenicline at high dose of 2.5 mg/kg resulted in a place aversion that was dependent on α5 nAChRs but not β2 nAChRs. Furthermore, varenicline (0.1 and 0.5 mg/kg) reversed nicotine withdrawal signs such as hyperalgesia and somatic signs and withdrawal-induced aversion in a dose-related manner.

Our results indicate that the α5 nAChR subunit plays a role in the effects of varenicline on nicotine reward in mice. Moreover, the mediation of α5 nAChRs, but not β2 nAChRs are probably needed for aversive properties of varenicline at high dose. Varenicline was also shown to reduce several nicotine withdrawal signs.

1. Introduction

Varenicline (Chantix®) was approved by the FDA for smoking cessation in 2006. Its clinical efficacy and advantage compared to bupropion and nicotine replacement therapies (NRTs) is well established (Gonzales et al., 2006; Jorenby et al., 2006). However, all these pharmacotherapies are of limited effectiveness since only about one-fifth of smokers are able to maintain long-term abstinence with any of these drugs (Health and Report, 2008; Schnoll and Lerman, 2006). Therefore, an increased understanding of the mechanisms through which varenicline reduces nicotine dependence could aid in the identification of new treatment targets, and better inform pharmacotherapy of smoking cessation.

In vitro binding and functional studies showed that varenicline is a α4β2* nicotinic receptor (nAChR) partial agonist (Mihalak et al., 2006; Rollema et al., 2007) [* denotes that these nAChRs may contain other α and β subunits as well [Reviewed in (Gotti et al., 2006)]. Partial agonism at α4β2* nAChRs is believed to be the mechanism by which varenicline reduces nicotine reward in the self-administration paradigm (George et al., 2011; O'Connor et al., 2010; Rollema et al., 2007) as well as nicotine-induced dopamine release in the nucleus accumbens (Reperant et al., 2010) in rodents. However, other subunits such as α5 or α6 can contribute to the α4β2* receptor heteromeric nAChRs and form subtypes such as

Abbreviations: ACh, acetylcholine; nAChR, nicotinic acetylcholine receptor; CPP, conditioned place preference; CPA, conditioned place aversion.

* Corresponding author. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, United States.
E-mail address: deniz.bagdas@vcuhealth.org (D. Bagdas).

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α4α5β2, which are present in the brain and are expressed on dopaminergic nerve terminals (Zoli et al., 2002). In fact, the agonist activity of varenicline is reduced by α4β2α5 receptors compared to high sensitivity α4β2 nAChR subtypes (Peng et al., 2013). In addition, varenicline has recently been demonstrated to be a full agonist at α7 and α3β4 nAChRs with a higher potency at these two subtypes than nicotine (Grady et al., 2010; Mihalak et al., 2006). However, the contribution of these nicotinic subtypes to varenicline's effects on nicotine reward is currently unknown. We therefore examined the role of α5 and α7 nAChR subunits in the effects of varenicline on nicotine reward using the conditioned place preference (CPP) test.

An important aspect of varenicline effectiveness as a smoking cessation agent is that it can reduce the severity of nicotine withdrawal (Gonzales et al., 2006; Jorenby et al., 2006; Nakamura et al., 2007). Emerging evidence from human studies indicates that co-occurring pain and pain-related cognitive processes may play a role in the maintenance of tobacco dependence (Ditre et al., 2016, 2011; LaRowe et al., 2017). Animal studies have long established that alterations in nociception and pain sensitivity occur after withdrawal from nicotine (Cohen et al., 2015; Damaj et al., 2003). Although current research in humans has not addressed the effects of varenicline on nicotine withdrawal-associated changes in pain sensitivity, animal models can be used effectively to evaluate such effects. As no studies have reported the effects of varenicline on nicotine withdrawal-induced hyperalgesia, another goal of the present study was to examine the aspects of varenicline that effect nicotine withdrawal and pain reactivity in mouse models of dependence. Additional experiments evaluated whether varenicline would reverse nicotine withdrawal-associated aversion, hyperalgesia, and other somatic signs in the same conditions.

2. Materials and methods

2.1. Animals

In the current study, ICR male mice (8 weeks upon arrival; Harlan Laboratories, Indianapolis, IN) were used to test the effect of varenicline on nicotine reward and withdrawal, unless noted otherwise. Genetically modified female and male mice (equal sexes in each group) for certain nAChRs were used to investigate possible mechanism in varenicline's effect. The genetically modified mice (null for the α5, β2 and α7 nAChR subunit and their wild-type littermates) were bred in an animal care facility at Virginia Commonwealth University (Richmond, VA) and are maintained on a C57BL/6J background with a backcrossing to at least N12 generations as described previously (Jackson et al., 2009). Male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and were used only for backcrossing.

Mice were housed four per cage with *ad libitum* access to food and water on a 12-h light cycle in a 21 °C humidity- and temperature-controlled room that was approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs

(−)-Nicotine hydrogen tartrate [(−)-1-methyl-2-(3- pyridyl) pyrrolidine (+)-bitartrate] and mecamylamine HCl (non-selective nAChR antagonist) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Varenicline (7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)(3)benzazepine) was supplied by the National Institute of Drug Abuse (NIDA Drug Supply Program, Bethesda, MD). Drugs were dissolved in physiological saline and administered subcutaneously (s.c.) and freshly prepared solutions were given to mice at 10 ml/kg. All doses are expressed as the free base of the drug. All studies were conducted by an experimenter blinded to treatment.

2.3. Conditioned place preference (CPP) studies

An unbiased five-day CPP paradigm was performed, as we previously described (Jackson et al., 2017). A three-chamber design CPP apparatus (ENV3013; Med Associates, St Albans, VT) was used to determine possible place preference or avoidance in testing nicotine and varenicline. On day 1, animals were allowed to freely move in all chambers (two conditioning chambers with a central acclimation chamber) for a 15-min duration and the baseline time spent for each chamber was recorded. A CPP box was consisted of three chambers: two outer chambers (20 × 20 × 20 cm each; white mesh & wall or black rod & wall) and a small grey chamber in the middle connected to each outer chamber with a door. White and black chambers were used to condition animals to test drug or vehicle. Based on the time spent in each conditioning chamber, animals were divided into equal group of mice whenever is possible. Mice were confined in differed chambers after vehicle or test drug administration for 20 min for a three-day conditioning period (days 2–4). These conditioning sessions were included two sessions as morning and afternoon for each day; animals were confined in one chamber (e.g. white) in the morning and in other chamber (e.g. black) in the afternoon. While control groups received saline in both morning and afternoon sessions, the drug group received nicotine, varenicline, or their combination in one session and saline in other session. Pretreatment times for nicotine and varenicline were as follows: 5 and 10 min, respectively. The drug-paired chamber was determined by randomization. Morning and afternoon sessions were 4 h apart from each other. All sessions were conducted by the same experimenter. On day 5, mice were given access to move freely in all chambers for a 15-min duration without any drug administration. The preference score was found by determining the difference between time spent in the drug paired side on day 5 versus the time in drug paired side on day 1. A significant positive response in time spent in the drug-paired chamber was interpreted as a CPP.

2.4. Nicotine precipitated withdrawal studies

In order to test whether varenicline is able to attenuate nicotine withdrawal, we used a mouse nicotine precipitated withdrawal model as previously described (Damaj et al., 2003). For this reason, mice were implanted with s.c. osmotic minipumps (model 2000; Alzet Corporation, Cupertino, CA) under isoflurane anesthesia and infused with 24 mg/kg/day nicotine or saline for 14 days (Jackson et al., 2008). Nicotine concentration was adjusted according to animal weight and minipump flow rate. On the morning of day 15, mice were pretreated with vehicle, varenicline (0.05, 0.1 and 0.5 mg/kg, s.c.) 10 min before challenge with the non-selective nAChR antagonist, mecamylamine (2 mg/kg, s.c.) to precipitate withdrawal. As described previously (Jackson et al., 2008), we evaluated the occurrence of somatic signs and hyperalgesia nicotine withdrawal signs 10 min after mecamylamine injection. Mice were put in an empty cage and observed for a 20-min period. Paw and body tremors, head shakes, backing, jumps, curls, and ptosis were considered somatic signs. First, the total number of somatic signs was evaluated for each mouse, then, the average number of observed somatic signs was determined for each test group. Hot plate test (Thermojust Apparatus, Richmond, VA) was used to