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The role of various nicotinic receptor subunits and factors influencing nicotine conditioned place aversion

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ABSTRACT

Affective nicotine withdrawal symptoms are of major motivational significance in contributing to relapse and continued tobacco use; thus, it is important to understand the molecular and receptor-mediated mechanisms that mediate affective withdrawal behaviors. Previous work using the conditioned place aversion (CPA) model has shown that nicotine withdrawal is associated with a negative affective state, and place aversion to previously neutral environmental stimuli represents a motivational component in the maintenance of drug use. Thus, the purpose of this study was to evaluate the role of genotype, sex, and age and to extend previous studies examining the role of various nicotinic receptor subtypes in the development of nicotine withdrawal aversion using the CPA model. Mice were chronically treated with nicotine and conditioned for two days with various nicotinic receptor antagonists. The major findings showed that mecamylamine and dihydro-β-erythroidine (DHβE), but not hexamethonium or methyllycaconitine citrate (MLA), precipitated significant aversion in the CPA model. This pharmacological data support our previous knockout mouse data suggesting that nicotine CPA is mediated by central β2-containing nicotinic receptors, but not α7 nicotinic receptors. Further, we show that sex and age are contributing factors to the development of nicotine CPA. Overall, the results of our study provide some insight into pharmacological and behavioral factors involved in the development of an aversive motivational component associated with nicotine withdrawal.

1. Introduction

It has been widely accepted that chronic nicotine use in humans leads to tolerance and a withdrawal syndrome consisting of both somatic and affective symptoms. While avoidance of somatic nicotine withdrawal symptoms contributes to the maintenance of smoking behavior, it is hypothesized that affective nicotine withdrawal symptoms are of greater motivational significance in contributing to relapse and continued tobacco use (Koob et al., 1993; Markou et al., 1998). Several groups have reported utilization of rodent models to assess the nicotine withdrawal syndrome by measuring physical and affective nicotine withdrawal signs. The relative lack of selective agonists and antagonists for the different nicotinic receptor subtypes has lead to the generation of several α-conotoxins, which have greater selectivity for specific nicotinic acetylcholine receptor (nAChR) subtypes, as well as genetically modified animals, such as knockout mice; thus, we have established nicotine withdrawal models in the mouse in order to use genetically modified mice to elucidate nicotinic receptor involvement in nicotine withdrawal. Previously, our laboratory characterized a nicotine withdrawal model in the mouse that allowed us to measure both physical and affective withdrawal signs in one setting (Damaj et al., 2003). More recently, we reported testing nicotinic receptor knockout mice in the conditioned place aversion (CPA) model, an affective measure of nicotine withdrawal (Jackson et al., 2008).

The CPA model measures the aversive state associated with nicotine withdrawal. It is a form of classic Pavlovian conditioning where the animal learns to avoid a compartment that was previously paired with an aversive stimulus. Previous work using this model in the rat has shown that nicotine withdrawal is associated with a negative affective state, and place aversion to previously neutral environmental stimuli represents a motivational component in the maintenance of drug use (Suzuki et al., 1996). Because the model also tests the animals in an antagonist-free state, it evaluates the important role of environmental stimuli in the maintenance of drug use. While the nicotine CPA model has been well defined in rats, this model has not been fully evaluated or characterized in mice. Indeed, the use of a mouse model would be advantageous in that it offers the possibility of exploring the

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underlying mechanisms of nicotine withdrawal through the use of genetically modified mice.

Previously, we tested β2, α7, and α5 knockout mice in the CPA model to identify the role of various nicotinic receptor subunits involved in affective nicotine withdrawal (Jackson et al., 2008); however, due to the issue of compensation in knockout animals, in the current study, we tested available antagonists for major nicotinic receptors to complement of our knockout data. Furthermore, we extended our pharmacological investigation by examining the role of various factors, such as genotype, sex, and age, on the development of nicotine CPA.

2. Methods

2.1. Animals

Male 129P3/J (129) and male and female C57Bl6/J mice were purchased from Jackson Laboratories. The mice were 8–10 weeks of age at the start of all studies. This is an adult age in the mouse, and published studies utilizing mice between 8 and 12 weeks of age do not report major behavior differences to nicotine in mice in this age range. Male adult and adolescent ICR mice were purchased from Harlan Laboratories. Adult mice were 8–10 weeks of age and weighed approximately 20–25 g at the start of the experiment. Adolescent mice were approximately 3 weeks of age [postnatal day (PND) 21] at the start of the experiment. Animals were housed in groups of five on a 12 h light/dark cycle with free access to food and water. Animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal care facility and the studies were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.2. Drugs

(−)-Nicotine hydrogen tartrate salt, mecamylamine hydrochloride, dihydro-β-erythroidine (DHβE) and methyllycaconitine citrate (MLA) were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Hexamethonium dichloride was purchased from Sigma/RBI (Natick, MA). All drugs were dissolved in physiological saline (0.9% sodium chloride) at a volume of 10 ml/kg body weight. Hexamethonium injections were administered intraperitoneally (i.p.). All other drugs were administered subcutaneously (s.c.). Doses are expressed as free base of the drug.

2.3. Chronic nicotine administration

Mice were implanted with Alzet osmotic mini pumps [model 2002 (14 days) or model 2004 (28 days) Durect Corporation, Cupertino, CA] filled with (−)-nicotine or saline solution. The concentration of nicotine was adjusted according to animal weight and the mini pump flow rate, resulting in 36 mg/kg/day for 14 or 28 days. Because the period of early adolescence lasts approximately from PND 21 to 36, adult and adolescent mice used for the age assessment were chronically infused with 48 mg/kg/day for 7 days to ensure a sufficient level of dependence. The mini pumps were surgically implanted subcutaneously under sterile conditions with sodium pentobarbital anesthesia (45 mg/kg, i.p.). An incision was made in the back of the animal, and a pump was inserted. The wound was closed with wound clips, and the animal was allowed to recover before being returned to its home cage.

2.4. Nicotine CPA

The mice were chronically exposed to nicotine for 7 or 14 days prior to initiation of testing to induce dependence. Infusion continued throughout the duration of testing. We used a biased and counterbalanced CPA protocol to measure aversion, as the biased nicotine CPA protocol is generally utilized for rat studies (Suzuki et al., 1996; Ise et al., 2002; Malin et al., 2006; O'Dell et al., 2007). The CPA apparatus consisted of a three-chambered box with a white compartment, a black compartment, and a center grey compartment separated by partitions. The black and white compartments also had different floor textures to help the mice further differentiate between the two environments. Day 1 of CPA testing (Day 8 or 15 of chronic nicotine administration) was the pre-conditioning day. The mice were placed in the grey center compartment for a 5 min habituation period, followed by a 15 min test period. During habituation, mice did not have access to the other compartments. During the test period, the partitions were raised and mice were allowed to roam freely between compartments. The CPA boxes were connected to a computer, which recorded the amount of time the mouse spent in each compartment. A pre-preference score was determined for each mouse and was used to pair the mouse with the antagonist to its initially preferred compartment. On days 2 and 3 of CPA testing, all mice received injections of saline in the morning and were immediately confined to their non-drug-paired compartment for 30 min. Four hours later, mice received an injection of antagonist and were immediately confined to their drug-paired compartment for 30 min. Day 4 was the antagonist-free test day. Mice moved freely between compartments as on day 1 and activity counts and time spent on each side were recorded via photosensors using Med Associates interface and software. Data were expressed as time spent on drug-paired side minus time spent on saline-paired side. A reduction in time spent in the initially preferred compartment was interpreted as aversion.

2.5. Statistical analysis

For all data, statistical analyses were performed using StatView® (SAS, Cary, NC, USA). All studies were analyzed with one-way ANOVAs [with treatment as the between subject factor] or two-way ANOVAs [with treatment and genotype, sex, or age as the between subject factors] using the Neuman–Keuls post hoc test. p values of less than 0.05 were considered significant.

3. Results

3.1. Acquisition of aversion in the CPA model

To determine the acquisition of aversion in the CPA model, C57BL/6 mice were subjected to one, two, or three conditioning sessions (day 2, days 2 and 3, or days 2, 3, and 4 respectively) with mecamylamine (3.5 mg/kg) before the antagonist-free test day. Test day was day 3, day 4, or day 5, based on the number of mecamylamine conditioning sessions. One mecamylamine conditioning session was sufficient to produce significant mecamylamine-precipitated aversion in chronic nicotine infused mice ($F_{(3,31)} = 6.098$, $p < 0.05$). A significant aversive response was also noted after two conditioning sessions ($F_{(3,31)} = 6.098$, $p < 0.05$); however, there was no significant effect after three days of mecamylamine conditioning (Fig. 1A). Mice were also placed in the

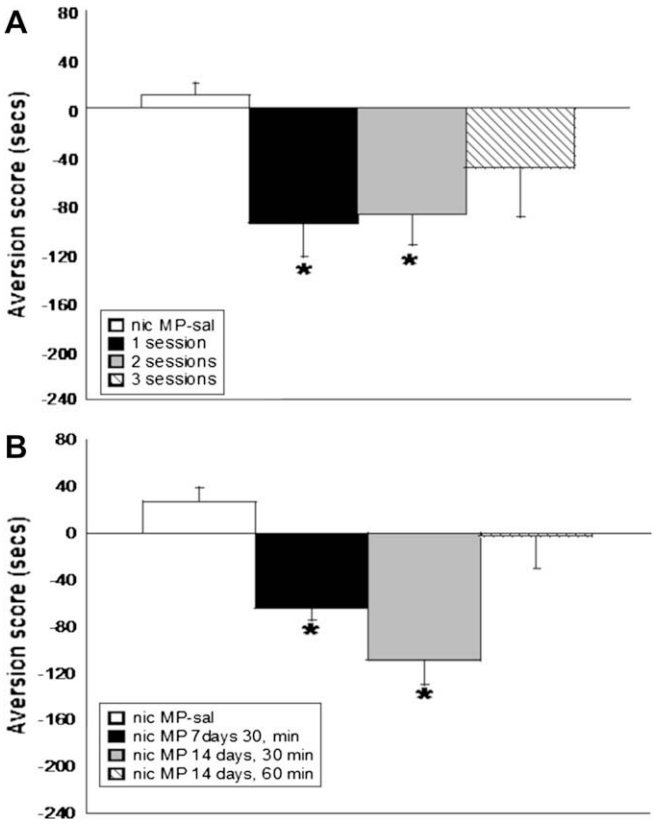

Fig. 1. Acquisition of aversion in the CPA model. (A) Mice acquire aversion in the CPA model after one and two conditioning sessions; however, no significant effect was observed after three conditioning sessions. (B) Aversion is precipitated in mice chronically infused with nicotine for 7 and 14 days prior to test initiation. 30 min conditioning sessions are sufficient to develop aversion. The effect is lost after 60 min conditioning sessions. Each point represents ±S.E.M. of 10–12 mice per group. * denotes $p < 0.05$. MP = mini pump, nic = nicotine, sal = saline.