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Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the α7 nicotinic acetylcholine receptor subunit

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Abstract

Withdrawal symptoms are a major deterrent when people try to quit smoking. The α7 subunit of the neuronal nicotinic acetylcholine receptor (nAChR) is highly expressed in the brain, and has been suspected to play a major role in nicotine addiction. We studied the influence of α7-containing nAChRs on nicotine withdrawal and tolerance, in wild type mice and mice null for the α7 nAChR subunit (α7 −/−). For withdrawal experiments, animals were implanted with osmotic minipumps delivering nicotine for 13 days. A single intraperitoneal injection of the nAChR antagonists mecamylamine (MEC) or methyllycaconitine (MLA) was used to precipitate withdrawal. In wild type mice, both MEC- and MLA-precipitated somatic signs of withdrawal such as increased grooming, scratching and shaking. In α7 −/− mice, the somatic effects of MEC-precipitated nicotine withdrawal were significantly reduced. Interestingly, the presumed α7-specific antagonist MLA also precipitated withdrawal. Tolerance, which was measured as a decrease in nicotine-induced hypolocomotion after subchronic nicotine treatment, was normal in α7 −/− mice. Finally, because anxiety and withdrawal symptoms are highly correlated in humans, we studied anxiety-like behaviors in α7 −/− mice using a battery of anxiety-related tests. The behavior of α7 −/− mice was indistinguishable from that of control mice. Our results point to the α7 subunit as one of the players in nicotine withdrawal, but not in nicotine tolerance or basal anxiety-like behavior.

Keywords: Nicotinic acetylcholine receptor; Knock out mice; α7 Subunit; Nicotine; Withdrawal; Tolerance; Anxiety; Mecamylamine; Methyllycaconitine

1. Introduction

Tobacco addiction is a prominent global health problem (Peto et al., 2000). The major addictive component of tobacco is nicotine, which acts at pentameric neuronal nicotinic acetylcholine receptors (nAChRs). These receptors comprise either α and β subunits, or α subunits only. To date, nine α and three β subunits have been cloned (Ferrari et al., 2002). Certain subunit combinations, such as α4β2, α3β4, or α7 only, predominate in neurons. Among these, the α4β2- and α7-containing combinations are the most widely expressed in the central nervous system (CNS) (Dani and De Biasi, 2001; Hogg et al., 2003).

Presynaptic α7-containing (α7*) nAChRs facilitate neurotransmitter release (Alkondon et al., 2000). Pharmacological experiments have implicated these receptors in several effects of nicotine such as aversion and reward (Laviolette and van der Kooy, 2003), anxiety-like behavior (Tucci et al., 2003) and working memory (Levin, 2002). The generation of mice carrying an α7 null mutation provided a new tool for investigating the role of this subunit in the CNS (Orr-Urtreger et al., 1997). So far, few phenotypes have been associated with this mutation in both basal conditions (Franceschini et al., 2000; Morley and Rodriguez-Sierra, 2004; Paylor et al., 1998; Stolerman et al., 2004) and in the presence of nicotine (Franceschini et al., 2002; Stolerman et al., 2004). To date, nicotine withdrawal-enhanced nociception is the only nicotine-related phenotype shown to be affected in α7 −/− mice (Grabus et al., 2005).

Abbreviations: nAChRs, nicotinic acetylcholine receptors.

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The appearance of withdrawal symptoms upon cessation of chronic nicotine exposure is one of the major factors precluding the majority of people from successfully quitting tobacco use. Understanding which type of nAChR is involved in the mechanisms underlying nicotine withdrawal is important in the quest for drugs that can treat nicotine addiction. We have shown before that β4* nAChRs have a major role in nicotine withdrawal as mice carrying a β4 null mutation display greatly reduced nicotine withdrawal symptoms (Salas et al., 2004). However, nicotine withdrawal can be precipitated in mice chronically treated with nicotine by antagonists with partial selectivity not only for α3β4-nAChR (mecamylamine: MEC), but also for α4β2-nAChR (di-hydro-β-erythroidine) or α7*-nAChR (Damaj et al., 2003). In particular, a major role for the α7 subunit in nicotine withdrawal in mice was suggested by the fact that systemic MLA precipitates withdrawal after chronic nicotine treatment (Damaj et al., 2003). Those results suggest that either multiple nAChRs participate in the withdrawal syndrome, that the subunit specificity of some of these drugs is less than usually assumed, or both.

Tolerance to the effects of drugs of abuse is commonly observed when a drug is repeatedly used, and the link among tolerance, addiction and withdrawal has been studied for more than a century (Lacaille et al., 1987). It is widely believed that tolerance plays a critical role in the development and maintenance of nicotine addiction. The α4 subunit might mediate tolerance to some of the effects of nicotine (Tapper et al., 2004), while the α7 subunit seems to have no effect on tolerance (Naylor et al., 2005).

Anxiety relief is another reason often cited by smokers when asked why they continue smoking despite the adverse consequences (Parrott, 1998). Because nAChR β4 −/− mice are insensitive to the somatic signs of nicotine withdrawal (Salas et al., 2004) and they show decreased basal anxiety-like behavior (Salas et al., 2003), we wanted to further explore the link between anxiety-like behavior and nicotine withdrawal. Others suggested that α7 −/− mice might have decreased anxiety-like behavior in the open field (Paylor et al., 1998) and therefore, we examined anxiety-related behavior in α7 −/− mice in more detail.

In summary, the present study takes advantage of α7 −/− mice to address the role of α7* nAChRs in mediating the somatic signs of nicotine withdrawal, nicotine tolerance, and basal anxiety-related behavior.

2. Materials and methods

2.1. Animals

Experiments were conducted on 2- to 5-month old wild type mice and age matched mice lacking the α7 nAChR subunit (Orr-Urtreger et al., 1997). Mice were back-crossed 9 or 10 times into a C57BL/6 background. Male and female mice were housed under a 12–12 light–dark cycle, with access to food and water *ad libitum*, and experiments were performed during the light phase. Genotypes were confirmed at the end of each experiment and were disclosed to the experimenters after completion of data analysis. All procedures were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee and followed the guidelines for animal intramural research from the National Institute of Health.

2.2. Nicotine withdrawal

Alzet pumps model 1002 (14 days, flow rate 0.25 μl/h, Durect, CA) were subcutaneously implanted according to manufacturer's instructions. Pumps were filled with either saline or nicotine tartrate in saline to deliver a 24 mg/kg/day dose of nicotine (as free base) for 13 days. The mouse nicotine withdrawal protocol, which was previously used in our laboratory (Salas et al., 2004), was based on research by Berrendero (Balerio et al., 2004) and Damaj (Damaj et al., 2003). After 13 days of either nicotine or saline infusion, mice were given intraperitoneal (ip) injections of either MEC (3 mg/kg) or MLA (7.5 mg/kg), and immediately placed in a cage where withdrawal signs were recorded for 20 min. Grooming, scratching, chewing, and shaking were the main parameters monitored. Less common behaviors (''other'' symptoms) such as cage scratching, head nodding, and wet dog shakes were also recorded (Salas et al., 2004).

2.3. Tolerance

For subchronic nicotine treatment in tolerance experiments, on day 1, mice were acclimated to the procedure by receiving two ip saline injections, 8 h apart. On days 2, 3 and 4, mice received three ip injections of either saline or 0.5 mg/kg nicotine, 8 h apart. On day 5, mice were injected with 0.5 mg/kg nicotine and locomotion was immediately measured over a 30 min session using a computer-operated tracking system (Any-maze, Stoelting, Wood Dale, IL).

2.4. Basal anxiety-related behavior

Open field activity test: drug-naïve mice were placed in the open field (clear Plexiglas, 40 × 40 × 40 cm) for 10 min. Total locomotor activity and distance moved in a center square (20 × 20 cm) were measured using a computer-operated Ethovision system (Noldus, Wageningen, The Netherlands; this system was also used in all of the other basal behavior experiments). The total/center distance moved ratio was taken as a measure of anxiety-like behavior (Paylor et al., 1998). One hour after the first exposure to the apparatus, mice were returned to the open field, but this time an unfamiliar plastic block was added to the center of the open field to measure response to novelty.

Light/dark box: this test was performed by placing the mouse in a cage (44 × 21 × 21 cm) that has two chambers: one chamber was bigger and lit, and the other was smaller and dark (Crawley and Goodwin, 1980). The animal was initially placed in the lit side, and transitions between the sides and the time spent in each division were recorded for 10 min.

Elevated plus maze: this maze consisted of four arms (25 × 7 cm, elevated 50 cm from the floor), of which two had high black walls (15 cm high), and two were without walls (Pellow et al., 1985). Mice were placed at the intersection between the arms, and the number of entries into all arms, as well as the time spent in the open arms was recorded for 5 min.

2.5. Statistical analysis

Data were examined by two-way ANOVA (genotype × treatment) followed by Duncan's post-hoc comparisons, or by Student's *t*-test, when appropriate using the Statistica (Statsoft, Tulsa, OK) package.

3. Results

3.1. Alpha 7 −/− mice show impaired MEC-precipitated nicotine withdrawal

During the 20 min of observation after injection of 3 mg/kg MEC to precipitate withdrawal, control mice chronically treated with nicotine exhibited significantly more somatic signs of withdrawal than saline-treated control mice (Fig. 1a). The behavior of saline-treated α7 −/− mice was