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Null mutation of the $\beta 2$ nicotinic acetylcholine receptor subunit attenuates nicotine withdrawal-induced anhedonia in mice

Astrid K. Stoker^{a,1}, Michael J. Marks^b, and Athina Markou^{a,*}

^aDepartment of Psychiatry, School of Medicine, University of California San Diego, La Jolla, CA 92093-0603, USA

^bInstitute for Behavioral Genetics and Department of Psychology and Neuroscience, 447 UCB, University of Colorado, Boulder, CO, USA

Abstract

The anhedonic signs of nicotine withdrawal are predictive of smoking relapse rates in humans. Identification of the neurobiological substrates that mediate anhedonia will provide insights into the genetic variations that underlie individual responses to smoking cessation and relapse. The present study assessed the role of $\beta 2$ nicotinic acetylcholine receptor (nACh receptor) subunits in nicotine withdrawal-induced anhedonia using $\beta 2$ nACh receptor subunit knockout ($\beta 2^{-/-}$) and wildtype ($\beta 2^{+/+}$) mice. Anhedonia was assessed with brain reward thresholds, defined as the current intensity that supports operant behavior in the discrete-trial current-intensity intracranial self-stimulation procedure. Nicotine was delivered chronically through osmotic minipumps for 28 days (40 mg/kg/day, base), and withdrawal was induced by either administering the broad-spectrum nicotinic receptor antagonist mecamylamine (i.e., antagonist precipitated withdrawal) in mice chronically treated with nicotine or terminating chronic nicotine administration (i.e., spontaneous withdrawal). Mecamylamine (6 mg/kg, salt) significantly elevated brain reward thresholds in nicotine-treated $\beta 2^{+/+}$ mice compared with saline-treated $\beta 2^{+/+}$ mice and nicotine-treated $\beta 2^{-/-}$ mice. Spontaneous nicotine withdrawal similarly resulted in significant elevations in thresholds in nicotine-withdrawing $\beta 2^{+/+}$ mice compared with saline-treated $\beta 2^{+/+}$ and nicotine-treated $\beta 2^{-/-}$ mice, which remained at baseline levels. These results showed that precipitated and spontaneous nicotine withdrawal-induced anhedonia was attenuated in $\beta 2^{-/-}$ mice. The reduced expression of anhedonic signs during nicotine withdrawal in $\beta 2^{-/-}$ mice may have resulted from the lack of neuroadaptations in $\beta 2$ nACh receptor subunit expression and function that may have occurred during either nicotine exposure or nicotine withdrawal in wildtype mice. In conclusion, individuals with genetic variations that result in diminished function of the $\beta 2$ nACh receptor subunit may experience less anhedonia during nicotine withdrawal, which may facilitate smoking cessation.

Keywords

Nicotine; Nicotinic Acetylcholine Receptor; Beta2; Intracranial Self-stimulation; Anhedonia

1. Introduction

Negative mood states, such as anhedonia, that characterize tobacco withdrawal (American Psychiatric Association, 2000) have been associated with craving and relapse to tobacco smoking in humans (Hatsukami et al., 1989; Hughes, 2007; Leventhal et al., 2014). Characterization of the influence of genetic variations on anhedonia-like signs of tobacco withdrawal may have predictive value for the incidence of relapse to smoking during a quit attempt for individuals. Such information may also lead to the identification of new targets for smoking cessation medications.

The reinforcing effects of nicotine, the main psychoactive ingredient in tobacco, are mediated by the activation of nicotinic acetylcholine receptors (nACh receptors). Neuronal nACh receptors are homomeric or heteromeric combinations of five subunits ($\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$; (Clementi et al., 2000)). The $\beta 2$ nACh receptor subunit is the focus of the present study and predominantly forms heteromeric nACh receptor structures with $\alpha 4$ nACh receptor subunits. The $\alpha 4\beta 2^*$ nACh receptor is an important mediator of the reinforcing effects of nicotine (Picciotto et al., 1998) and widespread throughout the central nervous system (Hill et al., 1993). Because of the high affinity of $\alpha 4\beta 2^*$ nACh receptors for nicotine (Parker et al., 1998), the effects of the first cigarette of the day, which human smokers often consider the most rewarding (Benowitz, 1996), can be largely attributed to activation of $\alpha 4\beta 2^*$ nACh receptors. Although the activation of $\alpha 4\beta 2^*$ nACh receptors results in long desensitization times (Fenster et al., 1997), nicotine concentrations that are physiologically relevant to human smokers result in the upregulation of $\alpha 4\beta 2^*$ nACh receptors (Abdollahi et al., 2010). Because nACh receptor upregulation is considered an important feature of nicotine dependence, the indication that $\alpha 4\beta 2^*$ nACh receptors may be upregulated in smokers further emphasizes the importance of the role of these receptors in nicotine dependence.

The involvement of $\beta 2$ nACh receptor subunits in the anhedonic signs of nicotine withdrawal has not been studied extensively. The present study assessed anhedonia-like behavior in $\beta 2$ knockout ($\beta 2^{-/-}$) and wildtype ($\beta 2^{+/+}$) mice during spontaneous nicotine withdrawal (i.e., withdrawal induced by the cessation of chronic nicotine administration) and precipitated nicotine withdrawal (i.e., withdrawal induced by administration of a broad-spectrum nACh receptor antagonist). The anhedonic signs of nicotine withdrawal can be assessed in mice using the intracranial self-stimulation (ICSS) procedure (Stoker and Markou, 2011). The ICSS procedure used in the present study assesses brain reward function with current-intensity thresholds. Nicotine withdrawal elevated current-intensity thresholds in wildtype C57BL/6J mice (Johnson et al., 2008; Stoker et al., 2008), which is indicative of anhedonia. Jackson et al. (2008) previously reported that the affective signs of nicotine withdrawal, measured with the conditioned place aversion and elevated plus maze procedures, were attenuated in $\beta 2^{-/-}$ mice. Based on these studies, we hypothesized that

nicotine withdrawal-induced anhedonia in the intracranial self-stimulation procedure will be similarly attenuated in $\beta 2^{-/-}$ mice.

2. Materials and Methods

2.1. Experimental subjects

The $\beta 2$ nACh receptor subunit knockout mouse line was generated as described previously (Picciotto et al., 1995). $\beta 2$ knockout and wildtype mice were bred at the animal facilities of the University of Colorado Boulder and tested at the University of California San Diego. The mice were maintained in groups of two to five until electrode implantation surgery, after which the mice were housed individually. The subjects were males, 12–18 weeks of age at the time of surgery. The animal facility in which the mice were housed was humidity- and temperature-controlled with a reverse 12 h/12 h light/dark cycle (lights off at 7:00 AM). The mice had *ad libitum* access to food and water except during testing. Testing occurred in the dark cycle with a red light that illuminated the testing room, with the exception of the 12 h post-pump removal time-point, which occurred during the light cycle. The animal care, breeding, and experimental protocols were in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and National Research Council's Guide for the Care and Use of Laboratory Animals. The animal care and breeding protocols were approved by the Animal Care and Utilization Committee of the University of Colorado, Boulder. The animal care and experimental protocols were approved by the University of California San Diego Institutional Animal Care and Use Committee.

2.2. Drugs

Nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline solution and infused through 28-day subcutaneous osmotic minipumps (model 2004, Alzet, Palo Alto, CA, USA). Mecamylamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline and injected subcutaneously (s.c.) in a volume of 0.1 ml/10 g. The nicotine doses are reported as the base, and the mecamylamine doses are reported as the salt.

2.3. Minipump implantation and removal

A mixture of isoflurane (1–3%)/oxygen was used to anesthetize the mice before aseptic subcutaneous insertion of the osmotic minipumps that contained nicotine or vehicle solution. An incision was made at the shoulder level, and the minipumps were implanted parallel to the spine with the flow moderator inserted away from the wound site. After 28 days of chronic nicotine or vehicle administration, aseptic surgery techniques were used to remove the minipumps under isoflurane anesthesia. Stainless steel wound clips (7 mm, Reflex, Cellpoint Scientific, Gaithersburg, MD, USA) were used for all wound closures.

2.4. Intracranial self-stimulation: surgery, apparatus, and procedure

ICSS surgery, the apparatus, and the procedure were described in detail previously (Gill et al., 2004; Stoker et al., 2008). The mice were anesthetized with a mixture of 1–3% isoflurane in oxygen and positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) for the implantation of stainless steel bipolar electrodes (0.20 mm diameter; 6 mm

length; Plastics One, Roanoke, VA, USA) into the lateral hypothalamus (coordinates: anterior/posterior, 1.58 mm; medial/lateral, 1.0 mm; dorsal/ventral, 5.3 mm from flat skull; (Paxinos and Franklin, 2001) as described previously (Gill et al., 2004; Stoker et al., 2008)). The electrode was kept in place by affixing four stainless steel screws (3.2 mm length, Plastics One, Roanoke, VA, USA) to the skull with a resin ionomer (Den-Mat, Santa Maria, CA, USA) and dental acrylic (Ortho-Jet, Lang Dental, Wheeling, IL, USA). The mice were given 1 week to recover from surgery before initiating ICSS training.

ICSS training and testing were conducted in eight operant chambers (30.5 × 24 × 27 cm; Med Associates, St. Albans, VT, USA) with a wheel manipulandum (5.5 cm diameter, 4 cm width) for operant responding. Constant-current stimulators (Stimtek model 1200c, San Diego Instruments, San Diego, CA, USA) provided intracranial stimulation through gold-contact swivel commutators (model SL2C, Plastics One, Roanoke, VA, USA) connected to the head-mounted electrode with flexible bipolar leads (Plastics One, Roanoke, VA, USA).

The mice were trained and tested using the current-intensity threshold procedure, in which the current intensities varied according to the classical psychophysical method of limits described previously (Kornetsky and Esposito, 1979; Stoker et al., 2008). All of the mice were originally presented with stimuli that consisted of 50 pulses (100 μ s duration) and an interpulse interval of 10,000 μ s, resulting in a 495 ms stimulation train duration with a frequency of 100 Hz. The initial current intensity presented to the mice was set approximately 30 μ A above the baseline threshold of each mouse. Current intensity thresholds were assessed with a series of four alternating descending and ascending trials. Each given current intensity was presented in a block of three trials, and the current intensity changed in steps of 5 μ A between blocks. The midpoint between the current intensity at which a mouse made two or more positive responses per block of trials and the current intensity at which the mouse made fewer than two positive responses defined the threshold, which was the average of the four ascending and descending series presented during each testing session. If the thresholds of a subject were below 70 μ A during any one of the first seven test sessions, then the stimulus parameters were set to half of the original frequency (25 pulses, 100 μ s duration, and 20,000 μ s interpulse interval, resulting in a stimulation train duration of 482.5 ms with a 50 Hz frequency). The lowering of these stimulus parameters was implemented to avoid potential floor effects. In addition to threshold values, response latency was assessed by averaging the time (in seconds) that elapsed between the delivery of the stimulus and operant response for all of the trials that resulted in a positive response.

2.5. Experimental design

Experimentally naive $\beta 2^{-/-}$ and $\beta 2^{+/+}$ mice were surgically prepared with electrodes implanted into the lateral hypothalamus. After successful training in the ICSS procedure, thresholds were stabilized for a minimum of 3 weeks. Upon the stabilization of performance in the ICSS procedure (defined as 10% variation in brain reward thresholds over three consecutive daily test sessions), 28-day subcutaneous osmotic minipumps that delivered vehicle or 40 mg/kg/day nicotine (base) were surgically implanted. The mice were tested once daily during chronic nicotine administration. Mecamylamine (0, 1.5, 3, and 6 mg/kg, salt) was administered subcutaneously on days 9, 11, 13, and 15 of chronic nicotine/vehicle

exposure using a within-subjects Latin-square design, and the mice were immediately placed into the operant chambers for testing. The minipumps were removed after 28 days, and withdrawal signs were tested in the ICSS procedure 3, 6, 8, 12, 24, 28, 48, 52, 72, 76, 96, and 100 h after pump removal.

2.6. Statistical analyses

The statistical analyses were performed using the PASW 18 Statistical Package (SPSS, Chicago, IL, USA). The data were analyzed using mixed-design three-way analyses of variance (ANOVAs), with *Genotype* and *Nicotine/Vehicle Treatment* as the between-subjects factors and *Administration Day*, *Withdrawal Day* or *Mecamylamine Dose* as the within-subjects factors. The level of significance was 0.05. Statistically significant interaction effects in the ANOVAs were followed by Newman-Keuls *post hoc* analyses. Thresholds and response latencies during the 28-day administration period of nicotine/vehicle are expressed as a percentage of the baseline value, which was defined as the 5-day average before minipump implantation. For mecamylamine-precipitated nicotine withdrawal, thresholds and response latencies are expressed as a percentage of the baseline values on the preceding day. The thresholds and response latencies during spontaneous nicotine withdrawal are expressed as a percentage of the baseline value defined as the average of the last five ICSS sessions before minipump removal. The data time-points were combined for the analysis of the spontaneous nicotine withdrawal data (3 + 6 h, 8 + 12 h, 24 + 28 h, 48 + 52 h, 72 + 76 h, and 96 + 100 h) for a robust and reliable assessment of nicotine withdrawal-induced anhedonia. The following numbers of mice were excluded from the analyses because of the loss of the electrode head mount or malfunctioning of one of the commutators: saline-treated $\beta 2^{+/+}$, $n = 3$; nicotine-treated $\beta 2^{+/+}$, $n = 2$; saline-treated $\beta 2^{-/-}$, $n = 1$; nicotine-treated $\beta 2^{-/-}$, $n = 1$.

3. Results

Chronic 28-day nicotine administration did not affect ICSS thresholds or response latencies in wildtype or knockout mice compared with their vehicle-treated counterparts, reflected by the absence of significant interaction or main effects in the ANOVA (Fig. 1).

The highest dose of 6 mg/kg mecamylamine significantly elevated brain reward thresholds in nicotine-withdrawing $\beta 2^{+/+}$ mice compared with vehicle-“withdrawing” $\beta 2^{+/+}$ mice and nicotine-withdrawing $\beta 2^{-/-}$ mice (Fig. 2). The three-way repeated-measures ANOVA revealed a statistically significant *Mecamylamine Dose* $\times$ *Genotype* interaction ($F_{1,63} = 4.961$, $p < 0.05$) and significant main effects of *Mecamylamine Dose* ($F_{3,189} = 6.26$, $p < 0.001$) and *Genotype* ($F_{1,63} = 4.965$, $p < 0.05$) but no three-way interactions or main effect of *Nicotine/Vehicle Treatment*. Preplanned comparisons indicated significant elevations in ICSS thresholds in nicotine-treated $\beta 2^{+/+}$ mice after 6 mg/kg mecamylamine administration compared with vehicle-treated $\beta 2^{+/+}$ mice treated with 6 mg/kg mecamylamine ($p < 0.05$) and nicotine-treated $\beta 2^{-/-}$ mice ($p < 0.05$). Response latencies were not significantly affected by mecamylamine administration, reflected by the absence of a significant *Nicotine/Vehicle Treatment* $\times$ *Mecamylamine Dose* $\times$ *Genotype* interaction (data not shown).

During spontaneous nicotine withdrawal, brain reward thresholds were elevated in nicotine-withdrawing $\beta 2^{+/+}$ mice compared with vehicle-“withdrawing” $\beta 2^{+/+}$ mice, nicotine-withdrawing $\beta 2^{-/-}$ mice, and saline-“withdrawing” $\beta 2^{-/-}$ mice (Fig. 3). The three-way repeated measures ANOVA revealed a significant *Nicotine/Vehicle Treatment* $\times$ *Genotype* $\times$ *Withdrawal Day* interaction ($F_{6,378} = 4.961, p < 0.05$). *Post hoc* analyses indicated significant elevations in ICSS thresholds in nicotine-treated $\beta 2^{+/+}$ mice on days 1 and 2 of withdrawal compared with vehicle-treated $\beta 2^{+/+}$ mice ($p < 0.01$) and nicotine-treated $\beta 2^{-/-}$ mice ($p < 0.01$). Response latencies were not affected by precipitated nicotine withdrawal (data not shown).

4. Discussion

The anhedonic signs of early nicotine withdrawal were significantly attenuated in $\beta 2^{-/-}$ mice compared with $\beta 2^{+/+}$ mice. Specifically, nicotine withdrawal precipitated by the broad-spectrum nACh receptor antagonist mecamylamine (6 mg/kg) resulted in significant elevations of brain reward thresholds in nicotine-treated $\beta 2^{+/+}$ mice compared with vehicle-treated $\beta 2^{+/+}$ mice and nicotine-treated $\beta 2^{-/-}$ mice, which remained at baseline levels after mecamylamine administration. Spontaneous nicotine withdrawal, induced by the cessation of chronic nicotine administration via subcutaneous osmotic minipumps, significantly elevated brain reward thresholds in nicotine-withdrawing $\beta 2^{+/+}$ mice but not $\beta 2^{-/-}$ mutants. Attenuation of the anhedonic signs of nicotine withdrawal is consistent with the results of a previous study that demonstrated that other affective signs of nicotine withdrawal (i.e., conditioned placed aversion and anxiety-like behavior in the elevated plus maze) were also attenuated in $\beta 2^{-/-}$ mice (Jackson et al., 2008).

Importantly, the attenuation of nicotine withdrawal-induced anhedonia in $\beta 2^{-/-}$ mice may have resulted from the lack of neuroadaptations that occur during either nicotine exposure or nicotine withdrawal in wildtype mice. $\beta 2$ knockout mice did not acquire nicotine self-administration intravenously (Picciotto et al., 1998) or into the ventral tegmental area (Maskos et al., 2005). Similarly, nicotine-induced conditioned place preference was absent in $\beta 2^{-/-}$ mice (Walters et al., 2006). The crucial role for $\beta 2$ nACh receptor subunits in the VTA in nicotine dependence is further suggested by the demonstration of the location of these nACh receptor subunits on dopaminergic neurons that extend from the VTA to nucleus accumbens (NAc; (Mameli-Engvall et al., 2006)), an important pathway in the regulation of nicotine dependence. The decrease in firing activity of this dopaminergic projection from the VTA to NAc in $\beta 2^{-/-}$ mice may explain the absence of nicotine self-administration in these mice. Importantly, however, although $\beta 2^{-/-}$ mice did not immediately acquire nicotine self-administration behavior in a two-bottle choice nicotine consumption procedure, these mice steadily increased their nicotine consumption over the course of several weeks (Levin et al., 2009). The results of this study that used a two-bottle choice procedure suggest that the $\beta 2$ nACh receptor subunit is not essential for the acquisition of nicotine self-administration, which is further supported by genetic linkage studies in humans that showed that genetic variation in the $\beta 2$ nACh receptor subunit is not linked to nicotine dependence or smoking behavior (Keskitalo-Vuokko et al., 2011; Lueders et al., 2002; Silverman et al., 2000).

The role of the $\beta 2$ nACh receptor subunit in nicotine withdrawal was suggested by the results of several studies in both humans and rodents. Specifically, in humans, the early stages of smoking cessation were characterized by increased levels of $\beta 2^*$ nACh receptors (Staley et al., 2006). These increases in $\beta 2$ nACh receptor subunit expression and function that occur during nicotine withdrawal in healthy individuals may result in the expression of anhedonic signs during nicotine withdrawal. Varenicline, a partial agonist at $\alpha 4\beta 2$ nACh receptors (Coe et al., 2005), attenuated anhedonia during spontaneous nicotine withdrawal in the ICSS procedure in rats (Igari et al., 2013), further suggesting the involvement of the $\beta 2$ nACh receptor subunit in the negative mood state induced by nicotine withdrawal. Nicotine withdrawal-induced conditioned place aversion and anxiety-like behavior in the elevated plus maze (Jackson et al., 2008) were also absent in $\beta 2^{-/-}$ mice. The somatic signs of nicotine withdrawal, which have been suggested to be regulated peripherally rather than centrally (Watkins et al., 2000); but see (Malin et al., 1997)), were unaffected in $\beta 2^{-/-}$ mice, in which nicotine withdrawal resulted in similar increases in somatic signs in knockout and wildtype mice (Salas et al., 2004b). In contrast, somatic signs were significantly attenuated in $\beta 4^{-/-}$ mice (Salas et al., 2004a; Stoker et al., 2012).

In conclusion, the attenuation of nicotine withdrawal-induced anhedonia in $\beta 2^{-/-}$ mice may have resulted from the absence of neuroadaptations in $\beta 2$ nACh receptor subunit expression or function that occurred during either nicotine exposure or nicotine withdrawal in wildtype mice. Studies in humans indicate that anhedonia during smoking cessation leads to craving and relapse (Hatzigiakoumis et al., 2011; Hughes, 2007). The results of the present study suggest that humans with genetic variations that result in diminished function of the $\beta 2$ nACh receptor subunit may experience less signs of anhedonia during nicotine withdrawal, which may facilitate smoking cessation in these individuals.

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Figure 1. Effects of chronic nicotine administration on ICSS thresholds in $\beta 2^{-/-}$ and $\beta 2^{+/+}$ mice
 Threshold data are expressed as the area under the curve $\pm$ SEM for days 1 to 8 and 16 to 27.
 Data from days 9 to 15 are not displayed because the nACh receptor antagonist mecamylamine was administered during these days to precipitate nicotine withdrawal.
 Thresholds in nicotine-treated $\beta 2^{-/-}$ or $\beta 2^{+/+}$ mice did not differ significantly from their respective saline-treated counterparts.

Figure 2. Effects of mecamylamine-precipitated nicotine withdrawal on ICSS thresholds in $\beta 2^{-/-}$ and $\beta 2^{+/+}$ mice

The data are expressed as the mean number of thresholds $\pm$ SEM. Asterisks indicate significant elevations in ICSS thresholds in nicotine-treated $\beta 2^{+/+}$ mice after administration of 6 mg/kg mecamylamine compared with vehicle-treated $\beta 2^{+/+}$ mice ($*p < 0.05$), nicotine-treated $\beta 2^{-/-}$ mice ($*p < 0.05$), and saline-treated $\beta 2^{-/-}$ mice ($p < 0.05$).

Figure 3. Effects of spontaneous nicotine withdrawal on ICSS thresholds in $\beta 2^{-/-}$ and $\beta 2^{+/+}$ mice
 The data are expressed as the mean number of thresholds $\pm$ SEM. Asterisks indicate significant elevations in ICSS thresholds in nicotine-treated $\beta 2^{+/+}$ mice on Days 1 and 2 of withdrawal compared with vehicle-treated $\beta 2^{-/-}$ mice (** $p < 0.01$), nicotine-treated $\beta 2^{-/-}$ mice (## $p < 0.01$), and saline-treated $\beta 2^{-/-}$ mice ($p < 0.01$).