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The α3β4* nicotinic acetylcholine receptor subtype mediates nicotine reward and physical nicotine withdrawal signs independently of the α5 subunit in the mouse

Kia J. Jackson [a], Sarah S. Sanjakdar [b], Pretal P. Muldoon [b], J. Michael McIntosh [c,d], M. Imad Damaj [b,*]

[a] Department of Psychiatry, Virginia Commonwealth University, 800 E. Leigh St., Biotech I, Suite 390A, Richmond, VA 23219, USA
[b] Department of Pharmacology and Toxicology, Virginia Commonwealth University, 1220 E. Broad St., Molecular Medicine Research Building, Box 980613, Richmond, VA 23298-0613, USA
[c] Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
[d] Department of Psychiatry, University of Utah, Salt Lake City, UT 84112, USA

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ABSTRACT

The 15q25 gene cluster contains genes that code for the α5, α3, and β4 nicotinic acetylcholine receptor (nAChRs) subunits, and in human genetic studies, has shown the most robust association with smoking behavior and nicotine dependence to date. The limited available animal studies implicate a role for the α5 and β4 nAChR subunits in nicotine dependence and withdrawal; however studies focusing on the behavioral role of the α3β4* nAChR receptor subtype in nicotine dependence are lacking. Because of the apparent role of the α3β4* nAChR subtype in nicotine dependence, the goal of the current study was to better evaluate the involvement of this subtype in nicotine mediated behavioral responses. Using the selective α3β4* nAChR antagonist, α-conotoxin AuIB, we assessed the role of α3β4* nAChRs in acute nicotine, nicotine reward, and physical and affective nicotine withdrawal. Because α5 has also been implicated in nicotine dependence behaviors in mice and can form functional receptors with α3β4*, we also evaluated the role of the α3β4α5* nAChR subtype in nicotine reward and somatic nicotine withdrawal signs by blocking the α3β4* nAChR subtype in α5 nAChR knockout mice with AuIB. AuIB had no significant effect on acute nicotine behaviors, but dose-dependently attenuated nicotine reward and physical withdrawal signs, with no significant effect in affective withdrawal measures. Interestingly, AuIB also attenuated nicotine reward and somatic signs in α5 nAChR knockout mice. This study shows that α3β4* nAChRs mediate nicotine reward and physical nicotine withdrawal, but not acute nicotine behaviors or affective nicotine withdrawal signs in mice. The α5 subunit is not required in the receptor assembly to mediate these effects. Our findings suggest an important role for the α3β4* nAChR subtype in nicotine reward and physical aspects of the nicotine withdrawal syndrome.

1. Introduction

A major in nicotine research is to gain a better understanding of the molecular and receptor mediated mechanisms of nicotine dependence and withdrawal in order to generate more effective smoking cessation therapies. The initial targets for nicotine are nicotinic acetylcholine receptors (nAChRs), ligand-gated ion channels which have been identified throughout the central and peripheral nervous systems, as well as at skeletal neuromuscular junctions. While animal studies implicate a significant role for β2-containing nAChRs in nicotine reward, dependence, and withdrawal (Picciotto et al., 1998; Walters et al., 2006; Jackson et al., 2008, 2009b), to date, the 15q25 gene cluster, which contains the CHRNA5/CHRNA3/CHRNB4 genes, coding for the α5, α3, and β4 nAChR subunits respectively, has shown the most robust findings in human genetic studies as a candidate region contributing to risk of heavy smoking, nicotine dependence, and smoking related diseases in humans (Berrettini et al., 2008; Bierut et al., 2008; Liu et al., 2010; Lips et al., 2010; Ware et al., 2011).

The α5 nAChR subunit co-assembles with α3β4* nAChR subtypes (where * denotes the possible inclusion of additional subunits) to form functional receptors in the peripheral ganglia, as well as centrally, in the medial habenula (MHb) and interpeduncular nucleus (IPN) (Wada et al., 1990; Zoli et al., 1995; Quick et al., 1999; Whiteaker et al., 2002). Despite findings of robust associations with the 15q25 gene cluster, rodent studies assessing the role of α3, β4, and α5 nAChRs are limited, though available studies do implicate a role for these nAChRs in nicotine mediated behaviors.

* Corresponding author. Tel.: +1 804 828 1676; fax: +1 804 828 2117.
E-mail address: mdamaj@vcu.edu (M.I. Damaj).

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In the central nervous system (CNS), the mesolimbic dopamine pathway contains some α3β4* (where * denotes the possible inclusion of additional subunits) subtypes; however, the majority of this receptor subtype resides in the medial habenula (MHb) and interpeduncular nucleus (IPN) (Wada et al., 1990; Zoli et al., 1995; Quick et al., 1999; Whiteaker et al., 2002). In these brain regions, the α5 nAChR subunit co-assembles with α3β4* nAChR subtypes to various degrees (15–35% of the α3β4* subtypes at the MHb and IPN, respectively) (Grady et al., 2009). Importantly, α5-containing nAChRs in the MHb and IPN have recently been implicated in nicotine self-administration and reward. α5 nAChR knockout (−/−) mice are less sensitive to the acute behavioral effects of nicotine, and maintain expression of a significant nicotine conditioned place preference (CPP) at nicotine doses that are aversive in wild-type (+/+) littermates (Jackson et al., 2010). An increased nicotine intake was also observed in α5 nAChR −/− mice. This effect was 'rescued' in −/− mice by re-expressing α5 subunits in the MHb, and recapitulated in rats through α5 subunit knockdown in the MHb (Fowler et al., 2011). In addition, mice null for the α5 nAChR subunit exhibit a reduction in nicotine withdrawal somatic signs (Jackson et al., 2008; Salas et al., 2009).

The role of α3 and β4 nAChR subunits in nicotine's behavioral effects is starting to emerge. Studies using nicotinic knock-out mice, α3 and β4 nAChRs were found to be necessary for nicotine-induced seizures in mice (Kedmi et al., 2004; Salas et al., 2004a,b), while β4 nAChRs partially mediate acute nicotine-induced hypolocomotion (Salas et al., 2004a,b), hypothermic response (Sack et al., 2005), and antinociception (Semenova et al., 2012). Furthermore, withdrawal somatic signs and hyperalgesia are reduced in β4 nAChR −/− mice after nicotine withdrawal (Salas et al., 2004a,b; Stoker et al., 2012). β4 nAChR subunits were also found to be involved in affective nicotine withdrawal measures, as β4 nAChR −/− mice do not display elevated intracranial self-stimulation thresholds following nicotine withdrawal (Stoker et al., 2012). Currently, evidence of a clear behavioral role for α3 nAChR subunits in nicotine withdrawal is lacking. Selective α3β4* antagonists are becoming available to delineate the role of α3β4* nAChR subtypes in nicotine dependence. For example, the peptide α-conotoxin AuIB (AuIB), which was originally isolated from the venom of the predatory cone snail, *Conus aulicus*, was shown to most potently block α3β4 nAChRs as expressed in *Xenopus oocytes* (Luo et al., 1998). The peptide was subsequently synthesized, and synthetic material was used in subsequent studies to block native CNS α3β4 nAChRs in hippocampus (Luo et al., 1998; Kulak et al., 2001), locus coeruleus (Fu et al., 1999), interpeduncular nucleus (Grady et al., 2001), and medial habenula (McCallum et al., 2012). More recently, AT-1001, a relatively selective α3β4* antagonist, was reported to dose-dependently block nicotine intravenous self-administration in rats after systemic injection (Toll et al., 2012).

Because of the apparent role of the α3β4* nAChR subtypes in nicotine dependence, the goal of the current study was to better evaluate the involvement of this subtype in nicotine mediated behavioral responses. Thus, we used the α3β4* selective antagonist AuIB to question α3β4* nAChR contributions in acute nicotine behaviors, nicotine reward using the CPP model, and physical (somatic signs, hyperalgesia) and affective (anxiety-related behavior) nicotine withdrawal using a spontaneous nicotine withdrawal model. As an additional measure of affective nicotine withdrawal, we assessed the effects of AuIB in the conditioned place aversion (CPA) model. Further, we determined the contributions of α5 subunits to the effects of the selective α3β4* antagonist AuIB to nicotine reward and somatic nicotine withdrawal by measuring expression of nicotine CPP and nicotine withdrawal somatic signs in α5 nAChR −/− mice after pre-treatment with AuIB.

2. Methods

2.1. Animals

Male adult C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice null for the α5 nicotinic receptor subunit (C57BL/6 background) and +/+ littermates were shipped from Baylor College of Medicine, Houston, Texas (see Salas et al., 2003 for information regarding initial breeders) and were subsequently bred in an animal care facility at Virginia Commonwealth University. Mutant and +/+ mice were obtained by breeding heterozygote pairs. Male adult α5 nAChR +/+ and −/− mice were used for all α5 studies. Animals were 8–10 weeks of age, were group-housed in a 21 °C humidity-controlled AAALAC-approved animal care facility with *ad libitum* access to food and water. Experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.2. Drugs

(−)-Nicotine hydrogen tartrate salt and mecamylamine hydrochloride were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). The α3β4*-selective antagonist AuIB was synthesized as described by Luo et al. (1998). AuIB, purified from the venom of the "court cone", *Conus aulicus*, blocks the α3β4* receptor subtype with >100 fold higher potency than other receptor combinations, such as α3β2* and α4β4* (Luo et al., 1998). The compounds were dissolved in physiological saline (0.9% sodium chloride). Nicotine was administered by subcutaneous (s.c.) injection or through minipump infusion. AuIB was administered via intracerebroventricular (i.c.v.) injection. AuIB doses were calculated based on the functional IC_{50} at α3β4 nAChRs (Luo et al., 1998).

2.3. I.c.v. surgery

C57Bl/6J and α5 nAChR +/+ and −/− mice were anesthetized with sodium pentobarbital (45 mg/kg i.p.) on the evening prior to testing, and a scalp incision was made to expose the bregma. Unilateral injection sites were prepared using a 26-gauge needle with a sleeve of polyurethane tubing to control depth of the needle at a site 2 mm rostral and 2 mm lateral to the bregma at a depth of 2 mm. Animals were sutured in such a way to enable an injection volume of 5 μl using a 26-gauge needle with a sleeve of polyurethane tubing into the lateral ventricle on the morning of testing. The needle was held in place for 20 s to ensure drug delivery.

2.4. Acute nicotine assessment

Naïve C57Bl/6J mice were injected i.c.v. with vehicle or AuIB (14 or 70 pmol) 5 min prior to a single s.c. injection with nicotine or saline. Antinociception using the tail-flick and hot-plate tests, changes in body temperature, and changes in locomotor activity were measured 5 or 30 min following nicotine or saline injection.

2.4.1. Antinociception

Antinociception was assessed by the tail-flick method of D'Amour and Smith (1941) and the hot plate test ($n = 6$ per group). In the tail-flick test, mice were lightly restrained while a radiant heat source was directed onto the upper portion of the tail. A control response (2–4 s) was determined for each mouse before treatment, and test latency was determined 5 min after nicotine administration (2.5 mg/kg, s.c.). The apparatus has an automatic cut-off of 10 s to minimize tissue damage. The response for the tail-flick was calculated as percentage of maximum possible effect (% MPE), where % MPE = [(test − control)/(10 − control)] × 100. In the hot plate test, mice were placed into a 10-cm wide glass cylinder on a hot-plate (Thermojust Apparatus, Columbus, OH). The hot plate is a rectangular heated surface surrounded by plexiglass and maintained at 55 °C. The device is connected to a manually operated timer that records the amount of time the mouse spends on the heated surface before showing signs of nociception (e.g. jumping, paw licks). A control response (8–12 s) was determined for each mouse before treatment, and test latency was determined 5 min after nicotine administration (2.5 mg/kg, s.c). The timer has an automatic cut-off of 40 s to avoid tissue damage. The hot plate response was calculated as percentage of maximum possible effect (% MPE), where % MPE = [(test − control)/(40 − control)] × 100. While the dose of nicotine used in these studies (2.5 mg/kg) does significantly impact locomotor activity of the animal in motor activity assessments, by our observation it does not impair the mouse to the point that it is unable to flick its tail or exhibit nociceptive behaviors (e.g. paw licking, jumping) in the antinociceptive tests.

2.4.2. Body temperature

Rectal temperature ($n = 6$ per group) was measured by a thermistor probe (inserted 24 mm) and digital thermometer (YSI Inc., Yellow Springs, OH). Readings were taken just before and at 30 min after nicotine injection (1.5 mg/kg, s.c.). The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory varied from 21 to 24 °C from day to day.