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Chronic treatment with varenicline changes expression of four nAChR binding sites in mice

Michael J. Marks [a, b], Heidi C. O'Neill [a], Kelly M. Wynalda-Camozzi [d], Nick C. Ortiz [a], Emily E. Simmons [a], Caitlin A. Short [a], Christopher M. Butt [d], J.Michael McIntosh [c], Sharon R. Grady [a, *]

[a] Institute for Behavioral Genetics, University of Colorado, Boulder, CO, USA
[b] Department of Psychology and Neuroscience, University of Colorado, Boulder, CO, USA
[c] Departments of Biology and Psychiatry, University of Utah, Salt Lake City, UT, USA
[d] DSM Nutritional Products Biological Sciences 4, Boulder, CO, USA

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ABSTRACT

Introduction: Chronic treatment with nicotine is known to increase the α4β2-nAChR sites in brain, to decrease α6β2-nAChR sites and to have minimal effect on α3β4-and α7-nAChR populations. Varenicline is now used as a smoking cessation treatment, with and without continued smoking or nicotine replacement therapy. Varenicline, like nicotine, upregulates the α4β2-nAChR sites; however, it is not known whether varenicline treatment changes expression of the other nAChR subtypes.

Methods: Using a mouse model, chronic treatments (10 days) with varenicline (0.12 mg/kg/h) and/or nicotine (1 mg/kg/hr), alone or in combination, were compared for plasma and brain levels of drugs, tolerance to subsequent acute nicotine and expression of four subtypes of nAChR using autoradiography.

Results: The upregulation of α4β2-nAChR sites elicited by chronic varenicline was very similar to that elicited by chronic nicotine. Treatment with both drugs somewhat increased up-regulation, indicating that these doses were not quite at maximum effect. Similar down-regulation was seen for α6β2-nAChR sites. Varenicline significantly increased both α3β4-and α7-nAChR sites while nicotine had less effect on these sites. The drug combination was similar to varenicline alone for α3β4-nAChR sites, while for α7 sites the drug combination was less effective than varenicline alone. Varenicline had small but significant effects on tolerance to acute nicotine.

Conclusions: Effects of varenicline *in vivo* may not be limited to the α4β2*-nAChR subtype. In addition, smoking cessation treatment with varenicline may not allow receptor numbers to be restored to baseline and may, in addition, change expression of other receptor subtypes.

1. Introduction

Although the incidence of tobacco use in many parts of the world has declined in recent years, many people continue to use tobacco, primarily through cigarette smoking. The health consequences of cigarette smoking are widely recognized and a significant number of current smokers express a desire to quit. However, the success rate for quitting is quite low.

It is well established that exposure to nicotine elicits changes in the expression of the neuronal nicotinic cholinergic receptors (nAChR). Increases in the most widely expressed nAChR subtype (α4β2*-nAChR) occurs in mouse, rat and human brain following exposure to nicotine (Marks et al., 1983; Schwartz and Kellar, 1983; Benwell et al., 1988; Perry et al., 1999; Marks et al., 2011). The time-course of reversal of this upregulation after cessation of treatment in mice is approximately a week in mice (Marks et al., 1985; Turner et al., 2011; Yohn et al., 2014). In addition, nicotine-induced decreases in the expression of the α6β2*-nAChR subtype have been observed in rodent and monkey brain following chronic nicotine treatment (Perez et al., 2008; Perez et al., 2012; Marks et al., 2014).

* Corresponding author. Institute for Behavioral Genetics, 447UCB, University of Colorado, Boulder, CO 80309, USA.
E-mail addresses: marksm@colorado.edu (M.J. Marks), heidi.oneill@colorado.edu (H.C. O'Neill), Kelly.Wynalda@DSM.com (K.M. Wynalda-Camozzi), nick.ortiz@colorado.edu (N.C. Ortiz), emily.evans.simmons@gmail.com (E.E. Simmons), caitlin.a.short@gmail.com (C.A. Short), chris.butt@dsm.com (C.M. Butt), mcintosh.mike@gmail.com (J.Michael McIntosh), sharon.grady@colorado.edu (S.R. Grady).

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While α4β2*- and α6β2*-nAChRs have been implicated in some smoking behaviors, other subtypes including α3, β4 and α5 subunits have been shown to be important for appetite, aversion and withdrawal (Salas et al., 2004; Frahm et al., 2011; George et al., 2011; Jackson et al., 2013; Picciotto and Kenny, 2013; Stoker and Markou, 2013).

Several forms of nicotine replacement (NRT), including nicotine in gum, patches and lozenges, are in clinical use to reduce tobacco withdrawal symptoms and replacement provides significant harm reduction. Although NRT has been somewhat successful, it has not generally achieved the desired level of long-term quit rates. Subsequently, varenicline (Chantix), which has activity at nAChR somewhat different from nicotine including partial agonist activity at the α4β2*-nAChR subtype, was developed and is now widely used as a smoking cessation aid. While varenicline treatment has been somewhat more helpful than NRT for some smokers, long-term quit-rates are somewhat disappointing (Stapleton et al., 2008; Kralikova et al., 2013). Combination treatment strategies including co-treatment with nicotine and varenicline (Ebbert et al., 2009; Hajek et al., 2011; Hajek et al., 2013) have been employed in attempts to improve the quit-rate.

Varenicline exhibits higher affinity than nicotine for the α4β2*-nAChR, and was initially considered selective for this subtype (Coe et al., 2005). However, varenicline also exhibits activity at additional nAChR subtypes, including α3β4*-nAChR and α7-nAChR (Grady et al., 2010; Papke et al., 2010; Campling et al., 2013). Studies using rodent and cell culture models to evaluate the effects of chronic varenicline exposure have demonstrated that, similar to nicotine, these treatments elicit an up-regulation of α4β2*-nAChR binding sites (Turner et al., 2011; Hussmann et al., 2012; Hussmann et al., 2014). In humans, at therapeutic doses, varenicline may have effects on nAChRs other than β2* (Campling et al., 2013). Effects of chronic varenicline on receptor expression of subtypes other than β2*-nAChR have not been reported.

Nicotine-induced changes in expression are indicative of receptor interaction. It is currently unknown whether changes in nAChR expression are maintained by all smoking cessation treatments, although some compounds under investigation appear to allow return to baseline for α4β2*-nAChR (Turner et al., 2010; Hussmann et al., 2012; Hussmann et al., 2014; Yohn et al., 2014). Whether reversal of these changes is an important aspect of a successful quit attempt is also not known.

We compared chronic varenicline to nicotine, as well as co-treatment with both compounds in a mouse model to investigate selectivity of upregulation as a marker for drug effects at four different subtypes of nAChR. These four nAChR binding sites were quantitated in multiple brain regions. The results show that nicotine or varenicline treatment alone elicits very similar effects on ligand binding to α4β2*-nAChR and α6β2*-nAChR sites, but that varenicline treatment elicits significantly greater changes in populations of α3β4*nAChR and α7-nAChR sites than does nicotine treatment. Co-treatment effects were increased for some sites, but not all.

2. Methods

2.1. Materials

[^{125}I]-Epibatidine (2200 Ci/mmol) and [^{125}I]-α-bungarotoxin (110 Ci/mmol) were obtained from Perkin–Elmer NEN, Boston, MA. [^{125}I]-α-conotoxin MII (2200 Ci/mmol) was synthesized as previously described (Whiteaker et al., 2000b). NaCl, KCl, $MgSO_4$, $CaCl_2$, Na_2HPO_4, NaH_2PO_4, bovine serum albumin, polyethyleneglycol, polyethylenimine, nicotine, and cytisine were obtained from Sigma Chemical Co., St. Louis, MO. Ketamine, xylazine, acepromazine and buphrenophine were obtained from MWI Veterinary Supply. Sucrose was obtained from Roche Diagnostics, Indianapolis, IN. HEPES and NaHEPES were products of Amresco, Solon, OH. Silastic tubing a product of Dow Chemical were obtained through VWR International. Glass filters Type B were products of MicroFiltration Systems, Dublin, CA and glass fiber filters Type A/E were products of Pall Life Sciences, Port Washington, NY. Nylon mesh and 22 gauge stainless steel tubing were obtained from Small Parts, Inc. 5I-Epibatidine was a generous gift of Dr. Kenneth Kellar, Georgetown University. Varenicline tartrate was synthesized and kindly supplied by Targacept, Inc. (Winston–Salem, NC). Varenicline internal standard (PF-00142282) was kindly supplied by Pfizer (Groton, CT). Deuterated nicotine standard (d_4 ± nicotine), ammonium hydroxide and ammonium acetate were purchased from Sigma–Aldrich (St Louis, MO). UPLC-grade acetonitrile, formic acid and methanol were purchased from VWR (Radnor, PA).

2.2. Mice

C57Bl/6J mice were bred and maintained at the Institute for Behavioral Genetics. After weaning, mice housed with same sex littermates had free access to food and water on a 12-hr light/dark cycle at 22 °C. All care and treatment protocols were approved by the Animal Care and Utilization Committee of the University of Colorado and followed the guidelines for the care and use of mice by the National Institutes of Health. All efforts were made to minimize the number of animals treated by using a preliminary dosing study and by analyzing all mice treated in the two compound study for tolerance as well as all four autoradiography binding protocols.

2.3. Chronic treatment

Methods previously described for continuous infusion (Marks et al., 1983; Marks et al., 2011) were followed with minor modifications. Mice were anesthetized by intraperitoneal injection of a ketamine (100 mg/kg)/xylazine (10 mg/kg). A cannula constructed of silastic tubing (0.30 mm inner diameter, 0.64 mm outer diameter) was inserted 8 mm into the vein and anchored to the underlying tissue with surgical silk thread. The silastic tubing was connected to 22 gauge stainless steel tubing attached to a nylon circle (1 cm diameter) which was affixed to the back of the mouse between the scapulae. Following surgery each mouse was injected with 0.1 mg/kg buprenorphine and placed in a freshly bedded cage. The mouse was warmed and monitored repeatedly until awakening.

The day after surgery the cannula was checked for free flow. The mouse was weighed and transferred to an infusion chamber (15 cm × 15 cm × 30 cm, l × w × h). The stainless steel tubing was connected to polyethylene tubing attached to a 1 ml syringe mounted on a Harvard Infusion pump that delivered isotonic sterile saline at a rate of 35 μl/h. Saline infusion was maintained for two days before beginning drug treatment. Four treatment groups were used: saline-infused (controls), 1.0 mg/kg/hr nicotine, 0.12 mg/kg/hr varenicline and 1.0 mg/kg/hr nicotine plus 0.12 mg/kg/hr varenicline. In addition, a preliminary experiment in which mice were treated with saline, 0.12 or 0.6 mg/kg/hr varenicline was performed to establish the appropriate varenicline dose for the larger study. All drug doses are free base.

Mice for analytical studies of blood/brain levels were treated as above with either 1.0 mg/kg/hr nicotine or 0.12 mg/kg/hr varenicline for 10 days.