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Short communication

Nicotine physical dependence in the mouse: Involvement of the α_7 nicotinic receptor subtype

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Abstract

Although chronic nicotine produces dependence in mice, the role of specific nicotinic receptors has not been examined in knockout animals. The present study utilized α_7 nicotinic receptor knockout mice to explore the role of this receptor subunit in nicotine dependence. Mice were chronically exposed to nicotine (0 or 200 μg/ml) in their drinking water and assayed for somatic withdrawal signs, hyperalgesia (tail-flick and hot-plate) and spontaneous activity following nicotine cessation. Nicotine withdrawal produced increased somatic signs in both strains and hyperalgesia in wild-type, but not in knockout animals. These results indicate that the α_7 nicotinic receptor subunit may mediate some aspects of nicotine dependence.

Keywords: Nicotine; Withdrawal; Hyperalgesia; Knockout

1. Introduction

Different animal models of nicotine dependence and withdrawal have allowed for assessments of the underlying mediators of these phenomena. In one model, antagonists are administered to nicotine-dependent animals to determine whether blockade of certain receptors precipitates withdrawal. Using this procedure, mecamylamine precipitates somatic withdrawal signs (Damaj et al., 2003; Grabus et al., 2005) and hyperalgesia (Damaj et al., 2003) in nicotine-dependent rodents. Increased somatic signs are also precipitated by other nicotinic receptor antagonists, such as dihydro-β-erythroidine (Malin et al., 1998) and methyllycaconitine (Damaj et al., 2003).

Although these studies suggest the involvement of multiple receptor subtypes in nicotine dependence, the specific roles of each subtype are unclear. For example, α_7 nicotinic receptors are present in the ventral tegmental area, which is a brain region implicated in dependence and withdrawal (Nomikos et al., 1999). Some researchers have found that α_7 nicotinic receptors are involved in specific nicotine withdrawal signs (Damaj et al., 2003), but other studies have failed to demonstrate such effects (Grabus et al., 2005; Markou and Paterson, 2001). Therefore, the role of the α_7 nicotinic receptor subunit in nicotine dependence needs further clarification.

Although antagonists have been utilized to study processes contributing to nicotine dependence, these compounds generally lack selectivity for one receptor type. An alternative method would be the use of knockout animals. However, developmental and compensatory issues complicate the interpretation of knockout studies (see Gingrich and Hen, 2000). Although each of these approaches possesses certain limitations, results from antagonism and knockout studies can complement one another. Specifically, models of nicotine dependence and withdrawal that utilize knockout animals can be compared to those exploring antagonist-precipitated withdrawal to better determine the specific role of the various receptor subtypes in these processes.

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One method to produce nicotine dependence in mice is through chronic exposure in the drinking water. Abrupt cessation of chronic oral nicotine results in increased numbers of somatic withdrawal signs and hyperalgesia in mice, with these behavioral indicators peaking around days 2–3 of withdrawal (Grabus et al., 2005). Chronic oral nicotine also increases plasma nicotine and cotinine levels (Pietilä et al., 1995), produces hyperactivity (Gäddnäs et al., 2000), upregulates nicotinic acetylcholine receptors (Sparks and Pauly, 1999), increases dopamine levels in specific brain regions (Pietilä et al., 1995) and produces nicotine tolerance (Sparks and Pauly, 1999).

Therefore, the goal of the present experiment was to examine withdrawal following chronic oral nicotine exposure in α_7 nicotinic receptor knockout and wild-type mice. Results from the present experiment will be compared to those in prior studies using methyllycaconitine, a selective α_7 nicotinic receptor antagonist, to further elucidate the mechanisms underlying nicotine dependence and withdrawal.

2. Materials and methods

2.1. Subjects

Experimentally naïve, male mice lacking the α_7 nicotinic receptor subunit (C57BL/6J background) and wild-type littermates were purchased from the Jackson Laboratories (B6.129S7-charna7tm1bay, number 003232). Breeding of homozygous knockout or wild-type mice produced progenies ($N=10$ per strain). Animals were 8–12 weeks of age and 20–30 g at the start of the experiment. They were housed five per cage, with each cage containing mice of only one genotype. Subjects were given free access to food.

2.2. Drugs

Nicotine free base was added to a 2% saccharin sodium solution and administered in drinking bottles as the sole source of fluid access. Compounds were purchased from Sigma Chemical Company (Milwaukee, WI).

2.3. Dependence induction

During a 21-day exposure period, experimental subjects were presented with orally available nicotine (200 μg/ml), and control animals received the 2% saccharin solution alone. This exposure period and nicotine concentration were based upon prior data from our lab indicating that 20–30 days access to 200 (but not 100) μg/ml oral nicotine produced dependence in the mouse (Grabus et al., 2005). Fluids were replenished, consumption levels measured and animals weighed every 2–3 days of chronic oral exposure. Experimenters were blind to nicotine exposure conditions during behavioral testing.

2.4. Spontaneous withdrawal assessments

Nicotine solutions were replaced with tap water on day 22. Withdrawal data were then collected for these animals 1, 2 and 3 days following nicotine cessation (i.e., repeated assessments) during which animals received tap water as their fluid source. Methods for all tests are described in Grabus et al. (2005). Assays were completed in the following order.

2.4.1. Somatic signs

Animals were observed (20 min) for paw tremors, backing and head shakes.

2.4.2. Hyperalgesia

Hyperalgesia was measured using two thermal models.

2.4.2.1. Tail-flick. Animals were lightly restrained while a radiant heat source was shone onto the tail, and latency to remove the tail from the heat source was recorded.

2.4.2.2. Hot-plate. Animals were placed on a heated surface (55.1±0.1 °C) and latency to lick a paw or jump was recorded.

2.4.3. Spontaneous activity

After a 5-min chamber acclimation period, horizontal locomotor activity was recorded (10 min).

2.5. Data analysis

Significant overall three-way, repeated measures analysis of variance (ANOVA) was followed by post hoc comparisons when appropriate (Fisher's protected least significant test). All statements of statistical significance are based on $P<0.05$.

3. Results

3.1. Effects of nicotine on body weight and fluid consumption

Prior to oral nicotine exposure, wild-type animals weighed on average 23.2 g, while knockout animals weighed an average of 22.8 g. At the end of the experiment (following chronic nicotine exposure), average weights for wild-type and knockout animals were 23 and 24 g, respectively. For weight changes (data not shown), the effects of Strain [$F(1,16)=0.481$, $P=0.4978$] and Concentration [$F(1,16)=0.271$, $P=0.6100$] were not significant, and there was no significant Strain × Concentration interaction [$F(1,16)=0.271$, $P=0.6100$]. In addition, the effect of Day [$F(6,96)=7.950$, $P<0.0001$] and the Day × Concentration interaction [$F(6,96)=2.772$, $P=0.0158$] were significant, but there were no significant Day × Strain

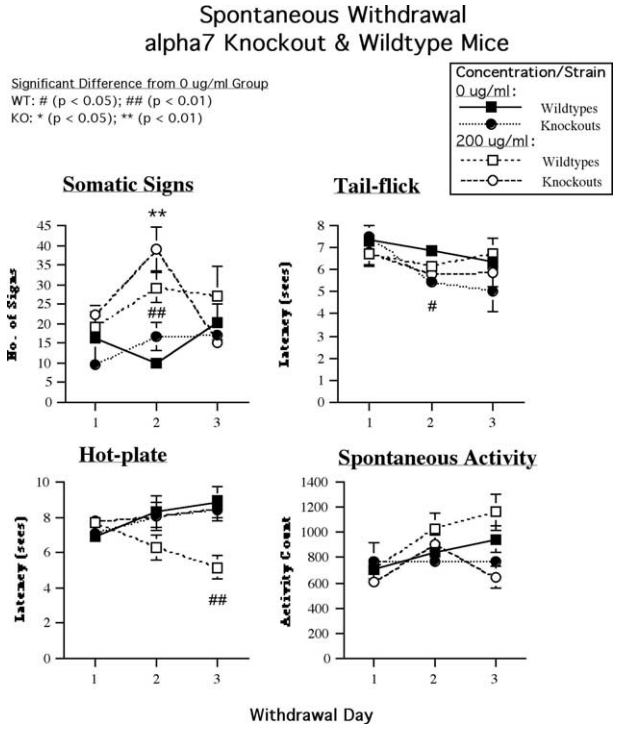

Fig. 1. Male α_7 nicotinic receptor knockout and wild-type mice were allowed free access to drinking water containing 0 or 200 μg/ml nicotine ($n=5$ per strain+concentration) for 21 days. Tap water replaced nicotine solutions on Day 22, and mice were assessed for spontaneous withdrawal 1, 2 and 3 days later. Results are presented as means±S.E.M. for each measure.

[$F(6,96)=0.497$, $P=0.8092$] or Day × Concentration × Strain [$F(6,96)=2.165$, $P=0.0530$] interactions. Therefore, groups did not differ from each other in weight changes, although there were within-group differences in weight loss/gain across days.

Because fluid consumption data consisted of one measurement per group on each day assayed, the effect of Day could not be assessed. Instead, a two-way ANOVA determined whether any group differences existed. For fluid consumption (data not shown), the effect of Concentration [$F(1,28)=29.708$, $P<0.0001$], but not Strain [$F(1,28)=0.233$, $P=0.6328$], was significant, and there was no significant Strain × Concentration interaction [$F(1,28)=0.911$, $P=0.3480$]. Within each strain, animals given 200 μg/ml nicotine drank significantly less than animals exposed to saccharin alone (P's<0.01). The effect of concentration was not strain-dependent in that, for either 0 or 200 μg/ml nicotine, wild-type animals did not differ from knockouts.

3.2. Effects of the α_7 nicotinic receptor subtype in spontaneous withdrawal

Withdrawal data in α_7 nicotinic receptor knockout and wild-type mice on days 1, 2 and 3 following cessation of 21 days of oral nicotine (0 or 200 μg/ml) are presented in Fig. 1. For ease of presentation, results from three-way, repeated measures ANOVAs are presented in Table 1, with significant results indicated. Therefore, this paragraph only discusses post hoc test results. Both α_7 nicotinic receptor knockout and wild-type animals chronically exposed to nicotine showed significantly greater numbers of somatic signs compared to their saccharin-exposed controls on withdrawal day 2 only (all P's<0.01). However, nicotine-exposed knockout and wild-type animals did not significantly differ from each other in this measure. In contrast, α_7 nicotinic receptor wild-type, but not knockout, animals chronically exposed to nicotine showed significant hyperalgesia, as indicated by decreased tail-flick latencies ($P<0.05$) on day 2 and decreased hot-plate responsivity ($P<0.01$) on day 3 when compared to their saccharin-exposed controls. Finally, withdrawal from chronic oral nicotine did not produce locomotor changes in either strain, given that there were no significant differences in the spontaneous activity assay.

4. Discussion

The present study demonstrates the differential involvement of the α_7 nicotinic receptor subtype in nicotine withdrawal. Our results show that genetic removal of this subtype attenuated withdrawal-induced hyperalgesia. These results are consistent with the finding that methyllycaconitine precipitates hyperalgesia in mice exposed to chronic nicotine infusions (Damaj et al., 2003).

Table 1
Results from three-way, repeated measures ANOVA

	Somatic signs	Tail-flick	Hot-plate	Spontaneous activity
Strain	$F(1,16)=0.001$; $P=0.9743$	$F(1,16)=3.917$; $P=0.0653$	$F(1,16)=3.198$; $P=0.0927$	$F(1,16)=0.390$; $P=0.5409$
Concentration	$F(1,16)=12.533$; $P=0.0027$*	$F(1,16)=0.083$; $P=0.7776$	$F(1,16)=2.719$; $P=0.1187$	$F(1,16)=0.981$; $P=0.3366$
Strain × Concentration	$F(1,16)=1.494$; $P=0.2393$	$F(1,16)=0.521$; $P=0.4810$	$F(1,16)=4.799$; $P=0.0436$**	$F(1,16)=0.141$; $P=0.7124$
Day	$F(2,32)=10.890$; $P=0.0002$*	$F(2,32)=6.482$; $P=0.0043$*	$F(2,32)=0.497$; $P=0.6132$	$F(2,32)=0.385$; $P=0.6836$
Day × Strain	$F(2,32)=9.287$; $P=0.0007$*	$F(2,32)=1.950$; $P=0.1589$	$F(2,32)=1.449$; $P=0.2498$	$F(2,32)=2.553$; $P=0.0936$
Day × Concentration	$F(2,32)=6.767$; $P=0.0035$*	$F(2,32)=1.771$; $P=0.1865$	$F(2,32)=5.314$; $P=0.0102$**	$F(2,32)=0.369$; $P=0.6940$
Day × Strain × Concentration	$F(2,32)=1.972$; $P=0.1557$	$F(2,32)=0.370$; $P=0.6936$	$F(2,32)=2.942$; $P=0.0672$	$F(2,32)=1.416$; $P=0.2576$

* $P<0.01$.
** $P<0.05$.

Although the α_7 nicotinic receptor subtype appeared to be implicated in withdrawal-induced hyperalgesia, it did not appear to play a role in other aspects of nicotine withdrawal, such as somatic withdrawal behaviors. These results are consistent with studies that failed to find a role for the α_7 nicotinic receptor in this component of withdrawal. Particularly, methyllycaconitine does not precipitate somatic withdrawal signs in rats made dependent upon nicotine via osmotic minipumps (Markou and Paterson, 2001) or in mice exposed to chronic oral nicotine (Grabus et al., 2005). However, one study found that methyllycaconitine precipitated increased somatic signs in mice made dependent upon nicotine via minipumps (Damaj et al., 2003). These discrepant results could be due to the different exposure routes and/or strains used.

Within the present experiment, it was not possible to examine the involvement of the α_7 nicotinic receptor subtype in withdrawal-induced locomotor changes, since nicotine-dependent wild-type animals did not differ from saccharin-exposed controls in this measure. Past studies have shown that α_7 nicotinic receptors are implicated in withdrawal-induced hypolocomotion, with intrategmental injections of methyllycaconitine producing significantly decreased locomotion (compared to saline-injected controls) in nicotine-dependent rats (Nomikos et al., 1999). Once again, the discrepant results could be due to the different species and/or routes of exposure used.

Although the present experiment indicated that the α_7 nicotinic receptor subtype mediates certain aspects of nicotine dependence, data must be interpreted with caution. First, results could be impacted by strain differences in pharmacokinetics. However, given that animals showed differences in one assay (i.e., hyperalgesia) but not another (i.e., somatic signs), pharmacokinetic factors may not play a major role. Another possible confound is that knockout animals may develop compensatory adaptations due to receptor losses. Although deletion of the α_7 nicotinic receptor subtype does not appear to produce any observable phenotypic abnormalities or changes in other nicotinic receptor populations (Orr-Urtreger et al., 1997), compensatory mechanisms cannot be ruled out.

However, our results do confirm findings from antagonism studies that suggest the involvement of multiple nicotinic receptors in nicotine dependence. Mecamylamine (Damaj et al., 2003; Grabus et al., 2005), an antagonist with preference for $\alpha_3\beta_4$* receptors (Papke et al., 2001), and dihydro-β-erythroidine (Malin et al., 1998; although see Grabus et al., 2005), a β_2 selective antagonist (Sharples and Wonnacott, 2001), precipitate increased withdrawal signs in nicotine-dependent rodents. These studies indicate that α_3- and β_2-containing receptors are implicated in nicotine dependence and withdrawal. Our results extend this observation by suggesting the involvement of the α_7 nicotinic receptor subtype in certain aspects of spontaneous withdrawal from nicotine (i.e., hyperalgesia). Results from experiments utilizing knockout animals, therefore, may provide specific conclusions regarding the roles of specific receptor subtypes in nicotine addiction and provide direction for more effective cessation therapies.

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