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Amitifadine, a triple monoamine re-uptake inhibitor, reduces nicotine self-administration in female rats

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ABSTRACT

A wider diversity of drug treatments to aid smoking cessation is needed to help tailor the most efficacious treatment for different types of smokers. This study was conducted to determine whether amitifadine, which inhibits re-uptake of dopamine, norepinephrine and serotonin, would decrease nicotine self-administration at doses that do not cause adverse side effects. Adult female Sprague–Dawley rats were trained to self-administer nicotine intravenous (IV) and were given acute doses of amitifadine in a repeated measures counterbalanced design. Effects of amitifadine on locomotor activity and food motivated responding were also evaluated. Chronic amitifadine effects were also examined. The 30 mg/kg amitifadine dose significantly reduced nicotine self-administration. The 5 and 10 mg/kg doses reduced nicotine self-administration during the first 15 min of the session when the greatest amount of nicotine was self-administered. The 30 mg/kg amitifadine dose, but not the lower doses caused a significant reduction in locomotor activity averaged over the one-hour session and reduced food motivated responding. The 10 mg/kg dose caused hypoactivity at the beginning of the session, but 5 mg/kg did not cause any hypoactivity. The effects of chronic amitifadine treatment (10 mg/kg) over the course of 15 sessions was also determined. Amitifadine caused a significant reduction in nicotine self-administration, which was not seen to diminish over two consecutive weeks of treatment and a week after enforced abstinence. Amitifadine significantly reduced nicotine self-administration. This prompts further research to determine if amitifadine might be an effective treatment for smoking cessation.

1. Introduction

Tobacco addiction is responsible for millions of premature deaths each year (Hatsukami et al., 2008). The current drug treatments which include nicotine replacement, as well as varenicline and bupropion are more effective than placebo, but fall short of being effective for the majority of tobacco users (Frishman et al., 2006). The variety of tobacco users may benefit from different types of treatments, which would entail having available a greater variety of treatments.

Nicotine potentiates the release of a variety of neurotransmitters, including the dopamine, norepinephrine and serotonin (Li et al., 1998) which are important for a variety of important neurobehavioral functions, including addiction. Dopaminergic innervation from the ventral tegmental area to the nucleus accumbens has been shown in many studies to be critically involved in the neural basis of addiction (Di Chiara et al., 2004). There is also evidence for involvement of norepinephrine and serotonin (Benowitz and Peng, 2000; Dudas and George, 2005; Frishman, 2007; Rezvani et al., 1990), which interact with each other and with dopamine. We have shown that the serotonin $5HT_{2c}$ agonist lorcaserin reduces nicotine self-administration (SA) in rats (Levin et al., 2011a). The noradrenergic α2 agonist clonidine has been shown to improve smoking cessation pharmacotherapy (Glassman et al., 1988). Nortriptyline, which blocks the re-uptake of norepinephrine and to a lesser degree dopamine, has been found to improve smoking cessation pharmacotherapy (Hughes et al., 2005). Selective serotonin re-uptake inhibitors have not been found to be effective (Hughes et al., 2005) in general, although they may be efficacious in helping depressed smokers (Hitsman et al., 1999).

This study evaluated the efficacy of acute doses of amitifadine (EB-1010, DOV 21947), a serotonin-preferring triple re-uptake inhibitor of serotonin, norepinephrine, and dopamine, with K_i values for inhibition of uptake of 96, 23 and 12 nM for dopamine,

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norepinephrine, and serotonin, respectively (Skolnick et al., 2003). Amitifadine in the dose-range selected for this study was found by Skolnick et al. (2003) to reducedepressive tendencies in rats as measured by reduction in the duration of immobility in the forced swim test. Amitifadine has been found to have no appreciable direct effect on neurotransmitter receptors, including nicotinic receptors (Bymaster, unpublished observation). *In vivo*, amitifadine was active with minimal effective doses of 5 mg/kg in the rat forced swim test and mouse tail suspension antidepressant models (Golembiowska et al., 2012; Skolnick et al., 2003). Consistent with triple re-uptake blockade, amitifadine inhibited *ex vivo* binding to dopamine, norepinephrine and serotonin transporters and robustly increased these transmitters in prefrontal cortex and dopamine in the striatum of rats (Golembiowska et al., 2012; Lengyel et al., 2008). Recently, amitifadine was found in humans to have robust antidepressant activity and be well tolerated (Tran et al., 2012).

This study examined the effects of both acute and chronic administration of amitifadine on nicotine self-administration (SA). Effects of amitifadine were also assessed on food-motivated responding and locomotor activity to assess ancillary effects. It was hypothesized that acute and chronic administration of amitifadine would significantly reduce nicotine SA in rats.

2. Materials and methods

2.1. Subjects

Young adult 3–5 month old female (Acute Study $N=10$; Chronic Study $N=10$ Controls and $N=12$ amitifadine treated) Sprague–Dawley rats (Taconic Lab, Germantown, NY, USA) were used in the present study. Female rats were used in order to compare the data from the current studies with our previous work (Levin et al., 2011a, 2010, 2008, 2011b, 2011c) in which we documented the effectiveness of treatments on related transmitter systems for reducing nicotine SA in female rats. Animals were individually housed in a temperature controlled vivarium room located adjacent to the nicotine SA testing room. Animals were maintained on a 12:12 reverse light–dark cycle so that experimental sessions occurred during the active part of the rats' diurnal cycle. Animals were given *ad lib* access to water at all times excluding experimental sessions, and were fed approximately 10–15 g of rat chow daily 20–30 min after the completion of their experimental session to keep the rats at a lean healthy weight. This study was conducted under a protocol approved by the Duke University Institutional Animal Care and Use Committee in accordance with USDA regulations.

2.2. Nicotinic receptor binding

To determine the possibility that amitifadine may have effects mediated *via* direct actions on nicotinic receptors we determined its affinity for α7 and α4β2 nicotinic receptors in radioligand binding assays. Inhibition of binding to α4β2 nicotinic receptors in SH-SY5Y cells was determined using the radioligand [^{3}H]cytosine at 0.6 nM concentration according to the method of Gopalakrishnan et al. (1996). Incubation was for 120 min at 4 °C and non-specific binding was determined with 10 μM nicotine. Inhibition of binding to α7 nicotinic receptors in SH-SY5Y cells was determined using [^{125}I] α-bungarotoxin (0.05 nM) according to the method of Sharples et al. (2000). Incubation was 120 min at 37 °C and non-specific binding was determined using α-bungarotoxin (1 μM). Specific binding of the radioligands was determined by scintillation spectrometry technology. The assessment was conducted by Cerep, Inc. (Poitiers, France)

2.3. Drug treatments

Nicotine bitartrate solutions were prepared in isotonic sterile saline. The dose used for SA (0.03 mg/kg/infusion) was calculated as a function of the nicotine free base weight. The pH of the nicotine solution was adjusted to 7.0 using NaOH and the solution was filtered in a Nalgene filter (Nalgene Nunc International, Rochester, NY, USA) for sterilization. Between sessions all nicotine was kept in a dark refrigerator. Amitifadine ((1*R*,5*S*)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0] hexane hydrochloride) was provided by Euthymics Bioscience, Inc., Cambridge, MA, USA.

Amitifadine solutions were prepared in sterile water for doses of 5, 10 and 30 mg/kg (p.o.). Water vehicle was used as the control. The volume of oral gavage was 4 ml/kg given 30 min before testing.

2.4. Acute dose study

In the acute amitifadine dose effect study, three doses of amitifadine along with vehicle (sterile water) were tested in a counterbalanced order in rats ($N=10$) with at least two days between consecutive injections. The entire dose-effect function was run twice (phase 1 and phase 2). The test of acute amitifadine on locomotor activity was conducted between the two phases, and the test of food motivated responding was conducted after phase 2. Both the locomotor activity and food motivated responding tests were conducted with the same dosing parameters as with the tests on nicotine SA.

2.5. Chronic dosing study

In a second set of rats trained in the same way as the rats in the first study, chronic effects of amitifadine were tested using a between-subjects design with control ($N=10$) and 10 mg/kg amitifadine ($N=12$) groups. The two treatment groups were matched for similar predrug rates of nicotine self-administration. Rats were administered 10 mg/kg of amitfadine by gavage 30 min before testing for ten sessions over a period of two weeks with a two-day break between the first and second five sessions. To test for efficacy of amitifadine in attenuating resumption of nicotine SA, an additional five sessions were run after a nine-day period of enforced abstinence. This phase was conducted to model treatment efficacy in attenuation relapse after a cessation attempt. The nine-day break in nicotine access modeled an initially successful attempt at smoking cessation. Controls received sterile water vehicle. Amitifadine or vehicle administration only occurred on days when the animals were tested.

2.6. Behavioral procedures

Before the start of nicotine SA sessions, all animals were trained to lever press in a standard dual-lever operant chamber (Med Associates, St. Albans, VT, USA) for food reinforcement. All rats had an equivalent three sessions of food training. All rats averaged more than 50 food reinforcements per session Each chamber was equipped with two levers, two cue lights located directly above each lever, a house light, and a tone generator. After lever pressing was established, animals experienced three sessions of lever pressing for food under a fixed ratio (FR) 1 schedule of reinforcement. Following the completion of their final training session with food reinforcement, animals were anesthetized with a mixture of ketamine (60 mg/kg) and dormitor (15 mg/kg) and a catheter (Strategic Application Inc., Libertyville, IL, USA) was implanted into their jugular vein. The jugular catheter was attached to a harness that could be tethered to the infusion pump during experimental sessions.