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Differential effects of non-nicotine tobacco constituent compounds on nicotine self-administration in rats

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ABSTRACT

Tobacco smoking has been shown to be quite addictive in people. However, nicotine itself is a weak reinforcer compared to other commonly abused drugs, leading speculation that other factors contribute to the high prevalence of tobacco addiction in the human population. In addition to nicotine, there are over 5000 chemical compounds that have been identified in tobacco smoke, and more work is needed to ascertain their potential contributions to tobacco's highly addictive properties, or as potential candidates for smoking cessation treatment. In this study, we examined seven non-nicotine tobacco constituent compounds (anabasine, anatabine, nornicotine, myosmine, harmane, norharmane, and tyramine) for their effects on nicotine self-administration behavior in rats. Young adult female Sprague–Dawley rats were allowed to self-administer nicotine (0.03 mg/kg/50 μl infusion) under a fixed ratio-1 schedule of reinforcement. Each self-administration session lasted 45 min. Doses of each tobacco constituent compound were administered subcutaneously 10 min prior to the start of each session in a repeated measures, counterbalanced order two times. Anabasine displayed a biphasic dose–effect function. Pretreatment with 0.02 mg/kg anabasine resulted in a 25% increase in nicotine self-administration, while 2.0 mg/kg of anabasine reduced nicotine infusions per session by over 50%. Pretreatment with 2.0 mg/kg anatabine also significantly reduced nicotine self-administration by nearly half. These results suggest that some non-nicotine tobacco constituents may enhance or reduce nicotine's reinforcing properties. Also, depending upon the appropriate dose, some of these compounds may also serve as potential smoking cessation agents.

1. Introduction

As the use of tobacco products constitutes the leading cause of preventable death worldwide, and at a cost of approximately 193 billion dollars annually in the United States alone, there continues to be a need to understand more intricately the nature of tobacco addiction as well as a need to develop a greater arsenal of pharmacological tools to effectively reduce its use among tobacco addicts. While much progress has been made in the development of pharmacotherapies to treat tobacco addiction, the majority of tobacco users are typically unsuccessful at remaining abstinent permanently, even after multiple cessation attempts (Benowitz, 2010). Many of the currently available smoking cessation agents focus on replacing the nicotine from tobacco products (nicotine patches, gum, nasal spray, lozenges), or targeting nicotinic acetylcholine receptors (varenicline). Bupropion, which inhibits monoamine reuptake, also has direct nicotinic effects (Lukas et al., 2010). This approach has been pragmatic and intuitive, as nicotine is widely accepted as the primary reinforcing agent found in tobacco that causes addiction in humans (Rose and Corrigall, 1997). However, there are over 5000 chemical compounds present in tobacco smoke, many of which likely exert their own effects on the brain (Rose, 2006). Indeed, previous studies have suggested that minor alkaloids found in the tobacco leaf augment the effects of nicotine in rats (Clemens et al., 2009), or are reinforcing in their own right (Bardo et al., 1999). These findings are made all the more intriguing when one considers that despite the high prevalence of nicotine addiction among the human population, in laboratory settings nicotine itself is viewed as a relatively weak reinforcer, particularly in comparison with other drugs of abuse (Bespalov et al., 1999; Manzardo et al., 2002). It remains a priority to elucidate which of these non-nicotine compounds found in tobacco may contribute to its highly addictive properties. Conversely, just as the nicotine itself contained in tobacco has been utilized as a treatment for nicotine addiction (Levin et al., 1994), these compounds should be evaluated for their potential to also serve as pharmacological treatment agents. Therefore, the purpose of this study was to determine the effects of acute treatment of seven of these non-nicotine tobacco compounds in a rat model of nicotine self-administration.

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Anabasine, anatabine, nornicotine, and myosmine are all minor alkaloids present in the tobacco leaf (Huang and Hsieh, 2007). Each of these compounds shares a chemical structure closely related to nicotine, and most have been shown to have affinity for nicotinic acetylcholine receptors (nAChRs) (Crooks and Dwoskin, 1997; Maciuk et al., 2008). Anabasine and nornicotine have been the most studied of these four compounds, each having been shown to at least partially substitute for nicotine in drug discrimination tasks (Brioni et al., 1994; Desai et al., 1999) and evoke midbrain dopamine release from rat striatal slices (Dwoskin et al., 1993, 1995). Nornicotine has also been found to support self-administration in rats on its own, although this was accomplished using very high concentrations of the compound (Bardo et al., 1999).

The β-carboline alkaloids harmane and norharmane are also found in the tobacco leaf and have been shown to bind to several neurotransmitter receptors in the brain (Adell et al., 1996; Husbands et al., 2001; Melchior and Collins, 1982). The two compounds may also be formed in cigarette smoke or *in vivo* by chemical reactions between indolamines and aldehydes (e.g., acetaldehyde) that are created by combustion of tobacco (poly)saccharides (Cao et al., 2007). Like the minor alkaloids discussed above, both compounds elicit dopamine efflux in mesolimbic dopamine neurons, in what has been described as a dose-dependent, U-shaped manner (Arib et al., 2010; Baum et al., 1996). However, with regard to tobacco use, harmane and norharmane have received much more attention for their ability to inhibit both isoforms of monoamine oxidase (MAO). It is generally accepted that harmane is an inhibitor of monoamine oxidase A (MAO-A) and norharmane inhibits monamine oxidase B (MAO-B), although there is evidence that norharmane is an inhibitor of both enzymes (for a review, see van Amsterdam et al., 2006). It has previously been shown that monoamine oxidase inhibition enhances nicotine self-administration in rats (Guillem et al., 2005), and it is believed that the abrupt discontinuation of this inhibition potentiates withdrawal symptoms in smokers who attempt to quit. Interestingly, the monoamine tyramine, another compound present in the tobacco leaf (Songstad et al., 1991), is a substrate for MAO-A. The inhibition of MAO-A in tobacco users by the β-carboline alkaloids could lead to the potentiation of tyramine's sympathomimetic effects in the periphery.

The current studies were conducted to determine the interactive effects of a group of tobacco constituents on nicotine self-administration in rats. The hypothesis was that compounds that impact nicotinic receptors directly or have effects of inhibiting MAO activity would significantly affect nicotine self-administration. The characterization of the interactions of nicotine with other tobacco compounds could help increase understanding of why tobacco use is so addictive.

2. Materials and methods

2.1. Subjects

Young adult female Sprague–Dawley rats (8 weeks old at the start of the study) were purchased from Taconic Laboratories (Germantown, NY, USA) and used in the self-administration studies. The rats were singly housed in a vivarium at Duke University adjacent to the testing room under standard laboratory conditions. Single housing for the rats was necessary to prevent catheter damage from cagemates. All animals were kept on a 12:12 reverse light/dark cycle so that behavioral testing was performed during the animals' active phase. Animals were allowed unlimited access to water while in their home cages and were fed a restricted diet of standard rat chow after completing each testing session. All testing procedures in this study were approved by the Duke University Animal Care and Use Committee and conducted according to AAALAC guidelines.

2.2. Materials

Nicotine hydrogen tartrate, tyramine HCl, anabasine, myosmine, harmane, and norharmane were purchased from Sigma-Aldrich (St. Louis, MO, USA). D,L-Nornicotine was purchased from Matrix Scientific (Columbia, SC, USA), and D,L-anatabine was purchased from Fisher Scientific (Pittsburgh, PA, USA). Nicotine, nornicotine, tyramine HCl, myosmine, anabasine, and anatabine were dissolved in 0.9% sterile saline (Hospira Inc, Lake Forest, IL, USA). Harmane and norharmane were both dissolved in a solution containing equal parts sterile saline and DMSO (Sigma-Aldrich), which also served as the vehicle for these two compounds. All tobacco constituent solutions were injected (s.c.) in a volume of 1.0 ml/kg body weight.

2.3. Surgical procedures

Using aseptic technique, a sterile catheter (SAI Infusion Technologies, Libertyville, IL, USA) was surgically implanted into the jugular vein of each animal. Animals were anesthetized with a combination of ketamine (60 mg/kg i.p.) and dexmedetomidine (0.15 mg/kg i.p.). Under general anesthesia, an incision was made lateral to the midline and the jugular vein isolated via dissection. The vein was then tied off distal to the desired area of nick incision. A small incision was then made in the jugular and the catheter inserted until just outside the heart. The cannula was sutured to deep muscle and the remaining portion was routed subcutaneously around the back such that it emerged between the scapulae. The cannula was then connected to an infusion harness (SAI Infusion Technologies, Libertyville, Ill). All surgical wounds were sutured using polypropylene sutures and treated with the topical analgesic bupivacaine. Each animal was administered ketoprofen (5.0 mg/kg, s.c.) for postoperative pain. After surgery, the catheters were flushed daily with a combination of sterile saline and heparin (0.25 ml/day). Upon completion of each self-administration session, the nicotine remaining in the harness ports was removed and replaced with a sterile lock solution containing heparinized saline and gentamicin (8 mg/ml, Butler Schein Animal Health, Dublin, OH, USA) as an antibiotic.

2.4. Behavioral procedures

Animals were initially trained to receive a food reward via lever response under an FR1 schedule of reinforcement. Once the animal reached criteria for lever response training (3 consecutive 30 min sessions of ≥50 pellets earned), catheterization surgery was performed and nicotine self-administration sessions begun. Sessions were conducted inside dual lever operant chambers (30.5 X 24.1 X 21.0 cm) (Med Associates, St. Albans, VT, USA) with a response on one of the levers resulting in the delivery of an infusion of nicotine (0.03 mg/kg/50 μl infusion) and a response on the other lever having no consequence. The dose of 0.03 mg/kg of nicotine per infusion is widely used and was chosen based on previous work, demonstrating that this dose produced the most robust self-administration response to nicotine in rats (Corrigall and Coen, 1989). An illuminated cue light placed above the response lever served as a visual secondary reinforcer by indicating an active lever. The active lever for each animal was the same lever on which the animal was trained to respond for food rewards. A response on the active lever resulted in the delivery of a nicotine infusion (50-μl over less than 1 sec) and the activation of a feedback tone for 0.5 s. Each infusion of nicotine was followed by a 1 min timeout period in which the cue light was extinguished and lever responses were recorded but no infusions were delivered. Each session lasted 45 min. All sessions were programmed and recorded using MED-PC software.

2.5. Acute treatment with tobacco constituents

Acute treatment with each compound began after the animals completed 5 baseline sessions of nicotine self-administration. Each