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Tobacco's minor alkaloids: Effects on place conditioning and nucleus accumbens dopamine release in adult and adolescent rats

Julie A. Marusich[a,*], Mahesh Darna[b], A. George Wilson[c], Emily D. Denehy[c], Amanda Ebben[b], Agripina G. Deaciuc[b], Linda P. Dwoskin[b], Michael T. Bardo[c], Timothy W. Lefever[a], Jenny L. Wiley[a], Chad J. Reissig[d], Kia J. Jackson[d]

[a] RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709, USA
[b] College of Pharmacy, University of Kentucky, Lexington, KY 40536-0596, USA
[c] Center for Drug Abuse Research Translation, University of Kentucky, Lexington, KY 40536-0509, USA
[d] US Food and Drug Administration, Center for Tobacco Products, 10903 New Hampshire Ave., Silver Spring, MD 20993, USA

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ABSTRACT

Tobacco products are some of the most commonly used psychoactive drugs worldwide. Besides nicotine, alkaloids in tobacco include cotinine, myosmine, and anatabine. Scientific investigation of these constituents and their contribution to tobacco dependence is less well developed than for nicotine. The present study evaluated the nucleus accumbens dopamine-releasing properties and rewarding and/or aversive properties of nicotine (0.2–0.8 mg/kg), cotinine (0.5–5.0 mg/kg), anatabine (0.5–5.0 mg/kg), and myosmine (5.0–20.0 mg/kg) through in vivo microdialysis and place conditioning, respectively, in adult and adolescent male rats. Nicotine increased dopamine release at both ages, and anatabine and myosmine increased dopamine release in adults, but not adolescents. The dopamine release results were not related to place conditioning, as nicotine and cotinine had no effect on place conditioning, whereas anatabine and myosmine produced aversion in both ages. While the nucleus accumbens shell is hypothesized to play a role in strengthening drug-context associations following initiation of drug use, it may have little involvement in the motivational effects of tobacco constituents once these associations have been acquired. Effects of myosmine and anatabine on dopamine release may require a fully developed dopamine system, since no effects of these tobacco alkaloids were observed during adolescence. In summary, while anatabine and myosmine-induced dopamine release in nucleus accumbens may play a role in tobacco dependence in adults, the nature of that role remains to be elucidated.

1. Introduction

Tobacco products are commonly used psychoactive drugs that have led to a high public health toll (HHS, 2014; WHO, 2013). Although nicotine is believed to be the primary addictive constituent in tobacco, there are > 8400 other constituents in tobacco smoke (Rodgman and Perfetti, 2008), and the contribution of these constituents to tobacco dependence is not yet understood. In addition to nicotine, other tobacco constituents evaluated for addictive potential include nicotine metabolites and minor alkaloids (e.g., cotinine, myosmine, and anatabine) (Clemens et al., 2009; Hall et al., 2014), flavor additives (e.g., menthol) that enhance nicotine's pharmacological effects (Alsharari et al., 2015; Biswas et al., 2016), and β-carbolines that inhibit monoamine oxidase (Smith et al., 2015). Pharmacological effects of tobacco products are mediated by this chemical cocktail (Henningfield and Zeller, 2003). Since initiation of tobacco use often occurs during adolescence (Lydon et al., 2014), it is important to understand the pharmacological effects of tobacco constituents during this developmental period.

Nicotine activates the mesolimbic dopamine reward pathway. Nicotine-evoked dopamine release in the nucleus accumbens (NAc) depends on activation of nicotinic receptors in the midbrain ventral tegmental area (Nisell et al., 1994). Moreover, the stimulant and rewarding effects of nicotine depend on ventral tegmental area-mediated dopamine release in the NAc (Gotti et al., 2010). In contrast to nicotine, however, no studies have examined the dose-effect relationships for other tobacco constituents on dopamine release in the NAc in rodents.

Nicotine-evoked dopamine release in NAc is thought to be the primary mechanism leading to hyperactivity and reward. To measure reward, place conditioning is often used to evaluate the degree of association between the rewarding (conditioned place preference, CPP) or

* Corresponding author.
E-mail address: jmarusich@rti.org (J.A. Marusich).

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aversive (conditioned place aversion, CPA) properties of a drug and the environment in which these properties are experienced repeatedly (Carr et al., 1989; Tzschentke, 1998). Tobacco constituent cues (e.g., rewarding, aversive, smell, taste) may become associated with environmental and social conditions in which tobacco is consumed, leading to associations that may persist in the tobacco-free state. This associative learning may impede attainment and maintenance of abstinence.

Nicotine CPP has been demonstrated in adult and adolescent rats (Le Foll and Goldberg, 2005; Natarajan et al., 2011; Vastola et al., 2002); however, negative findings also have been reported (Belluzzi et al., 2004; Shoaib et al., 1994; Shram et al., 2006; Vastola et al., 2002). To date, no studies have reported tobacco constituent CPP; however, nicotine CPP is less likely to develop in rats pre-exposed to tobacco smoke compared to rats pre-exposed to nicotine alone (de la Pena et al., 2014). This suggests that non-nicotine constituents may diminish the rewarding effects of tobacco.

While it is known that nicotine activates the mesolimbic dopamine reward system, it is unclear if other tobacco constituents have similar or different effects, or if dopamine activation differs between adolescence and adulthood. This study was designed to reduce this gap. Furthermore, locomotor activity and CPP were assessed to determine if age-dependent differences in dopamine activation among tobacco constituents were associated with differences in dopamine-relevant behaviors.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats, obtained from Harlan Laboratories (Indianapolis, IN, USA), were housed in age-matched pairs in polycarbonate cages. Rats arrived at two ages: adult [postnatal day (PND) 60 or greater] and adolescent (PND 21–23). Rats were maintained in a temperature-controlled environment with a 12-h light-dark cycle, and had free access to rodent chow and water in their home cages. All studies were carried out in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), the ARRIVE guidelines, and in accordance with the Institutional Animal Care and Use Committee (IACUC) of the Food Drug Administration (FDA) and with other federal and state regulations. Additionally, place conditioning and in vivo microdialysis studies were performed in accordance with the IACUCs associated with RTI and the University of Kentucky, respectively.

2.2. Drugs

(-)-Nicotine, (-)-cotinine, myosmine, and (+)-methamphetamine were purchased from Sigma-Aldrich (St. Louis, MO). (±)-Anatabine was purchased from Matrix Scientific (Columbia, SC). These compounds were dissolved in sterile saline (USP grade), and the pH was adjusted to approximately neutral (pH ~ 7), as necessary. Doses of tobacco constituents were expressed as mg/kg of free base, and methamphetamine was expressed as mg/kg of the HCl salt. Nicotine, cotinine, myosmine, anatabine, and methamphetamine were injected in a volume of 1 ml/kg. Acepromazine, xylazine, and ketamine were obtained from Butler Schein (Dublin, OH). Carprofen and isoflurane were obtained from Merritt (Ridgefield, CT) and Cardinal Health (Dublin, OH), respectively.

2.3. Equipment

Microdialysis experiments were conducted in Plexiglas chambers with a pine bedding floor measuring 25 cm × 44 cm × 38 cm for the experiments using adults, and measuring 25 cm × 44 cm by 24 cm for experiments using adolescents. A swivel and tether system (BAS, Indianapolis, IN) was attached to the side of the chamber and connected to a microsyringe pump (KD Scientific, Holliston, MA, Model KDS250). Microdialysis samples were analyzed for dopamine using high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD, ESA Inc., Chelmsford, MA) as previously described (Meyer et al., 2013). The computer-controlled HPLC-EC system consisted of a solvent delivery pump (ESA model 582), a Coulochem III 5200 A electrochemical detector, and an ESA 542 HPLC autosampler and a 5014B analytical cell and 5020 guard cell. The guard cell was set at + 350 mV, electrode 1 at − 150 mV, and electrode 2 at + 220 mV. The mobile phase consisted of 90 mM NaH_2PO_4 H_2O, 50 mM citric acid, 1.7 mM 1-octanesulfonic acid, 50 μM EDTA, and 10% acetonitrile (pH 3.0 adjusted with phosphoric acid; flow rate was 0.6 ml/min). Samples (20 μl) were auto-injected onto an analytical column (ESA MD 150 × 3, 150 mm × 3.2 mm) and peaks were compared with external standards using an ESA Chromatography Data System (EZChrom Elite, ESA Chelmsford, MA).

Place conditioning sessions were conducted in an automated system comprised of three-compartment chambers, with each side measuring 27.5 cm × 22 cm × 31.5 cm and the center measuring 14 cm × 22 cm × 31.5 cm. Chambers were surrounded by an array of 4 × 16 photocell infrared beams. The equipment was interfaced with San Diego Systems software (model 6610-001-A, San Diego, CA). Compartments were separated from each other by removable doors, and each compartment had distinct environmental cues. The equipment was verified to be unbiased for both adolescent and adult rats before conditioning sessions began, i.e., when averaged across animals of either age, amount of time spent was not different between side compartments, indicating lack of systematic side preference.

2.4. Experimental design

Upon arrival, rats were assigned to a single dose of a single compound or saline (n = 8–14/age/group). Nicotine (0.2–0.8 mg/kg), cotinine (0.5–5.0 mg/kg), anatabine (0.5–5.0 mg/kg), and myosmine (5.0–20.0 mg/kg) were evaluated. Also, methamphetamine (0.3–1.0 mg/kg) served as a positive control in the place conditioning study. Nicotine doses were chosen based on their ability to induced CPP in rats (Le Foll and Goldberg, 2005), and increase dopamine release in rat NAc (Adermark et al., 2015; Rahman et al., 2008; Silvagni et al., 2008). Minor tobacco alkaloid doses were chosen based on their ability to alter locomotor activity or intracranial self-stimulation (ICSS) threshold (Harris et al., 2015; Wiley et al., 2015), or substitute for nicotine in drug discrimination (Goldberg et al., 1989). Subcutaneous (s.c.) administration was used for all tobacco constituents to minimize first pass metabolism (Matta et al., 2007). Methamphetamine was administered intraperitoneally (i.p.).

2.5. Microdialysis surgery

Surgeries for microdialysis were performed under aseptic conditions on PND 76–77 (adults) or PND 30–31 (adolescents), using previously described methods (Meyer et al., 2013; Rahman et al., 2007). Anesthetic cocktails were based on age differences in pharmacokinetic effects of ketamine and xylazine (Veilleux-Lemieux et al., 2013). Adult rats were anesthetized by administering (i.p.) 0.44–0.54 ml/kg body weight of a cocktail containing 0.75 mg/kg acepromazine, 7.5 mg/kg xylazine, and 75 mg/kg ketamine. Adolescent rats were anesthetized by administering (i.p.) 0.15 ml of a cocktail containing 8 mg/kg xylazine and 60 mg/kg ketamine. Depth of anesthesia was monitored by eye blink response to corneal stimulation and muscle twitch response to strong pinch of the toe and tail. If responses were observed, supplementary anesthetic 1–4% isoflurane/oxygen inhalant was employed until responses were absent. Rats were placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL), and a guide cannula (MD-2251, 22 gauge, BAS, Indianapolis, IN) was implanted unilaterally in the NAc shell, with coordinates adjusted for each age (Paxinos and Watson, 1986). For