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Effects of nicotine-containing and "nicotine-free" e-cigarette refill liquids on intracranial self-stimulation in rats

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ABSTRACT

Background: Animal models are needed to inform FDA regulation of electronic cigarettes (ECs) because they avoid limitations associated with human studies. We previously reported that an EC refill liquid produced less aversive/anhedonic effects at a high nicotine dose than nicotine alone as measured by elevations in intracranial self-stimulation (ICSS) thresholds, which may reflect the presence of behaviorally active non-nicotine constituents (e.g., propylene glycol) in the EC liquids. The primary objective of this study was to assess the generality of our prior ICSS findings to two additional EC liquids. We also compared effects of "nicotine-free" varieties of these EC liquids on ICSS, as well as binding affinity and/or functional activity of nicotine alone, nicotine-containing EC liquids, and "nicotine-free" EC liquids at nicotinic acetylcholine receptors (nAChRs).
Methods and results: Nicotine alone and nicotine dose-equivalent concentrations of both nicotine-containing EC liquids produced similar lowering of ICSS thresholds at low to moderate nicotine doses, indicating similar reinforcement-enhancing effects. At high nicotine doses, nicotine alone elevated ICSS thresholds (a measure of anhedonia-like behavior) while the EC liquids did not. Nicotine-containing EC liquids did not differ from nicotine alone in terms of binding affinity or functional activity at nAChRs. "Nicotine-free" EC liquids did not affect ICSS, but bound with low affinity at some (e.g., α4ß2) nAChRs.
Conclusions: These findings suggest that non-nicotine constituents in these EC liquids do not contribute to their reinforcement-enhancing effects. However, they may attenuate nicotine's acute aversive/anhedonic and/or toxic effects, which may moderate the abuse liability and/or toxicity of ECs.

1. Introduction

Electronic cigarettes (ECs) are aerosol-producing devices designed to simulate the use of conventional tobacco cigarettes. Although ECs are often viewed as less addictive and safer than tobacco cigarettes, their abuse liability and other health consequences have not been well established (Brandon et al., 2015; Glasser et al., 2017; Walton et al., 2015). Nonetheless, ECs are becoming increasingly popular, especially among smokers and adolescents (e.g., Arrazola et al., 2015; Glasser et al., 2017). To address this growing health concern, the Food and Drug Administration Center for Tobacco Products (FDA CTP) recently extended their authority to regulate ECs in the same manner as cigarettes and other tobacco products (Food and Drug Administration, 2016). Development of appropriate methodology for evaluating abuse liability and other adverse effects of ECs is needed to inform FDA CTP regulation of these products.

Animal models are essential for tobacco product evaluation because they avoid limitations associated with human studies (e.g., inability to isolate the central nervous system (CNS) effects of nicotine and other tobacco constituents from peripheral sensory factors (e.g., taste, smell)) (Donny et al., 2012). An emerging approach for this purpose involves the use of extracts that are derived directly from tobacco or tobacco smoke and that contain a mixture of tobacco constituents (for review, see Brennan et al., 2014). In contrast to traditional preclinical models of tobacco addiction, which involve administration of nicotine alone or other isolated tobacco constituents, use of extracts provides insights

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into how the numerous constituents in a tobacco product act together to influence abuse liability. While such interactions can also be studied using exposure to actual cigarette smoke or EC aerosol (Bruijnzeel et al., 2011; Harris et al., 2010; Ponzoni et al., 2015; Small et al., 2010), these inhalational models do not allow dissociation of the direct CNS effects of smoke or EC aerosol from its sensory effects (e.g., taste, smell). Because extracts are administered systemically (i.p., s.c., or i.v.), they allow for the dissociation of these factors, as well as for more precise experimental control over dosing. Several studies have reported greater abuse liability for tobacco smoke extracts compared to nicotine alone (e.g., Brennan et al., 2015; Brennan et al., 2014; Costello et al., 2014), which may be due to the presence of certain constituents in the extracts (e.g., minor alkaloids, acetaldehyde) that can mimic or enhance nicotine's addiction-related effects when studied in isolation (e.g., Arnold et al., 2014; Bardo et al., 1999; Belluzzi et al., 2005).

Preclinical evaluation of EC liquids, which often contain a combination of nicotine and other behaviorally relevant constituents (e.g., minor alkaloids, acetaldehyde, and propylene glycol (Etter et al., 2013; Goniewicz et al., 2014; Han et al., 2016)), provides similar advantages as study of tobacco extracts. We recently found that low to moderate doses of nicotine alone and nicotine dose-equivalent concentrations of an EC liquid (Aroma E-Juice Dark Honey) were similar in terms of their ability to lower intracranial self-stimulation (ICSS) thresholds (LeSage et al., 2016), a putative measure of nicotine's ability to enhance the reinforcing effects of other stimuli ("reinforcement-enhancement") (e.g., Caggiula et al., 2009; Harrison et al., 2002; Huston-Lyons and Kornetsky, 1992). At high nicotine doses, nicotine alone elevated ICSS thresholds while EC liquid did not, suggesting a reduction in nicotine's acute aversive/anhedonic effects when delivered in EC liquid. Given that nicotine's aversive/anhedonic effects can limit its intake (see Fowler and Kenny, 2012; Fowler and Kenny, 2014; Fowler et al., 2011), reduction of these effects would be expected to increase EC consumption. However, we found no differences in i.v. self-administration (SA) of nicotine alone versus EC liquid (see LeSage et al., 2016 and below for further discussion). Alternatively, the ICSS findings might reflect a reduction in nicotine's toxic effects, which would be equally important given that product toxicity is a primary concern of the FDA CTP (Food and Drug Administration, 2016). Regardless of the interpretation of these findings, they suggest that at least this EC liquid contains behaviorally active levels of non-nicotine constituents as measured using ICSS. It is essential to evaluate the generality of these findings to additional EC liquids, which can differ substantially in levels of behaviorally relevant non-nicotine constituents including minor alkaloids (Etter et al., 2013; Goniewicz et al., 2014; Han et al., 2016).

The goal of this study was to further evaluate the acute effects of EC liquids on ICSS in order to understand the relative contribution of CNS effects of nicotine and non-nicotine constituents in EC abuse liability. Following an initial analysis of nicotine and minor alkaloid levels in 20 different EC liquids, we compared the ICSS threshold-altering effects of nicotine alone and nicotine dose-equivalent concentrations of EC liquids containing relatively high (Janty EC liquid) and low (NicVape EC liquid) levels of minor alkaloids relative to nicotine (Experiment 1). In contrast to the Aroma E-Juice EC liquid that we studied in LeSage et al. (2016), both Janty and NicVape EC liquids are available in a labeled nicotine concentration of 0 mg/ml. Therefore, Experiment 2 evaluated effects of these "nicotine-free" EC liquids on ICSS. To complement the behavioral data, Experiment 3 compared binding affinity of nicotine alone, nicotine-containing Janty and NicVape EC liquids, and "nicotine-free" Janty and NicVape EC liquids at several nicotinic acetylcholine receptors (nAChRs), including the α4ß2 and α3ß4 nAChR subtypes, which are implicated in nicotine addiction (De Biasi and Salas, 2008; Fowler et al., 2008). Functional effects of nicotine alone and nicotine-containing EC liquids at α4ß2 and α3ß4 nAChRs were also compared in a rubidium efflux assay.

2. Methods

2.1. Animals

Male adult Holtzman rats (Harlan/Envigo, Indianapolis, IN) weighing 300–350 g at arrival were individually housed in a temperature- and humidity-controlled colony room with unlimited access to water under a reversed 12 h light/dark cycle. Rats were food restricted to 18 g/day to facilitate operant performance and avoid detrimental health effects of long-term ad libitum feeding (Keenan et al., 1997; Keenan et al., 1999). Protocols were approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation in accordance with the 2011 NIH Guide for the Care and Use of Laboratory Animals and the 2003 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

2.2. Analysis of nicotine and minor alkaloids in EC liquids

2.2.1. Initial EC liquid alkaloid analysis

Concentrations of nicotine and the minor alkaloids nornicotine, anabasine, and anatabine were analyzed in 20 EC liquids using liquid chromatography-tandem mass spectrometry (LC–MS/MS) by modification of a previously described method (Rangiah et al., 2011). Briefly, each EC liquid was mixed with stable isotope-labeled nicotine and nornicotine, anatabine, and anabasine (internal standards), diluted with 10 mM ammonium acetate containing 5% methanol, and analyzed by LC–MS/MS on a Hypercarb column (Thermo Scientific), using 10 mM ammonium acetate (with 0.001% formic acid) and methanol as mobile phase. EC liquids were chosen based on their local popularity (e.g., TC Vape), their previous alkaloid characterization in Etter et al. (2013) (e.g., Johnson Creek), or because they were advertised as containing higher levels of minor alkaloids than other ECs (Aroma E-Juice). EC liquids were purchased in the Minneapolis area (TC Vape) or ordered online through their manufacturer (all other EC liquids). Following completion of the multi-product alkaloid comparison and selection of two EC liquids for the behavioral studies that contained relatively high and low levels of minor alkaloids relative to nicotine (i.e., Janty and NicVape, see below), nicotine and minor alkaloid levels in the "nicotine-free" variety of these products were analyzed in the same manner.

2.2.2. Routine nicotine assay

Nicotine concentrations in solutions of nicotine alone and Janty and NicVape EC liquid used in Experiments 2 and 3 were measured by gas chromatography (GC) with nitrogen phosphorus detection, according to standard protocol in our laboratory (Hieda et al., 1999),. The measured nicotine concentrations ± SEM for Janty and NicVape EC liquid vials used for dose preparation in Experiment 1 and 3 (labeled nicotine content = 24.0 mg/ml) were 22.08 ± 0.02 mg/ml (range 22.06–22.10 mg/ml) and 22.81 ± 0.29 (range 22.52–23.10 mg/ml), respectively. The average measured nicotine concentrations ± SEM for Janty and NicVape vials used for Experiments 2 and 3 (labeled nicotine content = 0 mg/ml) were 0.0058 ± 0.0017 mg/ml (range 0.0042–0.0075 mg/ml) and 0.0008 ± 0.0006 mg/ml (range 0.0001–0.0014 mg/ml), respectively.

2.3. Drugs

Nicotine bitartrate was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in sterile saline. Janty EC refill liquid (DK Port flavor) and NicVape EC refill liquid (Fruit Stripe Gum/Fruit Twist flavor) were obtained from Janty USA (http://www.usa.janty.com, Blasdell, NY) and NicVape (http://www.nicvape.com, Spartanburg, SC), respectively. According to the manufacturer, the Janty refill liquid contained 66.1% propylene glycol (PG), 15.0% vanillin tincture, 1.0%