Neuropharmacology xxx (2014) 1–12

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Reinforcing and neural activating effects of norharmane, a non-nicotine tobacco constituent, alone and in combination with nicotine

Monica M. Arnold [a, *], Sandra E. Loughlin [a], James D. Belluzzi [a], Frances M. Leslie [a, b]

[a] Department of Pharmacology, School of Medicine, University of California, Irvine, 92697, USA
[b] Department of Anatomy and Neurobiology, School of Medicine, University of California, Irvine, 92697, USA

ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form
14 May 2014
Accepted 23 May 2014
Available online xxx

Keywords:
Norharmane
Nicotine
Tobacco
Monoamine oxidase
Self-administration
cfos

ABSTRACT

Tobacco use is the leading cause of preventable death. Although the health risks are well known, cessation rates remain low. Whereas behavioral and neuroanatomical studies on tobacco addiction conventionally use nicotine, there is evidence that other constituents, such as monoamine oxidase inhibitors, may be important factors for modeling smoking. The aims of the present study were therefore to determine whether norharmane, a tobacco constituent and monoamine oxidase inhibitor, is self-administered alone and/or in combination with nicotine, and to evaluate the neural mechanisms underlying acquisition of self-administration of the two drugs. Sprague–Dawley rats were catheterized and allowed to intravenously self-administer either saline, nicotine (7.5 μg/kg/inj), norharmane (0.25 or 2.5 μg/kg/inj), alone or combined together (7.5 + 2.5 μg/kg/inj) for five days at fixed ratio (FR)1, two days each at FR2 and FR5, and one day at progressive ratio. Animals acquired self-administration of norharmane alone (2.5 μg/kg/inj), and the reinforcing effects of nicotine and norharmane were additive. For neuroanatomical analyses, rats self-administered the same treatments for six days at FR1, then brains were collected and processed by *in situ* hybridization for cfos mRNA expression. Treatment-specific profiles of regional cfos expression and correlations between cfos mRNA levels and behavioral responding were observed. Thus, not only was norharmane behaviorally reinforcing but, when combined with nicotine, resulted in patterns of neural activation distinct from that of norharmane or nicotine alone. This suggests that non-nicotine constituents can have central activating effects independent of nicotine, further substantiating the need for their inclusion in preclinical investigations of tobacco dependence.

1. Introduction

Smoking is highly addictive, increasing the risk for cancer, cardiovascular and respiratory diseases, and causing one out of five deaths each year (USDHHS, 2004). Approximately 95% of unaided quit attempts result in relapse within a year (Benowitz, 2008) and treatment success rates for those individuals using current pharmacotherapies are quite poor, with few people remaining smoke-free for extended periods (Reus and Smith, 2008; Jorenby et al., 2006). Such findings argue for improved preclinical models of tobacco use with which to test new treatment approaches.

Most animal studies that model tobacco use and dependence have largely focused on nicotine, the main psychoactive constituent in smoke considered responsible for tobacco addiction and dependence (Polosa and Benowitz, 2011). However, nicotine is only one of over 7000 constituents found in smoke (USDHHS, 2010), and has been shown to be weakly reinforcing compared to other abused drugs (Manzardo et al., 2002). Thus, findings from studies of nicotine alone may not accurately depict the central actions of tobacco.

There is evidence from preclinical studies that non-nicotine tobacco smoke constituents may enhance nicotine's effects. Monoamine oxidase (MAO) inhibitors are present in tobacco smoke, and MAO is significantly inhibited in the brains of smokers, an effect that appears to be a consequence of, not a risk factor for, smoking (Fowler et al., 2003). Since this enzyme metabolizes monoamines, MAO inhibition may result in increased synaptic levels of dopamine released by nicotine, thus potentiating its reinforcing effects (Berlin and Anthenelli, 2001; Fowler et al., 2003; Lewis et al., 2007). To that end, we and others have shown that peripheral injections of clinical MAO inhibitors, like tranylcypromine, induce significant increases in nicotine reinforcement in self-administration tests (Guillem et al., 2005, 2006; Villégier et al.,

* Corresponding author. Present address: Box: 356560, Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, 98195, USA. Tel.: +1 206 685 5380(office); fax: +1 206 543 9520.
E-mail addresses: monicamarnold@gmail.com, mmarnold@uw.edu (M.M. Arnold).

http://dx.doi.org/10.1016/j.neuropharm.2014.05.035
0028-3908/

2006, 2007). Furthermore, pretreatment with norharmane, a beta-carboline naturally found in tobacco leaf and smoke (Poindexter and Carpenter, 1962) that reversibly inhibits both MAO-A and MAO-B (Herraiz and Chaparro, 2005), can also augment nicotine self-administration (Guillem et al., 2005). However, it is not known whether norharmane alone is reinforcing or if it can enhance nicotine self-administration behavior when it is combined together with nicotine in the intravenous solution.

The aim of the present study was therefore to determine whether norharmane has reinforcing properties in the rat intravenous self-administration model that was designed to test the acquisition of self-administration rather than maintenance, and whether it potentiates the behavioral effects of nicotine. In order to elucidate the neuronal mechanisms underlying acquisition of self-administration behavior, regional cfos mRNA expression was also measured in the brains of rats that had self-administered nicotine or norharmane, alone and in combination. Our combined behavioral and neuroanatomical data show norharmane not only to have behavioral and neuronal activating effects of its own, but also to alter neuronal responses to nicotine. These findings further emphasize the need for inclusion of non-nicotine components in preclinical studies of tobacco dependence.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (380–400 g) were obtained from Charles River (Hollister, CA, U.S.A.) and maintained on a 12 h light/dark cycle (lights on at 07:00 am). Animals were group-housed and handled before surgery. After surgery, animals were single-housed. Food and water was available *ad libitum* with all testing occurring during the light phase. Rats recovered for three days post-operatively before experiments began and were housed in an AAALAC-accredited vivarium maintained by UCI University Laboratory Animal Resources personnel. All experimental procedures were performed in compliance with NIH Guide for Care and Use of Laboratory Animals (NIH No. 85-23, rev. 1985) and approved by the UCI Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Catheterization

Rats were anesthetized with equithesin (0.0035 ml/g) and a catheter was implanted into the right external jugular vein (Belluzzi et al., 2005). To maintain patency, catheters were flushed daily with heparinized saline (67 U heparin/0.2 ml). Catheter patency was tested with a quick-acting intravenous anesthetic, propofol (1 mg), on the day prior to self-administration, after the fifth test day and after the last test. Rats that did not display rapid anesthesia (5–10 s) were excluded from analysis.

2.3. Drug treatments

Nicotine di-tartrate and norharmane hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). Nicotine di-tartrate was dissolved in saline and the stock solution was raised to a pH of 7.2–7.4 with sodium hydroxide, with doses expressed as base. The 7.5 μg/kg/inj nicotine dose was chosen, rather than the more commonly used 30 μg/kg/inj dose, based on prior evidence of higher levels of responding for low doses of nicotine when nose pokes are used as the behavioral operandum, as was done in the current study (Clemens et al., 2010). Although the 7.5 μg/kg/inj nicotine dose may not be the norm for self-administration of nicotine alone, we and others have previously shown that pretreatment with clinical MAO inhibitors prior to behavioral testing causes a leftward shift in the nicotine dose response, with low nicotine doses including 7.5 μg/kg/inj more readily self-administered than the conventional 30 μg/kg/inj dose. (Guillem et al., 2005, 2006; Villégier et al., 2007).

Norharmane was dissolved in saline and doses were calculated as salt. The norharmane doses used (0.25 and 2.5 μg/kg/inj) were calculated based on norharmane plasma concentrations found in smokers after smoking one cigarette (116 ± 50.8 pg/ml; Rommelspacher et al., 2002). All intravenous drug solutions were made daily from stock solutions and filtered with a 22-μm Millipore® filter prior to being filled into glass syringes.

2.4. Self-administration

2.4.1. Experiment 1

Animals were tested in standard operant chambers (Med Associates, St. Albans, VT), equipped with nose-pokes. In order to investigate the ability of each drug to support acquisition of self-administration, rats received no prior operant training and were naïve to both drug and the behavioral paradigm before testing began. In experiment 1a, rats intravenously self-administered norharmane (0, 0.25 and 2.5 μg/kg/inj) during 2-h daily sessions over ten consecutive days. In experiment 1b, rats self-administered either saline, nicotine (7.5 μg/kg/inj), norharmane (2.5 μg/kg/inj), or nicotine + norharmane (7.5 μg/kg/inj + 2.5 μg/kg/inj) during 2-h daily sessions over ten consecutive days. Rats nose-poked in reinforced and non-reinforced holes, with nose-pokes in the reinforced hole yielding an intravenous 20-μl injection of saline or drug over 1.1 s. A cue-light over the reinforced hole illuminated for the duration of the infusion, followed by a 20-s timeout during which the house-light turned off and drug was unavailable. Nose-pokes in the non-reinforced hole were recorded but had no consequence and were used to assess overall locomotor activity. Drug injections were controlled and behavior was recorded with a Med Associates program (St. Albans, VT, U.S.A.) and PC computer system.

The first five 2-h test sessions were at a fixed ratio 1 (FR1) reinforcement schedule (one nose-poke delivered an injection), days six and seven at FR2, days eight and nine at FR5, and day ten at a progressive ratio (PR) schedule where the number of nose-pokes required to receive an infusion increased exponentially during the session (Richardson and Roberts, 1996: $[(5e^{(\text{number of infusions}-0.2)})-5]$). For the PR session, rats had 30 min to complete each ratio. If an animal did not meet the nose-poke requirement within that time, the behavioral session ended and the last completed ratio that resulted in an infusion was considered the breakpoint value.

2.4.2. Experiment 2

For anatomical analyses of neural mechanisms underlying acquisition of self-administration behavior, a separate group of rats self-administered saline, nicotine (7.5 μg/kg/inj), norharmane (2.5 μg/kg/inj), or nicotine + norharmane (7.5 μg/kg/inj + 2.5 μg/kg/inj) during 2-h daily self-administration sessions at FR1 over six consecutive days. This timecourse was chosen because it was determined to be the number of sessions required, on average, to observe acquisition of self-administration (unpublished observations). Immediately after the last session, animals were sacrificed, brains were promptly removed and flash frozen at −20 °C in a dry ice – isopentane mixture and stored at −80 °C until they were processed for measurement of regional levels of cfos mRNA expression.

2.5. In situ hybridization

Regional expression of cfos mRNA was measured by *in situ* hybridization as previously reported by Winzer-Serhan et al. (1999). Cfos mRNA levels were

Table 1
Brain areas analyzed for cfos mRNA expression.

Region	Subregion	Abbreviation
Cortical areas	Infralimbic	IL
	Prelimbic	PrL
	Anterior cingulate	AC
	Ventral/lateral orbital	VLO
	Agranular insular	AI
	Retrosplenial	RS
	Primary motor	M1
	Secondary motor	M2
	Primary sensory	S1
	Secondary sensory	S2
Caudate/putamen (Striatum)	Dorsolateral	dlCPu
	Ventrolateral	vlCPu
	Dorsomedial	dmCPu
	Ventromedial	vmCPu
Nucleus accumbens (Striatum)	Core	AcbC
	Shell	AcbS
Pallidum	External globus pallidus	EGP
	Ventral pallidum	VP
Amygdala & hypothalamus	Bed nucleus stria terminalus	BNST
	Basolateral amydgala	BLA
	Medial amygdala	MeA
	Central amygdala	CeA
	Hypothalamic paraventricular nucleus	PVN
Hippocampal complex	CA1 field	CA1
	CA2 field	CA2
	CA3 field	CA3
	Dentate gyrus	DG
	Lateral septum	LS
Thalamus	Ventoanterior/ventrolateral	VA/VL
	Mediodorsal	MD
	Paraventricular	PV
	Central	CTh
	Anteroventral	AV

Please cite this article in press as: Arnold, M.M., et al., Reinforcing and neural activating effects of norharmane, a non-nicotine tobacco constituent, alone and in combination with nicotine, Neuropharmacology (2014), http://dx.doi.org/10.1016/j.neuropharm.2014.05.035