Progress in Neuro-Psychopharmacology & Biological Psychiatry 69 (2016) 69–77

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Nicotine-specific and non-specific effects of cigarette smoking on endogenous opioid mechanisms

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 30 March 2016
Accepted 12 April 2016
Available online 17 April 2016

Keywords:
Smoking
PET
[11C]-Carfentanil
OPRM1
A118G

ABSTRACT

This study investigates differences in μ-opioid receptor mediated neurotransmission in healthy controls and overnight-abstinent smokers, and potential effects of the *OPRM1* A118G genotype. It also examines the effects of smoking denicotinized (DN) and average nicotine (N) cigarettes on the μ-opioid system. Positron emission tomography with ^{11}C-carfentanil was used to determine regional brain μ-opioid receptor (MOR) availability (non-displaceable binding potential, BP_{ND}) in a sample of 19 male smokers and 22 nonsmoking control subjects. Nonsmokers showed greater MOR BP_{ND} than overnight abstinent smokers in the basal ganglia and thalamus. BP_{ND} in the basal ganglia was negatively correlated with baseline craving levels and Fagerström scores. Interactions between group and genotype were seen in the nucleus accumbens bilaterally and the amygdala, with G-allele carriers demonstrating lower BP_{ND} in these regions, but only among smokers.

After smoking the DN cigarette, smokers showed evidence of MOR activation in the thalamus and nucleus accumbens. No additional activation was observed after the N cigarette, with a mean effect of increases in MOR BP_{ND} (i.e., deactivation) with respect to the DN cigarette effects in the thalamus and left amygdala. Changes in MOR BP_{ND} were related to both Fagerström scores and changes in craving.

This study showed that overnight-abstinent smokers have lower concentrations of available MORs than controls, an effect that was related to both craving and the severity of addiction. It also suggests that nicotine non-specific elements of the smoking experience have an important role in regulating MOR-mediated neurotransmission, and in turn modulating withdrawal-induced craving ratings.

1. Introduction

With over one billion smokers worldwide, nicotine dependence is a major health concern. There are more than 5 million deaths associated with tobacco each year, and tobacco smoking is the most prevalent cause of "preventable disease and death" in the United States (Graul and Prous, 2005). There are substantial lines of evidence pointing to a strong link between nicotine use and endogenous μ-opioid mechanisms, which may mediate some of nicotine's addictive properties and distress during withdrawal (for review see Pomerleau, 1998).

Animal and cell culture studies suggest that acute nicotine induces endogenous opioid release (Boyadjieva and Sarkar, 1997; Davenport et al., 1990). However, attempts to translate these initial findings into human studies have led to inconsistent results. Studies examining changes in MOR availability (binding potential, BP) after smoking denicotinized (DN) versus average nicotine (N) cigarettes with positron emission tomography (PET), an indirect measure of changes in neurotransmitter release and μ-opioid receptor activation, have found both reductions in BP (suggesting activation of neurotransmission) and increases (deactivation) in different regions of the brain (Domino et al., 2015; Scott et al., 2006). Alternatively, some studies have not found any significant differences in MOR binding after smoking N versus DN cigarettes (Kuwabara et al., 2014; Ray et al., 2011). Measures at baseline have also shown either lower MOR BP in smokers compared to nonsmoking controls (Scott et al., 2006), or no significant differences between groups (Kuwabara et al., 2014). The μ-opioid system is known to respond to positive expectancies, including the so-called placebo effect (Pecina et al., 2015a; Scott et al., 2008; Zubieta et al., 2005), which may impact the effects of both DN and N smoking. This effect

Abbreviations: PET, positron emission tomography; BP_{ND}, non-displaceable binding potential; MOR, μ-opioid receptor; CFN, carfentanil; RCL, raclopride; OPRM1, human μ-opioid receptor; BG, basal ganglia; NAC, nucleus accumbens; DN, denicotinized; N, nicotine; HC, healthy controls; VAS, visual analog scale.

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http://dx.doi.org/10.1016/j.pnpbp.2016.04.006
0278-5846/

could be particularly prominent in studies conducted after nicotine abstinence, when craving and positive expectancies are highest. This was initially suggested in a small pilot study (Scott et al., 2006), and could potentially contribute to inconsistencies in results across study designs.

To explore this possibility, the current study examined MOR non-displaceable BP (BP_{ND}) (Innis et al., 2007) at baseline, preceding and following DN and N cigarettes, and again during DN and N smoking using a single blind design. In this analysis, effects of DN and N cigarette smoking on subsequent BP_{ND} values were further controlled by their corresponding baseline values to provide a corrected measure of μ-opioid system activation (changes in BP_{ND}) after DN and N smoking. Smokers were studied after verified overnight abstinence when craving for cigarettes would be high. The effects of a common functional polymorphism of the MOR (*OPRM1*) gene, the A118G polymorphism, were also evaluated in these analyses. This substitution of an aspartic acid for asparagine in the MOR gene has been associated with lower levels of MOR mRNA and protein (Zhang et al., 2005; Kroslak et al., 2007). There has been a great deal of interest in whether this polymorphism is associated with aspects of addiction, but studies have given inconsistent results (Arias et al., 2006). The A118G polymorphism has been shown in previous PET studies to reduce both baseline MOR BP_{ND} (Pecina et al., 2015b; Ray et al., 2011; Weerts et al., 2013) and the activation of the μ-opioid system during positive expectations (Pecina et al., 2015b).

2. Materials and methods

2.1. Participants and study design

Twenty-four smokers and 22 healthy nonsmokers between the ages of 20 and 35 were recruited by advertisement for this study. All participants were male and right-handed, not on any medication, and had no history or current signs of psychiatric or physical illnesses. Participants were excluded if they used any drugs of abuse besides tobacco smoking. Written informed consent was obtained from all subjects, and the study was approved by the Institutional Review Board for Human Subject Research and the Radioactive Drug Research Committee at the University of Michigan.

Five individuals from the experimental group were excluded from the analyses. Three had missing elements of MRI or PET data, one subject was discovered to be a nonsmoker, and one tested positive for opioids at the time of his PET scan. The final sample was 19 male smokers between the ages of 20 and 35 (mean ± SD: 25.3 ± 4.4 years) and 22 nonsmokers (mean ± SD: 24.2 ± 3.7 years). Participants smoked between 6 and 30 cigarettes a day (mean ± SD: 18.4 ± 5.6), and had Fagerström scale of nicotine dependence scores ranging from 2 to 8.3 (mean ± SD: 5.5 ± 1.9) with 10 being most dependent (Heatherton et al., 1991).

Smokers were instructed to cease smoking the night before the 8:30 AM scans, resulting in 8–12 h of abstinence. Compliance was tested using a carbon monoxide (CO) detector (Vitalograph Breath CO Model BC1349, Vitalograph Inc., Lenexa, KS) with a requirement of CO levels <10 ppm prior to scanning (Domino and Ni, 2002).

In the overall protocol, PET scanning was conducted using the radiotracers ^{11}C-carfentanil (^{11}C-CFN) and ^{11}C-raclopride (^{11}C-RCL) targeting μ-opioid and dopaminergic D2/D3 receptors, respectively. Only the ^{11}C-CFN data is reported here. Smokers participated in the trials on two separate days, with two 90 min PET scans each day. For the first half of each scan the participants were simply instructed to lie still, providing a baseline measure. Between 43 and 53 min after tracer administration, smokers were directed to smoke either two DN cigarettes (0.08 mg nicotine/cigarette, 9.1 mg tar/cigarette) or two N cigarettes (1.01 mg nicotine/cigarette, 9.5 mg tar/cigarette) through a one-way airflow system. Smokers received the N cigarettes second in order to prevent the effects of the nicotine from carrying over into the DN condition. Each day, participants received one scan using the tracer ^{11}C-CFN and one using the tracer ^{11}C-RCL, separated by approximately 2 h. On the second day the participants came in, the tracer order was switched. Whether subjects received ^{11}C-CFN or ^{11}C-RCL first on their first scanning day was counterbalanced to control for any possible effects the earlier tracer or MOR turnover might have on the later scan. However, tracers were administered at subpharmacological doses and therefore should not have had any pharmacological effects, occupying less than 1% of available receptors (Zubieta et al., 2003). The half-life of carbon-11 is 20 min, resulting in complete decay between tracer administrations. The data from the ^{11}C-RCL scans has been previously reported (Domino et al., 2012, 2013), as has a different analysis of the ^{11}C-CFN data (Domino et al., 2015).

Prior to scanning, smokers were asked to rate a 1–10 visual analog scale (VAS) for "craving". They repeated this at 30 and 60 min into each of the scans (once before smoking and once after smoking). At the 30 and 60 min time points, participants also completed the Positive and Negative Affectivity Schedule (PANAS; Watson and Clark, 1999), Profile of Mood States (POMS; McNair et al., 1971), and Spielberger State Anxiety Inventory (STAI; Spielberger et al., 1983).

Six of the healthy controls were asked to smoke a sham cigarette (unlit cardboard cylinder) in place of either the DN or N cigarette. The remaining 16 controls simply underwent one 90 min baseline ^{11}C-CFN scan in which they were asked to lie in the scanner with no intervention.

2.2. Scanning protocol and data acquisition

Participants were placed in a Siemens HR+ scanner and data were collected as previously described (Domino et al., 2012; Scott et al., 2006). Briefly, scans were acquired in three-dimensional mode (reconstructed FWHM resolution ~5.5 mm in-plane and 5.0 mm axially, with septa retracted and scatter correction). Images were reconstructed using iterative algorithms (brain mode; FORE/OSEM four iterations, 16 subsets; no smoothing) into a 128 × 128 pixel matrix in a 28.8 cm diameter field of view. Attenuation correction was done using a 6 min transmission scan (^{68}Ge source) obtained before the radiotracer was injected. Image data was transformed into two sets of parametric maps: a tracer transport measure (K1) and a receptor related measure (BP_{ND}) using a modified Logan graphical analysis (Logan et al., 1996) with the occipital cortex as a reference region.

A light forehead restraint was used on each participant to reduce movement, and two intravenous (antecubital) lines were placed. ^{11}C-CFN was synthesized at high specific activity through the reaction of ^{11}C-methyliodide and a non-methyl precursor (Jewett, 2001). The tracer was administered through one of the intravenous lines, beginning with a bolus containing half of the tracer. The other 50% was administered continuously during the scan.

A high-resolution anatomical magnetic resonance image was obtained for each participant using a 3 T scanner (Signa, General Electric, Milwaukee, WI). The acquisition sequence used was an axial SPGR IRpPrep MR (TE = 5.5, TR = 14, TI = 300, flip angle = 20°, NEX = 1, 124 contiguous images, 1.5 mm thickness), followed by axial T2 and proton density images (TE = 20 and 100, respectively; TR = 4000, NEX = 1, 62 contiguous images, 3 mm thickness).

2.3. Bloods/genotyping/plasma nicotine

Before the first scan, 10 mL of venous blood was drawn from each participant. Blood was processed by the Michigan Center for Translational Pathology laboratory biorepository and Michigan Sequencing Core for analysis and subsequently reconfirmed (Domino et al., 2012). Each participant's genotype at the *OPRM1* A118G polymorphism was determined. Participants were divided into two groups based on whether they carried at least one rare G allele (*G) or whether they were homozygous for the A allele. Nicotine plasma levels were also determined by drawing blood samples just before smoking (43 min) and at five other time points after smoking initiation (49, 59, 65, 75,