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Tobacco smoking produces greater striatal dopamine release in G-allele carriers with mu opioid receptor A118G polymorphism

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ABSTRACT

Objective: To determine if carriers of the allelic expression of the G variant of the human mu opioid receptor (OPRM1) A118G polymorphism have greater increases in striatal dopamine (DA) release after tobacco smoking.
Methods: Nineteen of 20 genotyped male tobacco smokers, after overnight abstinence, smoked denicotinized (denic) and average nicotine (nic) containing tobacco cigarettes in a PET brain imaging study using [^{11}C]raclopride.
Results: The right striatum had more free D_2 receptors than the left striatum pre- and post-tobacco smoking. After smoking the nic cigarettes, mean decreased DA binding was observed in the left dorsal caudate (−14 6 11; t = 3.77), left and right ventral putamen (−26 3–8; t = 4.27; 28 2 1; t = 4.25, respectively), and right caudate (17 18 1; t = 3.92). The effects of A118G genotype on the binding potentials for these four regions were then analyzed. Carriers of the G allele had larger magnitudes of DA release in response to nic smoking than those homozygous for the more prevalent AA allele in the right caudate and right ventral pallidum ($t = 3.03$; $p = 0.008$ and $t = 3.91$; $p = 0.001$). A voxel by voxel whole brain SPM analysis using an independent samples t test did not reveal any other differences between genotype groups. In addition, the venous plasma cortisol levels of the volunteers from 8:30 a.m. to 12:40 p.m. were lower in the AG/GG allele carriers. Nic smoking increased plasma cortisol in both groups, but they were higher in the AA group.
Conclusion: This preliminary study indicates a difference in both brain striatal DA release and plasma cortisol in A118G polymorphic male tobacco smokers.

1. Introduction

It is well known that complex disorders such as drug addiction have important genetic components. Multiple opioid receptors are involved in alcohol (Herz, 1977), cocaine, narcotic (Kreek, 1996a,b,c; Kreek et al., 2004), and tobacco (Lerman et al., 2004; Munafo et al., 2007; Ray et al., 2006, 2007) dependence. Kroslak et al. (2007) have emphasized the most frequent coding variant of the human mu opioid receptor is the adenine to guanine transition A118G. Less common carriers of the G allele have less functional mu opioid receptors (OPRM1) which affect several neurotransmitter systems. Zhang et al. (2005) found that OPRM1-G118 variant has reduced mRNA and protein yield producing allelic expression imbalance.

Over 30 years have passed since Karras and Kane (1980) reported that naloxone reduced tobacco smoking and craving. Subsequent human tobacco smoking studies involving opioid antagonists have yielded inconsistent results. Nevertheless, endogenous opioid release by nicotine and tobacco smoking is well established. Several years ago in a pilot brain imaging study (Scott et al., 2007), we reported tobacco smoking modulation by both the mu opioid and DA systems. In addition to the DA and endogenous opioid release after tobacco smoking, genetic polymorphisms probably introduce individual variation in the degree of neurotransmission that occurs. In particular, the A118G polymorphism in the mu opioid receptor is known to affect several different aspects of smoking, addiction and reward related behavior (Ray et al., 2006, 2007, 2011).

The present report is a follow up study with a different group of tobacco smokers on the role of OPRM1 A118G in brain DA release following tobacco smoking, as measured by [^{11}C]raclopride displacement. In addition, venous plasma cortisol levels were obtained throughout the entire scanning sessions as a measure of scanner stress and response to nicotine.

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2. Materials and methods

2.1. Subjects and design

Twenty healthy mentally normal male subjects between 20 and 36 yrs of age (mean 25.8 ± 4.8), who smoked 15–40 cigarettes per day, were recruited for this project. The exclusion criteria included any previous mental or physical illness, or use of any drugs of abuse other than nicotine. In addition, urinary drug toxicological analyses were done to ensure compliance. Subjects were instructed to cease tobacco use overnight, approximately 8–12 h before the study, because it is well known that the first cigarette of the day is the most satisfying. During the experiment, they were supine and restrained in a PET scanner from about 8:00 a.m. to 12:40 p.m. A counterbalanced day 1, and within a week or so, day 2 design of PET scans with [^{11}C]carfentanil and [^{11}C]raclopride in trace non-pharmacological doses was used. Only the [^{11}C]raclopride data are reported herein because the [^{11}C]carfentanil data analysis is still in progress. The subjects smoked two of each cigarette for 5 min because of the unusual method used to comply with the "no smoking" rule of University Hospital. All subjects received both radiotracers in randomized and counterbalanced order. Although the radioisotopes were counterbalanced, the order of tobacco smoking was not. The smoking interval between denic and nic smoking was about 2 h. Subjects first smoked two denic (0.08 mg nicotine) cigarettes, then two nic (1.01 mg nicotine) cigarettes inserted into a one way airflow gallon bottle. The two different research cigarettes were obtained through the courtesy of Dr. Frank P. Gullota (retired) and Ms. Cynthia S. Hayes of the Philip Morris Research Center, Richmond, VA. The nic cigarettes were prepared with unextracted tobacco (nicotine 1.01 mg/cigarette and tar 9.5 mg/cigarette). The denic cigarettes were made with almost 100% extracted tobacco (nicotine 0.08 mg/cigarette and tar 9.1 mg/cigarette). Both cigarettes had identical filter tips and were made from the same blend of tobacco with no flavors added. Thus, their tar content was almost identical (9.5 vs 9.1 mg) and only the mg of nicotine per cigarette was markedly different (1.01 vs 0.08 mg). Unfortunately, these research cigarettes are no longer available.

2.2. Scanning protocol

PET scans were acquired with a Siemens HR + scanner in three dimensional (3-D) mode (reconstructed FWHM resolution ~5.5 mm in-plane and 5.0 mm axially), with septa retracted and scatter correction. Participants were positioned in the PET scanner gantry, and two intravenous (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. [^{11}C]Raclopride was synthesized at high specific activity (>2000 Ci/mmol) by the reaction of O-desmethyl raclopride with [^{11}C]methyltriflate. In each of the scans, 10–15 mCi was administered, with a total mass of raclopride of 0.089 ± 0.047 μg/kg per scan. This ensured that the compound was administered in tracer quantities, that is, subpharmacological doses occupying less than 1% of the available receptors. Fifty percent of the radiotracer doses were administered as a bolus, and the remaining 50% by continuous infusion for the remainder of the study. Heart rate and blood pressure were monitored during both scans.

2.3. Image and data acquisition

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 performed through a 6 min transmission scan (68Ge source) obtained before the PET study, also with iterative reconstruction of the blank/transmission data followed by segmentation of the attenuation image. Small head motions during emission scans were corrected by an automated computer algorithm for each subject before analysis, and the images coregistered to each other with the same software (Minoshima et al., 1993). Time points were then decay corrected during reconstruction of the PET data. Image data were then transformed on a voxel-by-voxel basis into two sets of parametric maps: (a) a tracer transport measure (K_1), and (b) a receptor-related measure (distribution volume ratio, DVR). To avoid the need for arterial blood sampling, these measures were calculated using a modified Logan graphical analysis (Logan et al., 1996) with the cerebellum (devoid D_2 receptors) as the reference region. With the partial bolus continuous infusion radiotracer administration protocol used, the Logan plot becomes linear by 5–7 min after the start of radiotracer administration, allowing the calculation of receptor measures early after each tracer administration. The slope of the Logan plot is equal to the (Bmax/Kd) + 1 for this receptor site (receptor concentration divided by its affinity for the radiotracer), and it has been referred to as the DVR. Bmax/Kd (or DVR − 1) is the 'receptor related' measure (BP_{ND}, or free receptor availability in vivo; Bmax = concentration of receptors, Kd = receptor affinity for the radiotracer). As changes in Bmax/Kd will cause a change in the slope of the Logan plot, we measured DVR during both the early and late phases of each scan. The slope during the early phase was estimated from 5 to 40 min post-injection, whereas the slope for the second phase was estimated from 45 to 90 min post-injection. Anatomical MRI scans were acquired before PET scanning on a 1.5 T scanner (Sigma, General Electric, Milwaukee, WI). Acquisition sequences were axial SPGR IR-Prep MR (TE = 5.5, TR = 14, T1 = 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). K_1 and DVR images for each experimental period and MR images were coregistered to each other and to the International Consortium for Brain Mapping (ICBM) stereotactic atlas orientation. Inasmuch as [^{11}C]raclopride is a good radioligand only for striatal D_2 receptors and not for the entire brain, only striatal binding was determined. Statistical parametric maps of differences between conditions (denic vs. nic) were generated by anatomically standardizing the T1-SPGR MRI of each subject to the ICBM stereotactic atlas coordinates, with subsequent application of this transformation to the DA receptor binding maps. The accuracy of coregistration and nonlinear warping algorithms was confirmed for each subject individually by comparing the transformed MRI and PET images to each other and the ICBM atlas template. The actions of nicotine were determined by subtracting the denic smoking effects from the nic effects.

2.4. Genotyping

Ten milliliters of venous blood was drawn from all 20 subjects prior to scanning for genotyping analyses. Whole blood was stored in Corning Polypropylene RNase-/DNase-free tubes frozen at −80 °C until testing. Blood was sent to the Michigan Center for Translational Pathology (MCTP) laboratory biorepository for genomic DNA extraction and purification. Extracted DNA was amplified via PCR using Roche's High Fidelity PCR kit according to manufacturer's instructions. Primer sequences were as follows: Forward: AGAGGAGAATGTCAGATGCTCAGC (5′-3′) and Reverse: ATGGAGTAGAGGGCCATGATCGTG (5′-3′). Amplified product of 430 bp was confirmed using 1% agarose gel electrophoresis. Samples that were amplified successfully were sent to the University of Michigan Sequencing Core for sequencing using the same primers used for PCR amplification. Sequence chromatograms were evaluated to manually determine the A118G allele at position ~283 within the amplified sequence for each subject, using FinchTV 1.4.0 software (Geospiza, Inc.). Subjects were divided into two groups according to whether they carried the rare (G) allele. Therefore, the group without the G allele were homozygous for the A allele (AA group; n = 14), and the group containing the G allele were either homozygous (n = 1) or