Original investigation

The Relationship of Varenicline Agonism of α4β2 Nicotinic Acetylcholine Receptors and Nicotine-Induced Dopamine Release in Nicotine-Dependent Humans

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Abstract

Introduction: Cigarette smoking continues to be one of the most important behavioral causes of morbidity and mortality in the world. Varenicline, an α4β2 nicotinic acetylcholine receptor (nAChR) partial agonist, has been shown to increase smoking quit rates compared with nicotine-based products. This human laboratory, double-blind, placebo-controlled study examined varenicline and placebo effects on α4β2-nAChRs occupancy, nicotine-induced change in [^{11}C]raclopride non-displaceable binding potential (BP$_{ND}$), and behavioral measures of cigarette smoking, nicotine craving, and withdrawal.

Methods: Current nicotine dependent daily smokers ($N = 17$) were randomized to varenicline 1 mg twice daily or placebo for 13 days. Using positron emission tomography), we characterized α4β2-nAChRs occupancy using [^{18}F]AZAN and dopamine receptor binding using [^{11}C]raclopride as well as behavioral measures of cigarettes smoked, craving, and nicotine withdrawal.

Results: Varenicline compared with placebo resulted in significant reductions in [^{18}F]AZAN BP$_{ND}$ in multiple brain regions including thalamus, midbrain, putamen, and ventral striatum. Following administration of a controlled-dose nicotine cigarette, dopamine release was significantly suppressed in the ventral striatum in the varenicline-treated compared with the placebo group. There was a significant relationship between α4β2-nAChRs BP$_{ND}$ measured in thalamus during the [^{18}F]AZAN scan and nicotine-induced change in raclopride BP$_{ND}$ in the ventral striatum.

Conclusion: This is the first human study to demonstrate a direct relationship between the extent of varenicline occupancy of α4β2-nAChRs and the magnitude of dopamine release following nicotine use.

Implications: It has remained unclear how nicotinic receptor blockade through partial agonist medications such as varenicline promotes smoking cessation. One hypothesized mechanism is downstream dampening of the mesolimbic reward dopamine system. For the first time in human smokers, we observed a direct relationship between the extent of varenicline blockade of α4β2-nACh nicotinic receptors and the magnitude of dopamine release following smoking. This has mechanistic and therapeutic implications for improving smoking cessation interventions.

Introduction

Nicotine, the primary substance responsible for continued tobacco use and dependence,[1] binds to nicotinic acetylcholine receptors (nAChRs),[2] which modulate mesolimbic dopamine.[3] Several pharmacological agents are currently approved to treat nicotine dependence. Varenicline, an $\alpha4\beta2$-nAChR partial agonist, has been associated with some of the highest smoking abstinence rates.[4]

This agent has dual mechanisms of action to treat cigarette smoking: (1) acting as a high-affinity agonist at $\alpha4\beta2$-nAChRs to decrease nicotine withdrawal and craving,[5–7] and (2) acting as an antagonist to dampen nicotine-induced dopamine release and reward.[8] To date, preclinical research has provided much of the mechanistic evidence. For example, a recent study using voltammetry in accumbal slices from rats demonstrated that varenicline increased dopamine release paired-pulse ratios more than nicotine in nicotine-dependent animals undergoing nicotine withdrawal.[9] There is still a paucity of mechanistic information available in humans despite substantial evidence of clinical effectiveness. Two human positron emission tomography (PET) imaging studies have examined varenicline effects, one on $\alpha4\beta2$-nAChRs and the other on dopamine levels. In daily smokers, following 48-hour cigarette abstinence, PET imaging using $\alpha4\beta2$-nAChR radioligand 2-FA demonstrated that a single, low dose of varenicline compared with placebo produced greater than 90% occupancy of $\alpha4\beta2$-nAChRs.[10] More recently, using the $DRD_{2/3}$ agonist $[^{11}C](+)$-PHNO in treatment-seeking smokers following overnight smoking abstinence, Di Ciano and colleagues[11] showed chronic varenicline treatment (10–11 days) increased basal dopamine levels in dorsal caudate and reduced cigarette craving.

This human laboratory, double-blind, placebo-controlled study examined mechanistic relationships between $\alpha4\beta2$-nAChR occupancy and mesolimbic dopamine activity following acute nicotine challenge during 13-day varenicline exposure. Specifically, we measured varenicline and placebo effects on $\alpha4\beta2$-nAChR non-displaceable binding potential (BP_{ND}), dopamine receptor BP_{ND} following a placebo cigarette, and dopamine release as measured by change in raclopride BP_{ND} between an active and placebo cigarette. We also obtained behavioral measures of cigarette smoking, nicotine craving, and withdrawal. In this study, $\alpha4\beta2$-nAChRs were measured using PET with (2)-2-(6-[18F]fluoro-2,39-bipyridin-59-yl)-7-methyl-7-aza-bicyclo[2.2.1]heptane ([^{18}F]AZAN), a novel radiotracer with high specific binding, low test–retest variability, and rapid and reversible kinetics in baboon[12] and human brain.[13] On the basis of earlier PET findings following a single varenicline dose,[10] we predicted that varenicline would occupy $\alpha4\beta2$-nAChRs and reduce [^{18}F]AZAN BP_{ND}. We further predicted that varenicline would attenuate nicotine-induced changes in raclopride BP_{ND} and blunt nicotine craving. We restricted analyses to a subset of brain regions previously shown to have high $\alpha4\beta2$-nAChR density[13] or be involved in nicotine reward.

Methods

Participants

Nicotine-dependent volunteers were recruited using media advertisements. Subjects were 18–60 years old, met *DSM-IV* criteria for nicotine dependence, and smoked 10–40 cigarettes/day. Exclusion criteria included positive urine toxicology; *DSM-IV* criteria for current alcohol or drug dependence; *DSM-IV* criteria for current or lifetime depressive, anxiety, or psychotic disorders and currently in need of or on psychotropic medications; body mass index less than 18.5 or weight greater than 350 lbs; serious medical condition; or abnormal magnetic resonance imaging (MRI) scan or history of significant closed head trauma. Pregnant or lactating subjects were excluded. The Johns Hopkins School of Medicine Institutional Research Board approved this study. Participants were compensated for study participation.

Procedures

Assessment

Callers were screened using a structured questionnaire and eligible individuals were invited to provide informed consent and complete an in-person interview. Smoking measures included the Fagerstrom Test for Nicotine Dependence),[14] Structured Clinical Interview for *DSM-IV* nicotine section (SCID; Non-Patient Edition),[15] and the Michigan Nicotine Reinforcement Questionnaire.[16] We also characterized lifetime and current smoking behaviors including age at first cigarette use, years of regular use, current number of cigarettes per day, and brand. Alcohol use and associated problems were evaluated using the Alcohol Use Disorders Identification Test,[17] the Timeline Followback for past 90-day drinking,[18] and the SCID alcohol section.[15] Drug use was assessed using the SCID drug section[15] and urine toxicology. The study Nurse Practitioner completed a medical history and physical examination, including electrocardiogram and laboratory tests.

Study Medication Administration

Maximal varenicline plasma concentration is reached within 3–4 hours following administration, and after multiple doses, steady-state concentration is reached within 4 days.[19] Johns Hopkins Hospital Investigational Drug Service randomized participants to medication condition. Drug was over-encapsulated and administered double-blind in blister packs labeled with participant ID number, date, and time of dose.

Varenicline was dosed using an escalating schedule: days 1–3, 0.50 mg at 08:00 AM; days 4–7, 0.50 mg at 08:00 AM and 08:00 PM; and days 8–14, 1.0 mg at 08:00 AM and 08:00 PM. Participants started study medication on an outpatient basis 7 days before scheduled clinical research unit (CRU) admission. Throughout the CRU stay, nurses directly observed medication ingestion to ensure compliance.

CRU Procedures

Participants had urine toxicology for illicit drug use and were breathalyzed for recent alcohol use at CRU admission. Female participants had a serum pregnancy test at CRU admission and before each PET scan.

Participants had unlimited access to their preferred cigarette brand; cigarettes were counted by nursing staff before and after each smoke break. On study days 11 and 13, smoking was restricted from 11:00 PM the night before to post-scan. The 15-hour smoking abstinence period ensured full onset of nicotine withdrawal symptoms[20–22] whereas balancing subject time and burden imposed by a residential stay and multiple PET scans. To monitor abstinence compliance, carbon monoxide levels were measured at ban start, upon rising on scan days, and when participants left the unit escorted by research staff.

Throughout the CRU stay, participants completed subjective report forms at 07:00 AM and 07:00 PM daily, rating current symptom

severity. Morning questionnaires included the Questionnaire of Smoking Urges[23] (QSU-brief), the Minnesota Nicotine Withdrawal Scale (MNWS)[24], Beck Depression Inventory,[25] Columbia Suicide Scale,[26] and a Medication Side-effects Questionnaire. The QSU and MNWS were completed in the evening.

Participants were discharged following the PET scan on study day 13 and provided with smoking cessation resources.

Magnetic Resonance Imaging

A three-dimensional magnetization-prepared rapid gradient echo (MPRAGE) sequence MR image of each subject was obtained for anatomic identification of the structures of interest using the following parameters: repetition time, 2,110 ms; echo time, 2.73 ms; flip angle, 8; slice thickness, 0.80 mm with zero gap; field of view, 24×18 cm^2; and image acquisition matrix, 288×320.

Acquisition and Reconstruction of PET data

Preparation

One hour before each PET scan, venous catheters were placed for radiotracer injection and for obtaining blood samples for measurements of radioactivity and analysis of radiotracer metabolites. Each subject had a custom-fitted thermoplastic facemask to reduce within-scan head motion. Scans were completed on the High Resolution Research Tomograph (Siemens/CTI, Malvern, PA).

[18F]AZAN PET Scan on Study Day 11

The radioligand was synthesized as reported previously.[27] Up to ~15 millicuries (mCi) of [18F]AZAN (a maximum mass cutoff of 3 mg) was injected into the participant as a bolus (9.6 mL of a total 18.0 mL) over 1 minute followed by continuous infusion at 4.6 mL/h over 90 minutes. These parameters were obtained by applying the published formula[28] to historical [18F]AZAN scans.[13] The subject was positioned in the scanner, and the emission scan started at 60 minutes from the bolus injection. A 6-minute attenuation scan was performed using a rotating ^{137}Cs point source after the emission scan. Vital signs and electrocardiogram were continuously monitored. Nicotine craving and withdrawal measures, the QSU-brief, and MNWS were completed before and after the PET session.

[11C]Raclopride Scans on Study Day 13

Participants smoked a denicotinized and nicotinized cigarette in fixed order during the [11C]raclopride PET session. Nicotine was administered using Quest cigarettes (Vector Tobacco, Miami, FL) containing genetically modified tobacco (0.6 mg nicotine; 10 mg tar) or Quest 0.05 mg nicotine "denicotinized" cigarettes. In a smoking chamber contiguous to the PET suite, participants smoked each cigarette through the mouthpiece of the Clinical Research Support System smoking topography device to ensure comparable inhalation volume between groups (Plowshare, Baltimore, MD). A measure of smoking urges (QSU-Brief) was obtained before and after cigarette administration.

Immediately following the denicotinized cigarette, the initial 40-minute emission scan began with a bolus injection of about 20 mCi [11C]raclopride over 1 minute (10.4 mL of a total 18.5 mL), followed by continuous infusion of the tracer (4.8 mL/h) for 90 minutes, as previously reported.[29,30] A 6-minute attenuation scan using a rotating ^{137}Cs point source followed the first emission scan. Participants then were removed from the scanner, had a 10-minute nicotinized cigarette administration in the smoking chamber, and

resumed the emission scan from 60 to 90 minutes post-tracer injection. A second attenuation scan then was obtained.

Reconstruction

Emission scans were reconstructed using the iterative ordered subset expectation-maximization algorithm[31] with radioactivity corrected for physical decay to scan start time after correcting for attenuation, scatter, and deadtime. Each PET frame consisted of 256 (left-to-right) by 256 (nasion-to-inion) by 207 (neck-to-cranium) voxels (1.27 mm cubic). Final resolution was expected to be about 2.5 mm full-width at half-maximum in three directions.[32] Frame schedules were three 10-minute frames for [18F]AZAN scans, and four 15-second, four 30-second, three 1-minute, two 2-minute, five 4-minute, and two 5-minute frames (a total of 30 frames in 40 minutes), and six 5-minute frames between 60 and 90 minutes for [11C]raclopride scans using separate transmission scans.

PET Data Analysis

Volumes of interest (VOIs) were obtained on MPRAGE MRIs with the software library tools of the Oxford Center for functional MRI of the Brain[33,34] for subcortical regions, and the Freesurfer software[35], and refined as needed. VOI for the midbrain tegmentum (MB) was defined manually.[13] The striatum VOIs (the whole putamen [Pu] and caudate nucleus) were divided into five subdivisions (per side),[36] as previously reported.[37] VOIs were transferred from MRI to PET spaces using parameters of PET-to-MRI coregistration given by statistical parametric mapping,[38] and applied to individual PET frames to obtain regional time-activity curves.

Regional values of BP_{ND} of [18F]AZAN[39] were obtained by the target-to-reference radioactivity ratios less one using the corpus callosum as reference region.[40,41] Regional estimates of BP_{ND} for [11C] raclopride were obtained for the denicotinized state (initial 40 minutes, postinjection) using the multilinear reference tissue method with 2 parameters (MRTM2),[42] setting the cerebellum as reference region.[43] Estimates of BP_{ND} of the post-nicotine state were obtained by the target-to-cerebellum radioactivity ratios less one using frames obtained from 60 to 90 minutes. The outcome variable, dopamine (DA) release (quantified as change in raclopride BP_{ND}), was calculated as follows: $(BP_{ND}\,B - BP_{ND}\,A)/BP_{ND}\,B$, and expressed in percentage, where superscripts B and A refer to denicotinized and nicotine states, respectively.

Data Analyses

Mean and standard deviations (SDs) of assessment characteristics were summarized; *t* tests were performed to examine unbalance between varenicline and placebo groups.

A mixed effect model with random intercept tested between-group difference in number of cigarettes smoked during the CRU stay; number of cigarettes smoked each day was the dependent variable and treatment condition and number of cigarettes smoked per day at assessment were independent variables. There was little daily variation in the outcome measure, so day was not included in the model as a covariate. The adjusted mean of the outcome measure was calculated for each treatment group and tested for statistical difference. A similar mixed effect model was also constructed to test for differences between the two groups on daily subjective report measures.

To test group differences related to [18F]AZAN and [11C] raclopride PET scans, we compared mean and SD of the injection activity, volume, mass, injected dose, and specific activity. There were

no significant group differences on any measure (see Supplementary Table 1); these variables were not included in the models.

Then we tested differences in AZAN BP_{ND} in selected brain VOIs using *t* tests. We also examined the associations of AZAN BP_{ND} in selected VOIs with the number of cigarettes smoked on study day 10 using linear regression models, with AZAN BP_{ND} as the independent variable and the number of cigarettes as the dependent variable. To examine for possible mediation by nicotine-induced dopamine release in the ventral striatum (vS), we ran these correlations with and without vS dopamine release (DAR) included as a covariate in the model, and also tested the correlation between vS DAR and AZAN BP_{ND}. Mediation was reported when there was significant correlation between vS DAR and AZAN BP_{ND}, and the relationship between AZAN BP_{ND} and number of cigarettes smoked was weakened by adding vS DAR to the regression model. We also tested the association between AZAN BP_{ND} and MNWS and QSU mean scores during CRU stay using linear regression models.

Next we tested group differences in dopamine release in selected VOIs using *t* tests. We used linear regression models to test the correlation between dopamine release and subjective measures obtained during the raclopride PET session.

Finally, we tested the association between AZAN BP_{ND} in selected VOIs rich in α4β2-nAChRs and dopamine release in the vS using linear regression models. All participants from both treatment groups were included in these models. Treatment group and the interaction term between treatment group and AZAN BP_{ND} were added as covariates to account for the different correlations in the placebo- and varenicline-treated groups.

Results

Participant Characteristics

Seventy-three persons provided informed consent. Of these, 41 were disqualified based on study exclusion criteria, including positive illicit drug toxicology, current psychiatric or medical disorders, and heavy alcohol use. Thirty-two persons were approved to participate in research procedures and 21 started study medication. Two persons withdrew due to medication side effects, and two dropped out prior to scanning. Seventeen participants (11 men, 6 women) completed at least one PET scan and were included in analyses.

There were no differences in baseline participant characteristics as a function of study medication condition (Supplementary Table 2). Mean age was approximately 40 years old. Average age at which participants began smoking was 19 years old, and they had been smoking regularly for a little over 20 years. All participants smoked on average 15–20 cigarettes daily. Mean Alcohol Use Disorders Identification Test score was 2, reflecting low risk for alcohol problems. This was in line with self-reported drinking on the Timeline Followback; participants drank on average 1 day/week and reported drinking on average two standard drinks on drinking days.

Cigarette Smoking and Nicotine Withdrawal During the CRU Stay

Smoking declined in both varenicline- and placebo-treated participants during their CRU stay; however, varenicline participants smoked significantly fewer cigarettes per day (adjusted mean = 6.25, standard error of the mean = 0.78) than placebo participants (adjusted mean = 8.45, standard error of the mean = 0.83; *p* = .027).

Mean nicotine withdrawal scores on both the QSU and the MNWS were not significantly different between the two groups

nor did they differ as a function of morning versus evening administration. Mean QSU scores were 25.06 (SD = 14.29) for varenicline subjects and 24.68 (SD = 12.39) for placebo subjects (*p* = NS). Mean MNWS scores were 8.16 (SD = 3.60) for varenicline subjects and 6.23 (SD = 2.92) for placebo subjects (*p* = NS).
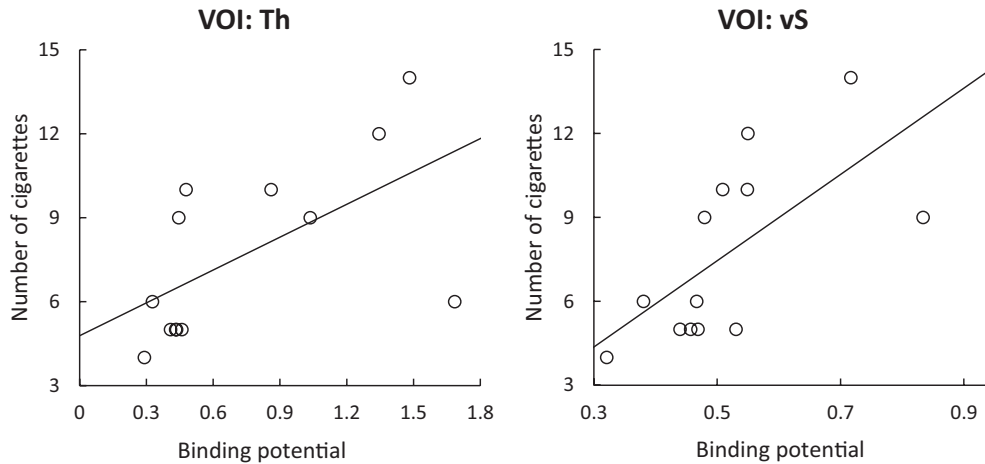
[18F]AZAN Binding Potential

Fifteen participants completed [18F]AZAN PET scan procedures. There were no group differences in AZAN injection activity, volume, mass, injected dose, or specific activity (Supplementary Table 1, upper panels).

As shown in Figure 1, AZAN BP_{ND} was significantly lower in the varenicline compared with placebo group in multiple brain regions. Differences in α4β2-nAChRs BP_{ND} between treatment groups were particularly notable in two brain regions rich in these receptors, including midbrain (MB; 0.64 [SD = 0.34] vs. 1.45 [SD = 0.32], *p* < .001) and thalamus (Th; 0.41 [SD = 0.07] vs. 1.37 [SD = 0.32], *p* < .001). Between-group differences also were observed in Pu (0.51 [SD = 0.10] vs. 0.73 [SD = 0.21], *p* = .022), and vS (0.46 [SD = 0.07] vs. 0.65 [SD = 0.19], *p* =.044). No group difference was observed in caudate nucleus.

We examined the association of α4β2-nAChR BP_{ND} and number of cigarettes smoked on study day 10 (day 3 of the CRU stay). We chose day 10 because medication ingestion had been observed over two full days ensuring compliance; no restrictions were placed on smoking because participants remained on the unit for the entire day, and it was the day immediately preceding the AZAN scan. Collapsing across both medication groups, we observed a positive association, with higher α4β2-nAChR BP_{ND} correlated with greater number of cigarettes smoked (Figure 2). This association was significant across multiple brain regions, including Th (β = 0.598, *p* =.031), MB (β = 0.684, *p* = .010), Pu (β = 0.787, *p* = .001), and vS (β = 0.652, *p* =.016). We examined whether these relationships were mediated by nicotine-induced dopamine release in the vS, and found a significant mediation effect only for the Th. There was no significant effect of medication condition on the relationship between number of cigarettes smoked and AZAN BP_{ND}. There was
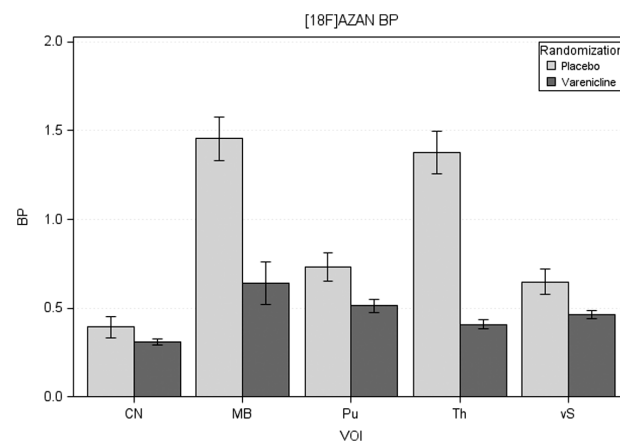
Figure 1. Mean and standard error of [18F]AZAN non-displaceable binding potential (BP_{ND}) in selected brain regions for varenicline- and placebo-treated groups. Light gray bars represented placebo-treated participants and dark gray bars represent varenicline-treated participants. Group differences in α4β2 nAChRs BP_{ND} were examined in midbrain (MB), thalamus (Th), caudate nucleus (CN), putamen (Pu), and ventral striatum (vS). Group differences were significant in all regions except CN.

Figure 2. Association of [18F]AZAN non-displaceable binding potential (BP_{ND}) and the number of cigarettes smoked on study day 10 in thalamus (Th) and ventral striatum (vS) for all study participants. Because there was no main effect of medication on these relationships, placebo- and varenicline-treated participants were combined for these analyses. There was a positive association, with higher α4β2-nAChR BP_{ND} correlated with greater number of cigarettes smoked in five of six regions. Two regions are shown: Th (β = .598, *p* = .031) and vS (β = .652, *p* = .016). VOI = volume of interest.

no association of α4β2-nAChR BP_{ND} and MNWS and QSU scores reported during the CRU stay.

Dopamine Release Following Cigarette Smoking by Medication Condition

Fifteen participants completed the [11C]raclopride PET. There were no group differences in raclopride injection activity, volume, mass, injected dose, or specific activity (Supplementary Table 1, lower panels). There also was no difference obtained in smoking volume (number of puffs × puff duration) for either the placebo or active cigarette during the PET procedure between the two medication groups (*p* = .763). No group differences in raclopride BP_{ND} were observed following placebo cigarette administration (all *p*s > .10).

As shown in Figure 3, dopamine release in the vS following active nicotine administration was significantly lower in the varenicline compared with placebo group (–10.9 [SD = 10.7] vs. 0.0 [SD = 5.8], *p* = .030). There was a trend for similar effects in caudate nucleus (5.4 [SD = 8.3] vs. 13.8 [SD = 7.7], *p* = .076) and Pu (2.4 [SD = 6.2] vs. 9.6 [SD = 7.8], *p* = .089). There was no association between dopamine release and participants' reports of nicotine craving.

Relationship Between [18F]AZAN Binding Potential and Dopamine Release Following Cigarette Smoking

There was a significant interaction between α4β2-nAChRs BP_{ND} in Th measured during the [18F]AZAN scan and nicotine-induced change in raclopride BP_{ND} in the vS as a function of medication condition (*p* = .011; Figure 4). Among placebo-treated participants, there was a positive association between [18F]AZAN BP_{ND} and nicotine-induced dopamine release in vS (*R* = .687; *p* = .131). In contrast, among varenicline-treated participants, higher AZAN BP_{ND} was associated with lower nicotine-induced dopamine release in vS (*R* = –.784; *p* = .065). Within-treatment group correlations did not reach significance due to small sample sizes.

Discussion

In this human laboratory study of varenicline effects on smoking, we observed a significant decrease in [18F]AZAN binding to
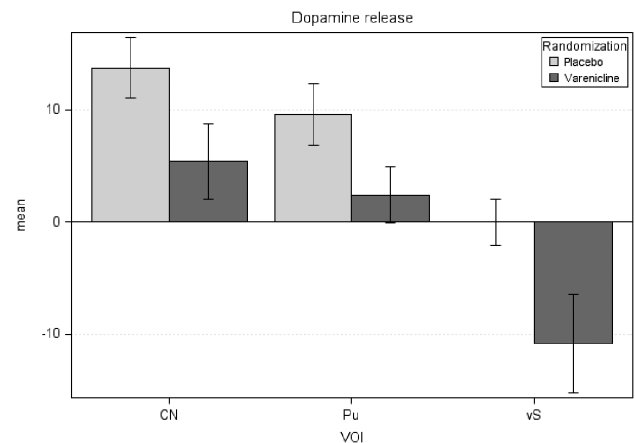
Figure 3. Histogram (mean boxes and standard error bars) of change in [11C] raclopride non-displaceable binding potential (BP_{ND}) in the active cigarette smoking scan relative to the placebo cigarette smoking scan for varenicline- and placebo-treated groups in three brain regions. Light gray bars represented placebo-treated participants and dark gray bars represent varenicline-treated participants. Change in binding potential was significantly lower in the ventral striatum (vS) for the varenicline compared with placebo group (–10.9 [SD = 10.7] vs. 0.0 [SD = 5.8], *p* = .030). There was a trend for similar effects in caudate nucleus (CN; 5.4 [SD = 8.3] vs. 13.8 [SD = 7.7], *p* = .076) and putamen (Pu; 2.4 [SD = 6.2] vs. 9.6 [SD = 7.8], *p* = .089). VOI = volumes of interest.

α4β2-nAChRs in varenicline-treated participants in key brain regions known to have high receptor density (Th, MB)[13] as well as regions involved in nicotine reward (vS, Pu).[29,44] Further, varenicline dampened nicotine-induced dopamine release in the vS and Pu. Importantly, among placebo-treated participants, higher AZAN BP in the α4β2-nAChR-rich Th was associated with greater nicotine-induced dopamine release in vS. This relationship was reversed among varenicline-treated participants

In this study, agonist properties of varenicline were evidenced in lower AZAN BP_{ND} in participants on active compared with placebo medication, indicating occupancy and stimulation of the nAChRs by varenicline. Variability across brain regions may be attributable to residual receptor occupancy following the 15-hour abstinence
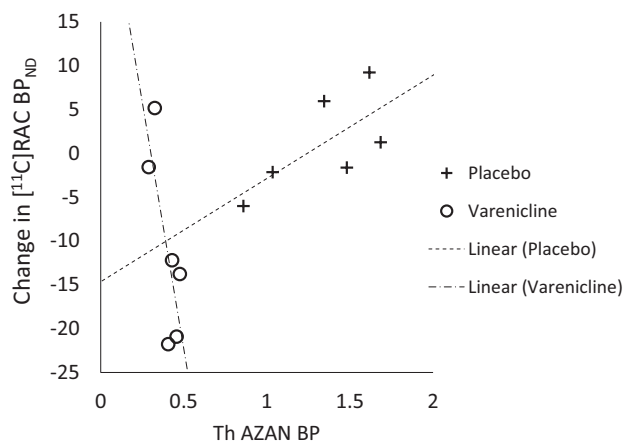
Figure 4. Relationship between α4β2-nAChR occupancy by AZAN in thalamus (Th) and nicotine-induced dopamine release in the ventral striatum (vS). There was a significant interaction between AZAN occupancy and dopamine release as a function of treatment condition (placebo-treatment [+]; varenicline-treatment [o]).

period prior to scanning. Notably, varenicline subjects had a greater reduction in smoking throughout their CRU stay compared with placebo subjects. Ashare et al.[45] reported a similar smoking reduction in non-treatment-seeking research participants receiving varenicline. Thus, there is evidence that research participants receiving active varenicline experienced its clinical benefits despite the fact that they were not treatment seekers interested in quitting smoking. Across both active and placebo participants, we observed a positive association between [18F]AZAN BP_{ND} following 15-hour smoking abstinence and the number of cigarettes smoked during the CRU day prior to the scan. This finding is in line with the predicted effects of varenicline. That is, the lower the [18F]AZAN binding potential, the greater the varenicline occupancy of α4β2-nAChRs, and the greater the smoking reduction. In clinical practice, this partial agonist effect may reduce nicotine craving and withdrawal, helping nicotine-dependent persons reduce their smoking. Interestingly, varenicline compared with placebo was associated with a reduction in the number of cigarettes smoked despite comparable scores for nicotine craving and withdrawal in both treatment groups. Because smoking was allowed on the unit, craving and withdrawal scores were quite low, thereby limiting the opportunity to observe a between-group medication effect. It is also possible that additional mechanisms contribute to smoking reductions independent of mechanisms that govern self-reported craving and withdrawal.

In this study, varenicline's antagonist properties were evidenced by dampening of nicotine-induced dopamine release in vS and Pu in active compared with placebo participants. We observed the most pronounced mitigation of dopamine release following nicotine administration in the vS, the well-established reward region within the mesolimbic system. Varenicline has a significantly higher affinity for α4β2-nAChRs than nicotine, thereby blocking nicotine access to the nAChRs and dampening the dopaminergic effects of nicotine.[8] In rats, when combined with nicotine, varenicline reduces nicotine-induced dopamine release to the level of the effect of varenicline alone, consistent with partial agonism.[46] Also, using a within-subject design, a recent PET study in active smokers reported a significant decrease in [11C](+)-PHNO binding in the dorsal caudate post-compared with pre-varenicline treatment, indicating greater endogenous dopamine occupancy of dopamine receptors

following steady state medication.[11] In this study, magnitude of dopamine release was not related to self-reported nicotine craving. In an earlier study of dopaminergic response to cigarette smoking in active smokers, Barrett et al.[47] also observed no relationship between change in dopamine release following smoking and self-reports of craving. Further, they observed considerable variability across participants in euphoric ratings, and variability across brain regions in the relationship between change in raclopride BP_{ND} and nicotine-induced euphoria, with no relationship in the vS and a negative relationship in caudate and Pu. In this study, we suspect that the blunting of dopamine release in the vS following the nicotinized cigarette was influenced by administration of a denicotinized cigarette at the start of the procedure, possibly limiting subsequent biological and subjective responses to the active nicotine cigarette. Finally, the type and preparation of cigarettes used in nicotine administration procedures (Quest, frozen, and defrosted) may have limited self-reported pleasant effects of smoking.

Perhaps most novel and important, this is the first human study to demonstrate a direct positive relationship between α4β2-nAChR occupancy and nicotine-induced dopamine release among the placebo-treated participants, and the disruptive effects of varenicline on this relationship. This finding may be key to understanding individual differences in nicotine sensitivity and medication efficacy. Genetic and environmental factors modify availability of nicotinic receptors. Individual differences are attributable to polymorphisms in loci that encode nicotinic receptor subunits including CHRNA4 and CHRNB2. Also chronic smoking upregulates nAChRs.[48–49] Recently, Brody et al.[50] demonstrated that heavy caffeine or marijuana use further increase nicotinic receptor availability in smokers. Among placebo-treated participants, our findings suggest that as nAChR availability increases in regions with high nAChR density (such as the Th), intermediary neurotransmitters such as glutamate are modulated,[51] resulting in downstream changes in dopamine release, particularly in reward centers such as vS. This phenomenon may contribute to the considerable difficulty that most chronic smokers experience during quit attempts. The finding also informs our understanding of varenicline's mechanism of action as a stop smoking agent. As previously reported,[10] the standard dosing regimen of varenicline resulted in a very high level of α4β2-nAChR occupancy and suppression of nicotine-induced dopamine release across participants. Interestingly, across all participants, the magnitude of dopamine release in the vS mediated the relationship between α4β2-nAChRs availability in the Th and number of cigarettes smoked, suggesting a direct link between these neurochemical associations and smoking behavior.

This study has notable strengths. First, it has been established that smoking a tobacco product devoid of nicotine can alter a variety of neurotransmitters, including an inhibition of monoamine oxidase (MAO) activity.[52] To control for these combustion effects in the current study, the Quest tobacco cigarettes matched the tobacco constituents while varying nicotine content between the denicotinized and nicotinized cigarettes. Second, we obtained three PET scans in each participant, enabling a within-subject approach to examining the relationship between α4β2-nAChR occupancy and nicotine-induced dopamine release. Although it would be ideal, it is not feasible to perform all three scans in one day; however, despite the separation in days, we observed a correlation between the AZAN and raclopride results. Third, our group has previously demonstrated the high specific binding, excellent test–retest characteristics, and rapid and reversible kinetics of [18F]AZAN in baboons

and humans, making this an optimal agent for nAChR scanning.[12,13] Fourth, participants resided on the CRU under continuous nursing supervision, enabling direct observation of medication ingestion, the ability to accurately count number of cigarettes dispensed and smoked, collection of repeated daily measurements of cigarette craving and withdrawal symptoms, and enforcement of smoking restrictions prior to PET procedures. Finally, we recruited smokers with no and/or low psychiatric comorbidity, thereby avoiding the neurochemical confounds introduced by substance use and other psychiatric disorders. Although this may limit generalization, it is a critical first step in clarifying mechanisms of action.

There also are study limitations. We had a relatively small sample size, but despite this, we observed the hypothesized relationships among the primary variables in brain regions selected a priori.[13] A second potential concern is the relatively brief 13-day varenicline dosing period; this was constrained for practical reasons in persons who were not treatment seekers. Notably, the period of directly observed medication ingestion was sufficient to achieve steady state prior to scanning.[19] During the raclopride PET procedure, the denicotinized and nicotinized cigarettes were smoked in fixed order. Although there are known limitations of using a fixed order design, we had no option when using the continuous infusion raclopride protocol. However, if anything, administering the denicotinized cigarette first could be expected to trigger a classically conditioned dopamine response, reducing the difference in dopamine response observed between the denicotinized and nicotinized scans. We did not obtain a direct measure of varenicline level before PET scanning; however, as participants ingested medication under nursing supervision and were healthy, it is likely that varenicline levels were reasonably consistent within the active treatment group. Finally, because of fiscal constraints of the funding mechanism, we did not measure nicotine or cotinine levels prior to the scans or nicotine metabolite ratio in participants; differential baseline levels and or rates of nicotine metabolism among the participants might also influence the results.

In summary, this is the first PET study in human smokers to demonstrate a significant relationship between α4β2-nAChR availability and nicotine-induced dopamine release. It is likely that this relationship has widespread influence on smoking behaviors, including risk for development of regular smoking, how heavily a person smokes, and the likelihood of successful quit attempts. Further, it is reasonable to predict that this relationship is altered by genetic and environmental factors that modify nicotinic receptor availability. One environmental factor shown to be significant in this study is the smoking cessation medication varenicline, highlighting the therapeutic importance of medication in improving success rates for smoking cessation.

Supplementary Material

Supplementary data are available at *Nicotine and Tobacco Research* online.

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Declaration of Interests

MEM, GSW, HK, RFD, and XX have no conflicts of interest to disclose. DW also has an association with Roche Neuroscience and Hoffman La-Roche, Lundbeck, Lilly (AVID), Five Eleven Pharma, Dart Pharmaceuticals, Addex Therapeutics, Intracellular Therapies, GE Heathcare, Janssen, Johnson and Johnson, and CDC/FDA contracts (tobacco products). The contents of the manuscript are solely the responsibility of the authors and do not necessarily represent the official view of the Johns Hopkins ICTR, NCATS or NIH.

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