Published in final edited form as:
Am J Psychiatry. 2013 April 1; 170(4): 399–407. doi:10.1176/appi.ajp.2012.12060793.

Effect of a nicotine vaccine on nicotine binding to the beta2-nAChRs in vivo in human tobacco smokers

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Abstract

Objective—Nicotine acts in the brain to promote smoking in part by binding to the beta2-containing nicotinic acetylcholine receptors (β_2*-nAChRs) and acting in the mesolimbic reward pathway. The effects of nicotine from smoking one tobacco cigarette are significant (80% of β_2*-nAChRs occupied for >6h). This likely contributes to the maintenance of smoking dependence and low cessation outcomes. Development of nicotine vaccines provides potential for alternative treatments. We used [^{123}I]5IA-85380 SPECT to evaluate the effect of 3′-AmNic-rEPA on the amount of nicotine that binds to the β_2*-nAChRs in the cortical and subcortical regions in smokers.

Method—Eleven smokers (36years (SD=13); 19cig/day (SD=11) for 10years (SD=7) who were dependent on nicotine (Fagerström Test of Nicotine Dependence score =5.5 (SD=3); plasma nicotine 9.1 ng/mL (SD=5)) participated in 2 SPECT scan days: before and after immunization with 4–400μg doses of 3′-AmNic-rEPA. On SPECT scan days, 3 30-min baseline emission scans were obtained, followed by administration of IV nicotine (1.5mg/70kg) and up to 9 30-min emission scans.

Results—β_2*-nAChR availability was quantified as V_T/f_P and nicotine binding was derived using the Lassen plot approach. Immunization led to a 12.5% reduction in nicotine binding (F=5.19, df=1,10, p=0.05). Significant positive correlations were observed between nicotine bound to β_2*-nAChRs and nicotine injected before but not after vaccination (p=0.05 vs. p=0.98).

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The data described herein was presented at the Society for Research on Nicotine and Tobacco in Houston TX on March 13–16, 2012.

Disclosures: Dr. Seibyl has equity interest in Molecular Neuroimaging, LLC. Dr. O'Malley: member, American College of Neuropsychopharmacogy workgroup, the Alcohol Clinical Trial Initiative, sponsored by Alkermes, Abbott Laboratories, Eli Lilly & Company, GlaxoSmithKline, Johnson & Johnson Pharmaceuticals, Lundbeck, and Schering Plough; partner, Applied Behavioral Research; medication supplies, Pfizer; contract, Nabi Biopharmaceuticals to conduct Phase III trials; Advisory Board, Gilead Pharmaceuticals; consultant, Alkermes, GlaxoSmithKline, Brown University, University of Chicago; Scientific Panel of Advisors, Hazelden Foundation. Dr. Hatsukami: contract, Nabi Biopharmaceuticals to conduct clinical trial. Dr. Esterlis: contract, Nabi Biopharmaceuticals for supply of nicotine vaccine.

The study was registered in the Clinicaltrials. gov with the following identifier NCT00996034. PI was Irina Esterlis, Ph.D., Yale University.

There was a significant reduction in the daily number of cigarettes and desire for a cigarette (p=.01 and p=.04, respectively).

Conclusions—This proof-of-concept study demonstrates that immunization with nicotine vaccine can reduce the amount of nicotine binding to β_2*-nAChRs and disrupt the relationship between nicotine administered vs. nicotine available to occupy β_2*-nAChRs.

Introduction

It is estimated that half a million individuals in the United States die of tobacco smoking related diseases yearly; however, an equal number become dependent on tobacco yearly. Smoking cessation rates are low, and the currently available FDA approved treatments for tobacco addiction are 10–30% effective at one-year follow up. The past decade has seen a different approach emerge for treating tobacco addiction: vaccines to block nicotine entry into brain, potentially the most addictive constituent in tobacco cigarettes. Vaccines are designed to stimulate the production of antibodies specific to the nicotine molecule. The composite of nicotine bound to these antibodies is too large to cross the blood-brain barrier, reducing the amount and rate of nicotine entering the brain, and the reinforcing and addictive effects (1). This strategy could potentially help prevent addiction to tobacco smoking in vulnerable individuals and facilitate smoking cessation in addicted smokers.

In the present study, we tested the efficacy of nicotine conjugate vaccine at reducing nicotine's entry into the brain and binding to the nicotinic acetylcholine receptors (nAChRs; primary binding site of nicotine in the brain) *in vivo* in human smokers (NicVAX [3′aminomethylnicotine conjugated to recombinant Pseudomonas exoprotein A (3′-AmNic-rEPA)]; manufactured by Nabi Biopharmaceuticals, Rockville MD). 3′-AmNic-rEPA has high affinity for nicotine (2) and prolongs nicotine elimination from the body in animal studies (3, 4). Four to five injections (400μg each) are safe, and the expected therapeutic effect of 3′-AmNic-rEPA is antibodies of >25μg/mL. (5, 6) Preclinical studies suggest that immunization results in ~30–90% less nicotine entering the brain after acute nicotine exposure (3, 7–10) and this is related to the observed decrease in locomotor (7, 8) and behavioral (11, 12) responses to nicotine. There is evidence that immunization slows nicotine elimination from the body (3, 4), which may contribute to reduction in smoking. This would be similar to the fact that slow nicotine metabolizers smoke less cigarettes, i.e. nicotine is available for longer period of time.

Chronic administration of nicotine upregulates the high affinity β_2*-nAChRs (asterisk denotes that β_2 may be coupled with α_4 or another subunit) (13) and nicotine from smoking cigarettes or from the nicotine inhaler occupies majority of these receptors (14–16). Although administration of the nicotine inhaler leads to a prolonged occupancy of the β_2*-nAChRs similar to that after smoking a cigarette, use of the inhaler does not alleviate craving symptoms as does smoking one cigarette (16). This is in part to the 10% lower nicotine binding at the β_2*-nAChRs after use of nicotine inhaler (14). Thus, in addition to the explicit differences between the nicotine inhaler and regular cigarettes (e.g., lack of other tobacco smoke ingredients, social impact), the 10% difference in the nicotine binding to the β_2*-nAChRs likely contributes to the poor ability of the nicotine inhaler to significantly reduce craving symptoms. The complexities of tobacco smoking dependence in human subjects and the current lack of highly efficacious treatments suggest the β_2*-nAChRs may be an excellent target for smoking cessation therapies.

The present proof of concept study evaluated whether immunization with 3′-AmNic-rEPA reduces the amount of nicotine that reaches the brain and occupies or binds to the β_2*-nAChRs in healthy human tobacco smokers. We used [^{123}I]5-IA-85380 ([^{123}I]5-IA) and single photon emission computed tomography (SPECT) imaging to quantify β_2*-nAChRs.

We administered 1.5mg/70kg nicotine intravenously (IV) to each subject, which is equivalent to the nicotine delivered from 1.5 cigarettes. We hypothesized vaccination with 3′-AmNic-rEPA would be associated with a significant decrease in nicotine binding to the β_2*-nAChR, indicating reduced entry into the brain by nicotine.

Materials and methods

Eleven non-treatment seeking tobacco smokers (7men, 4 women) signed consent and completed this study, approved by the Yale University School of Medicine, Veteran Affairs Health Care System, and University of Toronto Institutional Review Boards. Eligibility was evaluated via structured interview, behavioral assessments, physical examination, laboratory blood tests, urine drug screen, and an electrocardiogram.

Study design

All subjects participated in two [^{123}I]5-IA SPECT scan days 20 weeks apart and 4 3′-AmNic-rEPA injections between SPECT scan days (each 4 weeks apart). Subjects were instructed to abstain from tobacco cigarettes or any nicotine products for 5 days prior to each SPECT scan day to allow for any nicotine or metabolites to clear the brain because these may compete with radiotracer binding (17). Smoking abstinence was confirmed as previously (14). For the remainder of the study, subjects were instructed to smoke ad libitum but not to use any medications or NRTs. Smoking characteristics were recorded at each visit.

Assessments

The severity of nicotine dependence was assessed using the Fagerström Test of Nicotine Dependence (FTND) (18) at intake. Nicotine withdrawal symptoms were assessed using the Minnesota Nicotine Withdrawal Scale (MNWS) (19) and craving was assessed using the Tiffany Smoking Urges Questionnaire (20) at intake, during each period of smoking abstinence, and on each scan day before and after IV nicotine administration. The Tiffany Questionnaire of Smoking Urges brief (QSU-brief) (21) was used on SPECT scan days pre and post nicotine challenge. Two factors of Tiffany Smoking Urges Questionnaires were employed: desire (positive symptoms associated with wanting a cigarette) and relief (withdrawal relief expected if cigarette is smoked). Subsyndromal depressive symptoms were measured with the Center for Epidemiological Studies Depression Scale (CES-D) (22) and state and trait anxiety symptoms were measured with Spielberger's State-Trait Anxiety Inventory (STAI) (23) at intake and on both scan days.

3′-AmNic-rEPA

The active investigational product is purified 3′-aminomethylnicotine conjugated to *P. aeruginosa* r-exoprotein A (rEPA) (AMNic-rEPA). Each single-use syringe contained 3′-aminomethylnicotine conjugated to 400 μg rEPA adsorbed to 1.1 mg aluminum (Alhydrogel 85) in 1mL phosphate buffered saline (0.15 M NaCl, 0.002 M $NaPO_4$, pH 7.2, 0.01% polysorbate 80). All subjects were administered vaccines from the same lot.

Antinicotine Ab concentrations were measured using enzyme-linked immunosorbent assay (ELISA) as described previously (6). Because no national or international reference standards exist for nicotine antibodies, reference standards were developed by Nabi Biopharmaceuticals and prepared from pools of serum from human volunteers who were immunized. Nicotine-specific IgG antibody was quantitated by an ELISA in which antibody bound to nicotine-coated plates was quantitated against antibody bound by anti–Fab-coated plates. Here we report absolute concentrations of Ab, which are in units of mass/volume (μg/ml). Side effects of the vaccine were monitored as previously (5). Subjects' vital signs

(blood pressure, temperature, pulse, and respiration) were collected before and 30-min after vaccination. Following each vaccine appointment, subjects filled out a reactogenicity diary for 7 consecutive days to keep record of local and systemic reactogenicity and temperature, which was reviewed at the next administration date unless there was a notable reaction. Every subject was followed for two weeks after the last study date to review any symptoms or side effect.

Nicotine

Nicotine bitartrate (Siegfried CMS/Interchem) vials were prepared by mixing with saline to a concentration of 1mg/ml nicotine base, and were administered intravenously over 10 minutes.

Plasma nicotine and cotinine analyses

Venous blood samples for nicotine and cotinine analyses were drawn at intake and on each scan day. On the scan day, the samples were drawn prior to radiotracer administration, and after IV nicotine administration at 2mins, 5mins, 10mins, 20mins, 30mins, 60mins, 90mins, 120mins, 180mins, 240, and 300mins. Samples were processed as described previously (24). Plasma nicotine, cotinine (metabolite of nicotine) and 3-hydroxycotinine (metabolite of cotinine) were measured. Free nicotine was measured as it can cross the brain blood barrier and act on nicotinic receptors, and because the nicotine glucuronide is a minor metabolite which is rapidly cleared resulting in only a small fraction of the total nicotine in plasma being in the conjugated form. Free nicotine was measured by LC-MS/MS (25).

Using the sample data over time, we determined if there were changes in nicotine's half-life, volume of distribution and clearance as a result of treatment. Systematic clearance was determined by dividing the nicotine dose by the plasma $AUCt_{0\infty}$ (extrapolated using terminal time points). The nicotine half-life was estimated using a regression analysis of the concentration versus time. Nicotine's apparent volume of distribution was estimated by multiply its half-life by clearance then dividing by 0.693.

Immunogenicity samples

Serum samples were collected for immunogenicity measurements at 5 time points (before each of 4 vaccine administrations and on 2nd SPECT scan day). Antinicotine antibody concentrations were measured using enzyme-linked immunosorbent assay and subjects reported any adverse events as described previously (6).

MRI and [^{123}I]5-IA SPECT Imaging

MRI—Each subject participated in one magnetic resonance imaging (MRI) scan prior to SPECT scanning as previously on a Signa 1.5T system (General Electric Co, Milwaukee, Wis) (14).

SPECT scans and IV nicotine administration—All emission scans were obtained on a Phillips PRISM 3000 XP (Cleveland, OH) SPECT camera, and [^{123}I]5-IA was synthesized and administered as previously (26) using a bolus plus constant infusion paradigm with a ratio of 7.0h (SD=0.04) scan day 1 and 7.0h (SD=0.02) scan day 2, and a total injected dose (accounting for decay) of 358.7 MBq (SD=30.1) scan day 1 and 352.6 MBq (SD=31.4) scan day 2. Another antecubital venous catheter was placed into the opposite arm or hand to collect blood for protein binding and metabolism. Six hours following [^{123}I]5-IA injection, a simultaneous transmission emission protocol scan and 3 equilibrium emission scans were obtained. Subjects were removed from the camera and IV nicotine was administered through a butterfly catheter. Thereafter, up to additional 9 30-min emission scan were acquired to

evaluate nicotine-induced displacement of [^{123}I]5-IA. Blood samples were collected at the midpoint of each set of post-nicotine scans to quantify total parent and f_p, to correct for individual differences in metabolism and protein binding of [^{123}I]5-IA.

Image analysis

SPECT emission images were analyzed as previously (26). Regional [^{123}I]5-IA uptake was determined by V_T/f_p for the following brain regions: frontal, parietal, anterior cingulate, temporal and occipital cortices, thalamus, striatum, and cerebellum.

Determination of receptor occupancy (or nicotine binding)

V_T/f_P data from the pre-nicotine baseline and post-nicotine scans were analyzed by use of Lassen plots (14, 15, 27). Receptor occupancy (Ro) by nicotine was derived for each subject across all brain regions for each post-nicotine scan (compared to baseline) on each scan day, and the final result represents the average across scans for each subject.

Determination of nicotine reduction in brain

Concentration of nicotine in tissue can be calculated

$$C=\frac{Ro \cdot IC_{50}}{1-Ro}$$

Where C is concentration of nicotine in tissue; IC_{50} is concentration of nicotine in tissue at Ro=50%. In order to obtain the percent reduction of nicotine in tissue from time 1 (before immunization) to time 2 (after immunization), we divided concentration at time 2 (C_2) by concentration at time 1 (C_1) and subtracted the result from 1: $\%\Delta=(1-C_2/C_1)*100$.

Statistical analyses

All statistical analyses were performed using SPSS version 17.0 (SPSS Inc. Headquarters, Chicago, IL). To assess whether immunization reduces the overall amount of nicotine that reaches the brain and binds to receptors, analysis of variance with repeated measures was performed at the time maximal displacement of radioligand was achieved (3–4 hrs after IV nicotine injection). Statistical significance was set at $p\leq0.05$, two-tailed. Paired samples t-tests were also used to assess within-subject differences in mood, smoking and craving variables before to after immunization; as well as to assess differences in nicotine pharmacokinetic parameters. Nonparametric correlational analyses (Spearman rho correlation coefficient) were used to examine the relationship between receptor occupancy and nicotine variables on SPECT scan days.

Results

Participants

Healthy tobacco smokers were 36.1 years (SD=12.9), smoked an average of 19.5 cigarettes/day (SD=11.20) for 8.7 years (SD=6.2) and were moderately dependent on nicotine (FTND score 5.3 (SD=2.9). Smoking status was verified by plasma nicotine (9.1 ng/mL (SD=5)), urine cotinine (909.1 ng/mL (SD=126.1)) and breath CO (17.3 ppm (SD=5.3)) levels at screening. Mood and smoking craving parameters are described in Table 1.

SPECT scan day 1 (before immunization)—Participants abstained from smoking for 4.9 days (SD=0.8) prior to SPECT scan day (verified by urine cotinine (214.0 ng/mL (SD=346)) and carbon monoxide (2.9ppm (SD=2.5)). One participant was not able to

abstain from smoking and smoked the night prior to SPECT scan (hence higher urine cotinine levels than in our previous studies) but we proceeded with scan day procedures. This same subject was also not able to abstain from smoking for the second scan day and smoked the night before. Since this is a within subject design and the nicotine plasma level prior to the IV nicotine challenge on either SPECT day was below 0.1 ng/mL (analyzed as described in the results), we included this subject in the analyses.

Plasma nicotine levels: Nicotine concentration values for each subject are in Table 2 and Fig 1. After nicotine administration, the plasma nicotine concentration (Cmax) reached 9.6 ng/mL (SD=2.8) at 17.0 min (SD=10.3) (Tmax) and the area under the curve (AUC) was 1722 ng. min/ml (SD=951).

Receptor occupancy by nicotine before vaccination: Equilibrium, defined as ≤5% change in receptor availability per hour, was achieved between 6–8 h after injection on each scan day. Subjects were placed back in the camera at 59.4 min (SD=21.9) min post initiation of IV nicotine challenge. Maximal displacement of [^{123}I]5-IA was achieved 3–4hr post nicotine administration (56.2% (SD=11.1) (Fig 2). The range of maximal occupancy was 47.1%–68.3% across subjects (Table 2). There was a significant positive correlation between nicotine injected and nicotine bound (r=0.60, n=11, p=0.05) (Fig. 3).

Plasma antibodies levels: Titer levels were collected prior to administration of the vaccine at each vaccination appointment and on SPECT scan day 2 (Table 2). Prior to initiation of the vaccination schedule, none of the subject had detectable antibody levels. There was a significant increase in antibodies levels over the course of treatment, average of 75.9 μg/mL (SD=30.5) on scan day 2 (2 weeks after 4th injection), and all of the subjects acquired >25 μg/mL after vaccination schedule. No unexpected issues or adverse events were reported. Most commonly reported were the expected mild tenderness and ache at the injection site. None of the subjects required follow up past the standard 2-week end of study follow up.

SPECT scan day 2 (after immunization)

Smoking characteristics: At the time of the 4th vaccine (1–2 weeks prior to initiation of the 2nd smoking abstinence) participants smoked 11.7 cigarettes/day (SD=11), ~ 50% reduction from baseline. Prior to the second SPECT scan, participants abstained from smoking for 4.7 days (SD=0.6), and this reduction was verified by urine cotinine (162 ng/mL (SD=234)) and carbon monoxide (3.5 ppm (SD=4.2)) levels. On the morning of the second scan day, there was a significant reduction only in desire for cigarette (from morning of scan day 1 to morning of scan day 2) measured by both Tiffany QSU and QSU brief, t=2.36, df=10 p=0.04 and t=3.54, df=10, p=0.005, respectively) (Table 1).

Plasma nicotine concentration: Comparing scan day 2 to scan day 1, there was a significant increase in plasma nicotine Cmax after IV nicotine administration (t=−3.5, df=9, p=0.007) but not in the AUC or Tmax (Table 2). We also observed a significant effect of vaccine treatment on volume of distribution (V_D) and clearance (C_l) of nicotine such that both decreased from scan 1 to scan 2 (V_D: t=5.59, df=9, p=0.000; C_l: t=4.15, df=9, p=0.002). There were no significant differences in the ratio of free nicotine to antibody-bound nicotine immediately following nicotine administration as compared to 3 hours post nicotine. To examine the differences in metabolism of nicotine before as compared to after vaccination, AUCs were calculated for cotinine (Cot) and 3-hydroxycotinine (3HC) on each scan day to determine the ratio of 3HC/COT. There were no differences in the ratios of AUC $3HC_1$/ AUC Cot_1 (0.12 (SD=0.07)) to AUC $3HC_2$/ AUC Cot_2 (0.12 (SD=0.07); p=0.34) and no significant change in the overall half life of nicotine (138.8min (SD=78.2) vs. 132.2min

(SD=89.6); $p>0.05$). There were no significant correlations between any of the nicotine outcome measures and antibody levels on the day of the 2nd SPECT scan.

Receptor occupancy by nicotine after vaccination: There were no significant differences in baseline β_2*-nAChR availability before as compared to after vaccination (p>0.10). After baseline scans were obtained and IV nicotine was administered, subjects were placed back in the camera at 62.1min (SD=3.6) post initiation of IV nicotine challenge. Maximal displacement of the radioligand was achieved 3–4hr post nicotine administration (49.4% (SD=9.5)) (Fig 2). Maximal range in displacement was 34.5–66.5% across subjects (Table 2). Immunization was associated with a significant 12.5% decrease in receptor occupancy by nicotine (F=5.19, df=1,9, p=0.049) with an estimated reduction in brain nicotine of 23.6%. After removing subject #2 (who was not able to abstain from smoking prior to each scan), the statistical significance of the decrease in receptor occupancy by nicotine was reduced to p = 0.068 (F=4.45, df=1,8). Importantly, the positive correlation between nicotine binding to the receptor and amount of nicotine injected prior to immunization (r=0.60, n=11, p=0.05 or r=0.73, n=10, p=0.03 without subject #2) was not observed (r=0.01, n=11, p=0.98) following immunization (Fig 3). No significant correlations were observed between titer levels and change in in receptor occupancy by nicotine after immunization.

Discussion

This was a proof of concept study designed to evaluate the effect of the nicotine vaccine 3′-AmNic-rEPA on the ability of nicotine to enter the brain and bind to the high affinity β_2*-nAChRs in healthy tobacco smokers. The primary findings confirm immunization with 3′-AmNic-rEPA leads to a significant reduction in nicotine's ability to enter the brain and bind to β_2*-nAChRs. We observed a 12.5% decrease in β_2*-nAChRs occupancy by nicotine associated with a 23.6% decrease in available nicotine to enter the brain after vaccination.

All subjects had titer levels indicating that antibodies for nicotine had been developed. Consistent with the preclinical literature (4), administration of IV nicotine after immunization was associated with at least two-fold higher plasma nicotine concentrations compared to before immunization, as well as with altered nicotine clearance, volume of distribution and decreased ability for nicotine to enter the brain. Unlike in the rodent studies (4), immunization did not appear to prolong nicotine's terminal half-life. This is likely due to 1) The proportional change in clearance and volume of distribution, which would not alter nicotine half life. In rodent studies these variables were not proportional. 2) Rats and humans have similar but not identical nicotine clearance and volume of distribution, and the effects of vaccination on half-life could differ. 3) In the rodent studies, the titer levels achieved were much higher than in the current study and this likely affected the pharmacokinetics of nicotine (28).

Maximal nicotine binding to the β_2*-nAChR before immunization was 56.2% and was lowered significantly to 49.4% after immunization (12.5% reduction). This reduction in receptor occupancy by nicotine was associated with an estimated 23.6% reduction in the available nicotine in the brain. Vaccination disrupted the straightforward association between amount of nicotine administered and nicotine bound to β_2*-nAChRs, although we did not detect significant associations between achieved antibody levels and the reduction in receptor occupancy by nicotine post immunization. The lack of association between antibody levels and reduction in nicotine's occupancy of the receptors could be due to several reasons including a small sample size, the fact that all subjects achieved optimal antibody levels, or physiological differences between our subjects and those in rodent studies. A significant positive relationship between the amount of nicotine administered and β_2*-nAChR occupancy by nicotine has been previously shown by our group (14, 15) and

others (16). Thus the disruption in this association after immunization is remarkable and strongly implicates the vaccine's role at altering distribution of nicotine to the brain and occupancy of β_2*-nAChRs. These results are in line with findings by Satoskar and colleagues (29) showing that in vaccinated rats there was a significant reduction in the amount of nicotine reaching the brain compared to non vaccinated rats.

Clinical changes that accompanied the 12.5% reduction in bound nicotine were a 50% reduction in cigarette use and significantly less craving for cigarettes from baseline to completion of immunization. This difference in the amount of bound nicotine from baseline to post immunization is comparable to previous study where the 10% difference in bound nicotine partially contributed to the differential effects on craving (14). The clinical results in the present study may appear discrepant since Phase III clinical trials for this vaccine did not show efficacy. There are several potential explanations for this. First, the differences between the outcomes may be due to the fact that levels of antibody titers may have been suboptimal in the majority of the smokers in the clinical trial. Second, the 12.5% reduction in occupancy may not be sufficient to lead to improved abstinence rates. Third, the population in the present study was composed of non-treatment seeking smokers. Lastly, the clinical trials for 3′-AmNic-rEPA assessed smoking cessation outcomes months after the vaccination schedule compared to a placebo control, whereas the present study concentrated on the period immediately following immunization.

The study has limitations. The lack of a placebo control group limits clinical interpretation; however, this study was a proof of concept that nicotine vaccine does reduce amount of nicotine to the brain and affects nicotine pharmacokinetics *in vivo* in human subjects. The small sample size limits our ability to examine potential variables that may play a role in receptor response to vaccine such as gender. As described previously (15), use of radiotracer imaging limits interpretation of temporal findings. The slow kinetics of the radiotracer might not accurately model the correct time period for maximal occupancy of β_2*-nAChR by nicotine since [^{123}I]5-IA is characterized by a slow dissociation of the receptor-ligand complex and slow clearance from brain (30–32). This means that radioligand binding to the receptor does not instantaneously match the quantity of available receptors, and the maximal occupancy detected here at 3–4 hrs after nicotine administration is likely achieved sooner in the brain. Faster radioligands, which may provide a better representation of the effects of nicotine at the β_2*-nAChR, are currently under development.

To conclude, we showed that immunization with 3′-AmNic-rEPA significantly reduces β_2*-nAChR occupancy by nicotine by sequestering nicotine in the blood and reducing entry into the brain. Moreover, immunization was associated with significant reductions in cigarette use and craving in non-treatment seeking smokers. This study provides evidence for mechanisms involved in the use of vaccination against nicotine dependence in human tobacco smokers.

Acknowledgments

We thank the technologists at the Institute for Neurodegenerative Disorders for conducting the scanning protocol and Louis Amici (Yale University) for metabolite and protein binding analyses of the radiotracer. Special thank you to Dr. Paul Pentel (Minnesota University) for intellectual contribution. This manuscript is written in memory of Dr. Julie K. Staley who was instrumental at experimental design.

Funding: Support contributed by NIMH K01 MH092681 and VA Career Development Award –1, K12 DA000167, NIDA R21 DA024388, CIHR MOP 86471 and NIH DA 020830. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Alcohol Abuse and Alcoholism, the National Institute on Drug Abuse, or the National Institutes of Health.

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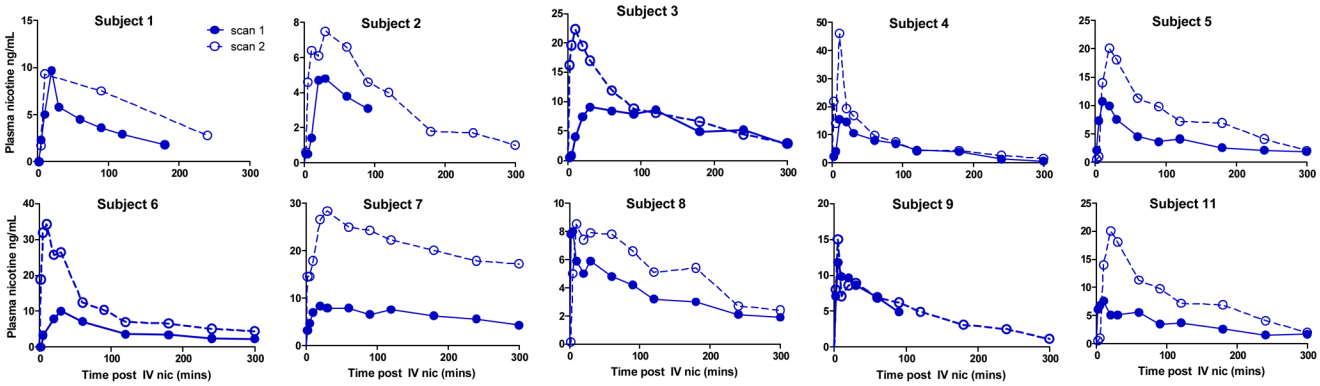

Figure 1.

Plasma nicotine levels after IV nicotine administration on two SPECT scan days for each subject: 1. before immunization (closed circles) and 2. after immunization (open circles) for each subject. Notably, average maximum concentration across subjects (Cmax; 9.1 vs 22.3ng/mL) and area under the curve (AUC; 1853 vs 2537 ng. min/ml) were significantly higher after immunization.

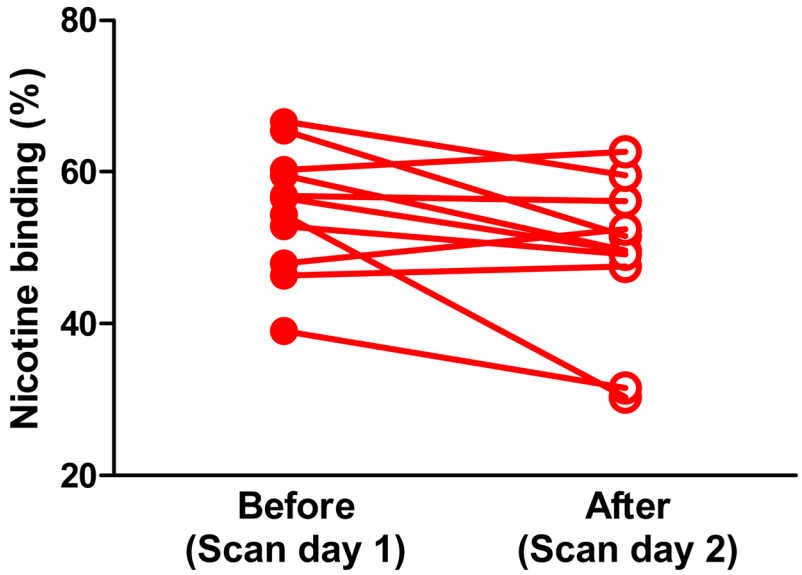

Figure 2.

β_2*-nAChR occupancy by nicotine after IV nicotine administration on two SPECT scan days: 1. before immunization (left – closed circles) and 2. after immunization (open circles) connected by a line to represent each subject. There was a significant average 12.5% decrease in nicotine's binding to the receptors after the immunization. Difference in binding was calculated as $[Ro_{\text{after}} / Ro_{\text{before}}] * 100$= % difference.

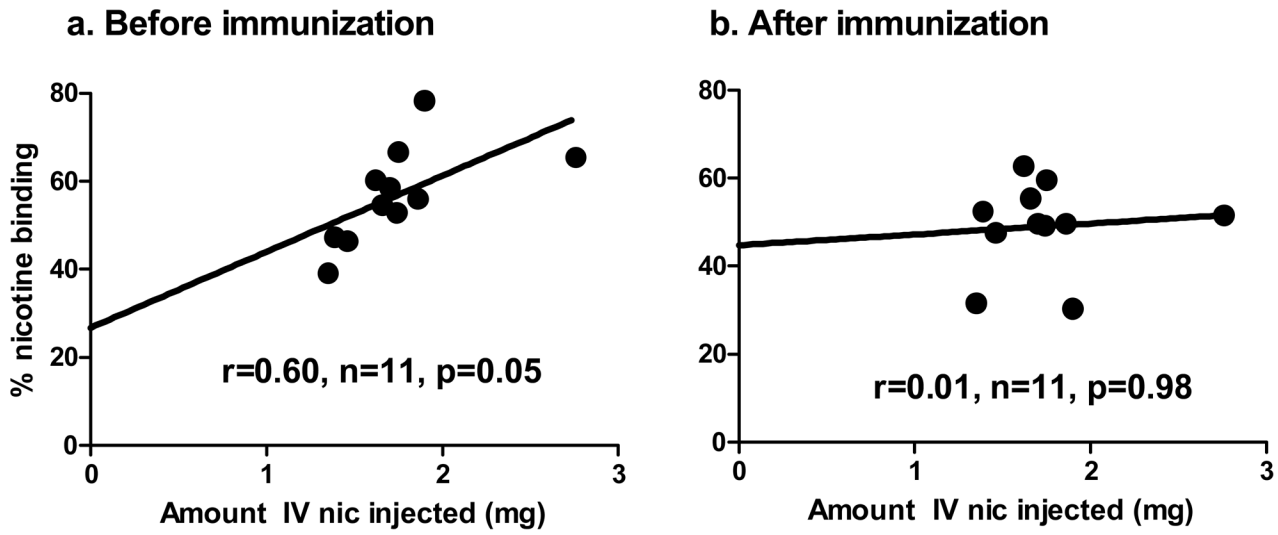

Figure 3.

Association between nicotine binding to the receptor and amount of IV nicotine administered on each SPECT scan day. Each subject received the same amount of IV nicotine (1.5 mg/70kg) on SPECT scan days. We observed a positive correlation between nicotine binding and amount of IV nicotine administered on the first SPECT scan day (a. Before 3′-immunization) but not on the second SPECT scan day (b. After immunization).[1]

[1]The positive correlation between nicotine binding to the receptor and amount of nicotine injected prior to immunization (r=0.60, n=11, p=0.05) was not observed (r=0.01, n=11, p=0.98) following immunization.

Table 1

Mood and smoking characteristics at baseline and on scan days

Measure	Baseline		Scan day 1				Scan day 2			
			Before IV nic		After IV nic		Before IV nic		After IV nic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CES-D	5.7	8.9	6.0	9.1	5.5	7.8	5.1	7.4	4.5	6.8
STAI-State	28.4	9.4	27.5	8.0	29.0	7.0	30.3	12.7	30.2	11.0
MNWS	4.5	7.2	3.8	3.7	1.6	1.6	3.5	7.8	2.1	3.7
Tiffany Desire	30.1	7.2	31.5	6.8	25.6	11.6	24.0	10.8	20.5	10.1
Tiffany Relief	16.2	8.6	16.7	8.3	14.5	8.8	13.1	7.8	9.8	4.2
QSU Brief Desire	9.3	4.5	9.2	3.3	7.0	4.4	7.2	4.6	4.2	3.5
QSU Brief Relief	6.5	4.2	7.2	4.5	6.1	4.3	5.9	3.7	4.2	1.8

CES-D – Center for Epidemiologic Studies Depression Scale; STAI – State – Spielberger State-Trait Anxiety Inventory; MNWS – Minnesota Nicotine Withdrawal Scale; Tiffany– Tiffany Questionnaire for Smoking Urges; QSU Brief – Questionnaire for Smoking Urges Brief.

Table 2

Plasma nicotine and receptor occupancy before and after treatment with 3′-AmNic-rEPA.

#	Ab (μg/mL)	IV nic (mg)	% Occupancy		AUC $t_{0-\infty}$ (ng. min/ml)		Cmax (ng/mL)		Tmax (min)		C_l (mL/min)		V_D (L)		Half life (min)	
			T1	*T2*	*T1*	*T2*	*T1*	*T2*	*T1*	*T2*	*T1*	*T2*	*T1*	*T2*	*T1*	*T2*
1	59.8	1.66	56.9	56.2	920	1994	9.7	9.3	20.0	10.0	1804	833	233511	154433	90	129
2*	45.3	1.90	54.4	30.3	742	1165	4.8	7.5	30.0	30.0	2560	1630	351398	213721	95	91
3	85.4	2.76	65.5	51.5	2496	2979	9.1	22.3	30.0	10.0	1106	926	263278	141204	165	106
4	64.6	1.86	56.6	49.7	1514	2413	15.5	46.1	10.0	10.0	1228	771	78755	132457	44	119
5	75.9	1.46	46.4	47.6	1421	2622	10.7	20.1	10.0	20.0	1027	557	172453	75759	116	94
6	127.9	1.75	66.6	59.6	2097	3994	10.0	34.2	30.0	10.0	835	438	295992	159289	246	252
7	64.6	1.35	39.1	31.5	4001	15718	8.3	28.4	20.0	30.0	337	86	161987	46904	333	378
8	41.7	1.62	60.3	62.7	1489	2083	8.0	8.5	5.0	10.0	1088	778	261298	177543	166	158
9	65.0	1.70	59.5	49.7	1205	1524	11.8	15.0	5.0	5.0	1411	1115	151182	154616	74	96
10**	NA	1.74	52.8	49.1												
11	129.2	1.39	48.0	56.2	1337	2625	7.6	20.1	10.0	20.0	1040	530	216448	72835	144	95
Mean		1.9	54.9	49.1	1722	3712	9.6	21.2	17.0	15.5	1244	766	218630	132876	147	152
SD (±)	75.9	0.5	8.7	10.1	951	4291	2.8	12.4	10.3	9.0	596	418	79657	52169	87	93
Sig			F=5.2, p=.05		t=−1.8, p=.10		t=−3.5, p=.01		t=0.1, p=.41		t=5.6, p=.00		t=4.2, p=.002		t=0.34, p=.74	

*Subject was not able to abstain from tobacco smoking for the required period.

**Poor venous access during and post IV nicotine challenge for subject #10 thus no nicotine plasma levels obtained on either scan day.

AUC – area under plasma nicotine concentration time curve, Cmax – maximal concentration of plasma nicotine, Tmax – time to reach Cmax, C_l – clearance of nicotine, V_D – volume of distribution. Pre – data from SPECT scan day prior to treatment with 3′-AmNic-rEPA; Post – data from SPECT scan day after treatment with 3′-AmNic-rEPA. SD – standard deviation.

Ab (μg/mL) – antibody concentration levels on scan day 2.

T1 – at time 1 (before immunization); T2 – at time 2 (after immunization)