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SPECT IMAGING OF NICOTINIC ACETYLCHOLINE RECEPTORS IN NONSMOKING HEAVY ALCOHOL DRINKING INDIVIDUALS

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Abstract

Background—The high rate of comorbidity of tobacco smoking with alcohol drinking suggests common neural substrates mediate the two addictive disorders. The beta₂-* containing nicotinic acetylcholine receptor (β₂*-nAChR) has recently emerged as a prime candidate because some alpha and beta subunit genes have been linked to alcohol consumption and alcohol use behaviors. We hypothesized that β₂*-nAChR availability would be altered by alcohol in heavy drinking nonsmokers.

Methods—Eleven heavy drinking (mean age 39.6±12.1y) and 11 age and sex-matched control (mean age 40.8±14.1y) nonsmokers were imaged using [¹²³I]5-IA-85380 ([¹²³I]5-IA) single photon

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Contributors

Irina Esterlis and Kelly P. Cosgrove conducted all MRI and SPECT scan appointments. Irina Esterlis also conducted image and data analysis, literature search, and wrote the manuscript and Kelly Cosgrove aided with manuscript preparation. Ismene L. Petrakis aided in study conceptualization and manuscript preparation. Sherry A. McKee supervised statistical analysis and aided in manuscript preparation. Frederic Bois conducted all the radiolabeling and supervised metabolite analyses. Erica Krantzler conducted data processing and image analysis, as well as data entry. Stephanie M. Stiklus recruited study participants: conducted telephone screens and in-person interviews, and helped with data entry. Edward B. Perry supervised medical evaluations of the potential subjects. Gilles D. Tamagnan aided with radiolabeling and study design, as well as manuscript preparation. John P. Seibyl supervised SPECT data acquisition and aided with medical issues. John H. Krystal aided with study design and completion, study related questions, and manuscript preparation. Julie K. Staley supervised the initial literature search, wrote the protocol, supervised all aspects of the study and aided with manuscript preparation. All authors contributed to and have approved the final manuscript.

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Conflict of interest

Conflict of interest from John H. Krystal: During the period of 2007-2009, he served as a scientific consultant to the following companies: Abbott Laboratories, AstraZeneca Pharmaceuticals, Bristol Meyers Squibb, Cypress Bioscience, Forest Laboratories, Glaxo SmithKline, Lohocla Research Corporation, HoustonPharma, Eli Lilly and Company, Pfizer Pharmaceuticals, Schering-Plough Research Institute, SK Life Sciences, Takeda Industries, and Transcept Pharmaceuticals. He hold less than \$1000 in exercisable warrant options with Tetragenex Pharmaceuticals. He is the principal investigator of a multicenter study in which Janssen Research Foundation has provided a drug and some support to the Department of Veterans Affairs. He is a co-sponsor for two patents under review for glutamatergic agents targeting the treatment of depression.

The remaining authors declare no conflict of interest.

emission computed tomography (SPECT). Heavy alcohol drinkers drank varied amounts of alcohol (70-428/month) to facilitate exploratory linear analyses of the possible effects of alcohol.

Results—Heavy drinkers consumed on average 9.1 ± 7.3 drinks/occasion; whereas controls drank 1.2 ± 0.9 drinks/occasion. Heavy drinkers were imaged 2.0 ± 1.6 days after last alcoholic beverage. Overall, there were no significant differences in β_2^* -nAChR availability between the heavy drinking and control nonsmokers. Exploratory analyses of other factors that may be uniquely regulated by alcohol suggested no effects of age, number of alcohol drinks, years drinking, severity of drinking, craving or withdrawal.

Conclusions—These preliminary analyses do not suggest a decrease in receptor availability in heavy drinking nonsmokers as compared to control nonsmokers. However, a larger study is warranted to explore effects of heavy alcohol drinking on other variables, such as sex, smoking, and genetic make up.

Keywords

heavy alcohol drinking; β_2^* -nicotinic acetylcholine receptors; SPECT imaging

1. INTRODUCTION

The cholinergic system can be altered following alcohol exposure and has been implicated in alcohol use disorders (Hunt and Majchrowicz, 1983, Kochlar and Erickson, 1986), with findings of altered cortical acetylcholine levels in animal and human subjects (Arendt et al., 1983, Beracochea et al., 1986), as well as reduced acetylcholinesterase activity in ethanol-fed rats (Miller and Rieck, 1993). Specific to our research interests, the β_2 -subunit containing nicotinic acetylcholine receptors (β_2^* -nAChR) (Yoshida et al., 1992, Robles and Sabria, 2006), as well as the gene (CHRNA4) that encodes the α_4 subunit (Butt et al., 2003), have been implicated in animal models of ethanol use disorders; however, no study has reported examination of this receptor in human alcohol drinkers.

While a specific ethanol binding site on the β_2^* -nAChR has not been identified, ethanol enhances the association rate of nicotinic agonist binding to nAChRs and can stabilize the nAChR in a nonfunctional, desensitized state (El-Fakahini et al., 1983). The affinity of agonist binding to the nAChR is enhanced in the presence of ethanol (Forman et al., 1989, Wood et al., 1991). Pretreatment with dihydro-beta-erythroidine (DhbetaE, $\alpha_4\beta_2$ -nAChR competitive antagonist) inhibits alcohol uptake (Kuzmin et al., 2008). In rodents, acute ethanol treatment does not alter nAChR number (Collins et al., 1988, Ribeiro-Carvalho et al., 2008); however, prolonged ethanol treatment (5 months) can enhance binding to nAChRs in thalamus and hypothalamus, but decrease binding in hippocampus in male rats (Yoshida et al., 1992). One study reported a 30% increase in nuclear $\alpha_4\beta_2$ nAChR in hippocampus after a 21 week treatment with ethanol (10% solution w/v) in rats (Robles and Sabria, 2006).

Studies on relationship between ethanol and nAChR in humans are sparse: an examination in postmortem tissue from human alcoholics failed to demonstrate a difference in [3 H]nicotine binding in thalamus and frontal cortex (Hellstrom-Lindahl et al., 1993). These findings were confounded by assessment of limited brain regions, excessively long postmortem interval (48 ± 4 h) and poor control for comorbid neuropsychiatric disorders including tobacco smoking. This presents a major caveat because the nicotine in tobacco smoke has very potent regulatory effects on β_2^* -nAChR, and if nicotine is still present in the brain (which it is up to 7 d post smoking) it interferes with radiotracer binding.

Human laboratory studies provide additional line of support for role of nAChR in alcoholism. Administration of mecamylamine, a nonselective nicotinic antagonist, interferes with the

stimulant and euphoric effects of alcohol (Blomqvist et al., 2002, Chi and Wit, 2003, Young et al., 2005). Varenicline, a partial nAChR agonist, reduces alcohol seeking and choice in rats (Steensland et al., 2007), and alcohol craving and self-administration behavior in heavy drinking smokers (McKee et al., 2009). In addition, the CHRNA2 and CHRNA4 are related to subjective response to alcohol (Ehringer et al., 2007) and enhancement of nAChR function by ethanol (Butt et al., 2003), respectively.

Presently, we explored the regulatory effects of alcohol on β_2^* -containing nAChRs. Based on preclinical and postmortem literature that suggest an upregulation and no change in β_2^* -nAChR availability, respectively, we hypothesized that heavy drinking nonsmokers would exhibit altered β_2^* -nAChR availability compared to control nonsmokers. These preliminary studies were limited to alcohol drinking nonsmokers to eliminate the possible confounding effects of nicotine. β_2^* -nAChR availability was measured using [123 I]5-I-A-85380 ([123 I]5-IA) and single photon emission computed tomography (SPECT) imaging, described previously (Staley et al., 2005a).

2. MATERIALS and METHOD

2.1 Participants

This study was approved by the Human Investigational Review Committees at Yale University and West Haven VACHS. Eligibility was evaluated via structured interview, physical examination, laboratory blood tests, urine drug screen, and electrocardiogram. None of the heavy drinking or control subjects had history or evidence of serious medical or neurological illness, psychiatric disorder or substance abuse (except for alcohol dependence/abuse in heavy drinkers), or used psychotropic substances for at least 1 year and no marijuana for at least 1 month preceding the study. None of the subjects reported taking any prescription medications within 6 months prior to SPECT study, nor had ever taken any medications known to act at the nAChRs, e.g., mecamylamine or varenicline. In order to participate in the study, heavy drinkers had to consume >70 standardized drinks per month based on Timeline Follow Back Interview (Sobell and Sobell, 1993), and had to have their last alcoholic beverage within 30 days of their SPECT scan. Based on NIH guidelines of percent alcohol by volume of each drink, one standardized drink contains 16.8mL of alcohol (NIAAA, 2008); therefore, one 12oz (336mL) beer, one 5oz (140mL) wine, or 1.5oz (42mL) liquor are considered as one standardized drink. Control subjects were defined as those who drank <20 drinks per month and no more than 4 drinks per occasion. Other drinking characteristics (age subjects began drinking, total number of drinking years, and family history of alcoholism) were documented. Heavy drinkers were offered a treatment referral upon completing study participation.

Nonsmoking status was defined as smoking < 40 cigarettes/lifetime and no cigarette use in the past 6 months, and confirmed by breath carbon monoxide levels (<11 ppm) and negligible plasma (≤ 50 ng/mL) and urinary (<100 ng/mL) cotinine levels at intake and scan days. Women could not be pregnant or breastfeeding to participate in the study. Estrogen and progesterone levels were obtained on the day of SPECT scan.

2.2 Assessments

At baseline screening appointment we administered the Structural Clinical Interview for DSM-IV (SCID-I), and questionnaires to assess alcohol and nicotine dependence and alcohol-related behavior (Fagerstrom Test of Nicotine Dependence - FTND, Tiffany Alcohol Craving Questionnaire, Retrospective Withdrawal Questionnaire), and depressive symptoms (Center for Epidemiological Studies Depression Scale - CES-D). Alcohol withdrawal/craving and depressive symptoms were assessed again on SPECT scan day. Family history of alcoholism was assessed via the self-report (defined positive if 1st degree relatives were diagnosed with

alcohol abuse or dependence). Subjects were negative for signs of withdrawal at the time of screening and the SPECT scan day as assessed by the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar).

2.3 [^{123}I]5-IA SPECT and MRI imaging

SPECT—Participants were imaged as described previously (Staley et al., 2005a). Briefly, [^{123}I]5-IA was administered using bolus plus constant infusion paradigm. The average yield was a $55.3\% \pm 14.7\%$ ($n=22$ preparations) and its radiochemical purity was $98.1\% \pm 1.9\%$. Three SPECT scans (30 min each) were obtained between 6–8 h of infusion, and plasma samples were collected in the middle of second scan to quantify total parent and free fraction (f_p) of parent tracer in plasma (Zoghbi et al., 2001) and correct for individual differences in metabolism and protein binding of [^{123}I]5IA (Cosgrove et al., 2007).

MRI—Magnetic resonance imaging was performed on a Signa 1.5T system (General Electric Co, Milwaukee, Wis). Axial images were acquired parallel to the anteroposterior commissural line with an echo time of 5 milliseconds; repetition time of 24 milliseconds; matrix 256×192 ; number of excitations of 1; field of view of 24 cm; and 128 contiguous slices with a thickness of 1.3 mm.

Image analysis: SPECT emission images were analyzed as described previously (Staley et al., 2005a). Regional β_2^* -nAChR availability was determined by V_T/f_p (regional activity/plasma free parent) (Innis et al., 2007), a highly reproducible outcome measure (Staley et al., 2005a). Standard regions of interest were placed on coregistered MRIs and transferred to SPECT images. Regions of interest were chosen based on those known to contain β_2^* -nAChRs, and included thalamus, striatum (average of caudate and putamen), cerebellum, and parietal, frontal, anterior cingulate, temporal, and occipital cortices. The average of two raters is used for all analyses. Interrater was $<10\%$ variability between raters across regions.

Statistical analyses: All analyses were performed using SPSS version 15.0 (SPSS Inc. Headquarters, Chicago, IL). Differences in plasma and brain outcome measures between heavy drinkers and controls were evaluated first using a multivariate analysis of variance (MANOVA) in order to control for experiment-wise Type I error. Correlations between regional brain [^{123}I]5-IA uptake (V_T/f_p) and drinking variables were conducted using Spearman rho (ρ) correlation.

3. RESULTS

3.1 Demographics

Demographic variables are reported in Table 1. Eleven (6 men, 5 women; mean age = 39.6 ± 12.1) heavy alcohol drinking nonsmokers and eleven sex and age-matched (mean age = 40.8 ± 14.1) control nonsmokers provided written informed consent to participate in this study. Of the heavy drinkers, nine subjects met criteria for current alcohol abuse, and two subjects met criteria for current alcohol dependence. Heavy drinkers were imaged 2.0 ± 1.6 days (mean $\pm$ SD; range 1–5 days; one person was imaged at 18 days) after their last alcoholic beverage. Heavy drinkers drank on average 9.1 ± 7.3 drinks per episode and drank 4.9 ± 2.3 days out of the week. One heavy drinking subject and three control subjects had first-degree family history of alcohol dependence or abuse. None of the subjects reported significant level of depression.

3.2 β_2^* -nAChR availability and relationship to drinking variables

There was no difference in total radiotracer dose administered between heavy drinking (368.9 ± 2.4 MBq) and control nonsmokers (357.7 ± 36.3 MBq). [^{123}I]5-IA uptake did not differ significantly between heavy drinkers and controls (see Figure 1), suggesting no overall effect

of heavy drinking on β_2^* -nAChR availability. We observed no association between [^{123}I]5-IA uptake and drinking measures (years of heavy drinking, standardized drinks per month, and days since last drink) in the overall sample of heavy drinkers.

4. DISCUSSION

The present study suggests that there are no significant differences in β_2^* -nAChR availability between heavy drinking nonsmokers and control nonsmokers in the thalamus, striatum, cerebellum and cortical regions. These findings differ from preclinical studies that suggest an increase in agonist binding to the nAChR after prolonged alcohol exposure (Yoshida et al., 1992, Robles and Sabria, 2006). This difference may be due to differences in regional subunit composition between species. Our results are consistent with a postmortem study that showed no evidence of alteration in [^3H]nicotine binding to thalamus or cortical nAChRs in deceased alcoholics compared to controls (Hellstrom-Lindahl et al., 1993). Additionally, we did not detect a relationship between drinking variables (years of heavy drinking, standardized drinks per week, and days since last drink) and β_2^* -nAChR availability. Some preclinical literature suggests there may be a difference between length of alcohol consumption and agonist binding to the nAChRs (Collins et al., 1988, Ribeiro-Carvalho et al., 2008, Yoshida et al., 1992), but this does not appear to be the case in the present sample.

The major limitation of the current study is low sample size. However, this study was designed to be exploratory to determine if there was a possible effect of alcohol, and if receptor availability may relate to alcohol use behaviors or delineate a unique subgroup of drinkers. The study design allowed flexibility in the number of drinks (70-428) consumed per month and in the number of days since last drink (up to 30), to examine associations between β_2^* -nAChR availability and drinking quantity and frequency, and if there was a change in β_2^* -nAChR availability over time since last drink. Only nonsmokers were studied to simplify the interpretation of the data because it has been established that the nicotine from tobacco smoke if still in the brain (up to 7-9 d), blocks binding to the receptor and would interfere with a reliable measurement (Staley et al., 2006). However, in a future study we plan to study the effects of alcohol on β_2^* -nAChR availability in smokers, since some studies show that smoking may have neuroprotective qualities against alcohol's harmful effect on brain neurochemistry (Staley et al., 2005b, Tizabi et al., 2004, Hetzler and Martin, 2006).

In this study we did not find a relationship between β_2^* -nAChR availability and days since last drink. The subjects who participated in the current study were imaged during early abstinence (on average within 2 days of last alcoholic beverage). However, long-term abstinence effects of alcohol on β_2^* -nAChR availability are not known and may differ by smoking status. For example, previous findings from our group showed an upregulation in β_2^* -nAChR availability in recently abstinent tobacco smokers compared to nonsmokers (Staley et al., 2006); however, this effect is reversed by 6-12 weeks of abstinence when the β_2^* -nAChR availability appears to normalize (Cosgrove et al., 2009).

Though the genes associated with encoding the nAChRs have been associated with alcoholism, due to the small sample size, we were not able to investigate the relationship between genetic factors and receptor availability in heavy drinking nonsmokers. Specifically, the CHRNA2 and CHRNA4 are related to subjective response to alcohol (Ehringer et al., 2007) and enhancement of nAChR function by ethanol (Butt et al., 2003), respectively, and CHRNA3, but not CHRNA5, is involved in acute locomotor response to alcohol (Kamens et al., 2008). An evaluation of the genetic contribution to β_2^* -nAChR expression in alcohol use disorders is warranted in a larger sample.

To our knowledge, this is the first human neuroimaging study that examines the β_2^* -nAChR as a possible neurochemical substrate of alcoholism. Though in the current small sample we did not detect a difference in β_2^* -nAChR availability between heavy drinkers and controls, evaluation of this receptor in a more chronic alcoholic sample is warranted.

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Figure 1.

Scatterplots illustrating individual $[^{123}\text{I}]$ 5-IA regional distribution volume (regional activity/free plasma parent) determined from the region of interest analyses for each individual subject is illustrated for the thalamus, striatum, and mean cortex (average of parietal, frontal, anterior cingulate, temporal and occipital cortices). Drinkers were imaged on average 2d after last drink. There were no significant differences between the total control and heavy drinking nonsmokers.

Table 1

Demographic characteristics for control and alcoholic nonsmokers

	Controls N=11	Heavy Drinkers N=11
<i>Age</i>	40.8 ±14.1	39.6 ±12.1
<i>Race</i>	8C, 3AA	9C, 2AA
<i>Years heavy drinking</i>	0	15.5 ±13.8
<i>Drinks per episode</i>	1.2 ±0.9	9.1 ±7.3
<i># Drinking days/week</i>	0.3 ±0.8	4.9 ±2.3
<i>Days since last drink</i>	370.7 ±1080.4	2 ±1.6
<i>CES-D</i>	4.0 ±5.5	6.2 ±4.2
<i>FH+</i>	3	1

Abbreviations: Race: AA – African American, C – Caucasian; CES-D - Center for Epidemiological Studies Depression Scale; FH+ - family history positive for alcohol

*Data are presented as mean ± SD.

**Race was self-reported by each subject.