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Changes in the $\alpha 4\beta 2^*$ Nicotinic Acetylcholine System During Chronic Controlled Alcohol Exposure in Nonhuman Primates*

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Abstract

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Contributors

TEB operated the cyclotron for radioisotope production. ATH, DWW, PJJ, and TEB performed the [¹⁸F]nifene radiochemistry. LMR and JAL supervised animal care, anesthesia, measurement of ethanol consumed by subjects, and processing of the ethanol consumption data. EOA conducted image data acquisition, assisted by LMR and JAL. ATH, DWW, and PJJ reconstructed and processed the imaging data. ATH performed image registration and creation of BPND maps. DLT performed statistical analysis. Literature search and manuscript preparation was performed by ATH, DLT, AKC, CFM, MLS, and BTC. The subjects in this study were part of a grant awarded to MLS including AKC, CFM, and BTC, all of whom oversaw aspects of research procedures. All authors contributed to and have approved the final manuscript.

Conflict of Interest

The authors have no conflict of interest to report.

Background—The precise nature of modifications to the nicotinic acetylcholine receptor (nAChR) system in response to chronic ethanol exposure is poorly understood. The present work used PET imaging to assay $\alpha 4\beta 2^*$ nAChR binding levels of eight rhesus monkeys before and during controlled chronic ethanol intake.

Methods— $[^{18}\text{F}]$ Nifene PET scans were conducted prior to alcohol exposure, and then again after at least 8 months controlled ethanol exposure, including 6 months at 1.5 g/kg/day following a dose escalation period. Receptor binding levels were quantified with binding potentials (BP_{ND}) using the cerebellum as a reference region. Alcohol self-administration was assessed as average daily alcohol intake during a 2 month free drinking period immediately following controlled alcohol.

Results—Significant decreases in $\alpha 4\beta 2^*$ nAChR binding were observed in both frontal and insular cortex in response to chronic ethanol exposure. During chronic alcohol exposure, BP_{ND} in the lateral geniculate region correlated positively with the amount of alcohol consumed during free drinking.

Conclusions—The observed decreases in nAChR availability following chronic alcohol consumption suggest alterations to this receptor system in response to repeated alcohol administration, making this an important target for further study in alcohol abuse and alcohol and nicotine codependence.

Keywords

PET Imaging; Chronic Alcohol Exposure; Rhesus Monkey; Alcohol and Nicotine Comorbidity; $\alpha 4\beta 2$ Nicotinic Acetylcholine Receptor

1. INTRODUCTION

An interaction of prolonged alcohol exposure with the nicotinic acetylcholine receptor (nAChR) system has been evidenced in an array of behavioral and *in vitro* experiments (see Larsson and Engel, 2004 for review). Additionally, the high comorbidity of nicotine and alcohol abuse suggests the nAChR system may play a role in alcohol dependence (Meyerhoff et al., 2006). Conflicting findings from *in vitro* studies cloud current understanding of the precise nature of this interaction. In rats, *in vitro* binding studies demonstrated increases in thalamic receptor density (B_{max}) but decreases in hippocampal B_{max} following prolonged alcohol exposure (Yoshida et al., 1982; Robles and Sabria, 2008). Conversely, a study in fibroblast and neuroblastoma cell lines found decreases in $[^3\text{H}]$ nicotine binding, whereas $\alpha 4$ mRNA levels increased and $\beta 2$ mRNA levels were unaffected after extended ethanol exposure (Gorbounova et al., 1998). Microdialysis experiments in rats demonstrated increases in acetylcholine (ACh) levels following the administration of ethanol (Stancampiano et al., 2004; Larsson et al., 2005). These mixed findings from *in vitro* experiments accentuate the importance of *in vivo* imaging studies to noninvasively assay subject-specific longitudinal changes to receptor systems. One previous study using the $\alpha 4\beta 2^*$ specific SPECT radioligand $[^{123}\text{I}]5\text{-IA-85380}$ in rhesus monkeys found no changes in binding levels immediately following extended free alcohol consumption, however, significant decreases in binding in the cortex and thalamus following 5–13 weeks alcohol abstinence were observed (Cosgrove et al., 2010).

The primary goal of the present study was to utilize PET imaging with [^{18}F]nifene, a PET radioligand with high affinity for $\alpha 4\beta 2^*$ nAChRs and fast kinetic properties, to assay $\alpha 4\beta 2^*$ binding levels before and during uniform chronic alcohol exposure. An additional objective was to assess associations of subject-specific binding with *ad libitum* alcohol consumption measured following controlled alcohol exposure. Eight alcohol-naïve rhesus monkey (*Macaca mulatta*) subjects were scanned under baseline conditions, consumed identical alcohol doses over at least 8 months, rescanned, and then allowed to freely drink alcohol for 2 months. These studies provide insight into individual nAChR system changes in response to uniform chronic alcohol exposure in subjects with varying alcohol self-administration rates.

2. METHODS

All procedures were in accordance with the NIH 'Guide for the Care and Use of Laboratory Animals' and approved by the University of Wisconsin-Madison Animal Care and Use Committee. These experiments examined eight adult rhesus monkeys (*Macaca mulatta*; 4M, 4F; 19.6–19.8 yr; 7.5–15.3 kg). All subjects were members of a cohort exposed to prenatal alcohol and/or prenatal stress and were reared under otherwise identical conditions (Schneider et al., 2013). A first [^{18}F]nifene PET scan ('baseline' scan) was conducted while subjects were alcohol-naïve. Subjects were then exposed to fixed ethanol concentrations for a total of 9 months as illustrated in Figure 1, based on a paradigm established by Vivian and colleagues (2001). Alcohol was provided as a 4% ethanol/water solution. Food pellets were delivered at regular time intervals, and only ethanol/water solution (no pure water) was available until the target daily alcohol dose was achieved. Subjects consumed 0.5 g/kg/day ethanol for one month, followed by 1.0 g/kg/day ethanol for one month, followed by 1.5 g/kg/day ethanol for 7 months. A second [^{18}F]nifene PET scan ('post' scan) was conducted during the last month of controlled alcohol exposure. Subjects were then allowed to consume alcohol *ad libitum* for 2 months, and average daily alcohol consumption was used to quantify alcohol self-administration.

Alcohol consumption was suspended 20–24 hours prior to imaging procedures such that no alcohol was present in the subject's system while scanning. On the day of PET scans, high specific activity [^{18}F]nifene was synthesized following previous methods (Hillmer et al., 2012). Subjects were anesthetized before imaging procedures with ketamine (10 mg/kg i.m.) and maintained on 1.0%-1.5% isoflurane throughout PET sessions. Scans were performed with a microPET P4 scanner (Tai et al., 2001) (Siemens, Knoxville, TN). A 512 s ^{57}Co transmission scan was first acquired, followed by a 60 min emission scan initiated with an 83–135 MBq bolus injection of [^{18}F]nifene. Regulated alcohol consumption was resumed on the day following the PET imaging session.

PET data were reconstructed and processed as described previously (Hillmer et al., 2012). Baseline and post images from each subject were aligned to the same space with FSL FLIRT (Jenkinson et al., 2002) to ensure identical intrasubject region identification. Regions of interest, selected and delineated by their elevated $\alpha 4\beta 2^*$ nAChR concentrations, were hand drawn for each subject to delineate the anteroventral thalamus (AVT), lateral geniculate region (LG), frontal cortex (FC), insular cortex (IC), and subiculum (SB), a substructure of

the hippocampus. [^{18}F]Nifene binding potentials (BP_{ND} , see Innis et al., 2007) for both baseline and post scans were generated with MRTM2 (Ichise et al., 2002), utilizing the cerebellum as a reference region.

Due to small sample size and detection of some outliers and skewness for several BP_{ND} metrics, non-parametric analyses were performed. Wilcoxon's signed rank test was used to test the null hypothesis that the distribution of the difference between baseline BP_{ND} and post BP_{ND} for each region is centered at zero. Spearman's rho rank correlation coefficient was computed for each region to test the null hypothesis of no association between different binding metrics and average alcohol consumed during free drinking. The binding metrics assessed with the correlation analysis included baseline BP_{ND} , post BP_{ND} , and change in BP_{ND} .

3. RESULTS

Statistically significant differences between baseline BP_{ND} and post BP_{ND} were detected in the FC ($V=35$; $p=0.015$) and IC ($V=33$; $p=0.039$), shown in Figures 2A and 2B. Median BP_{ND} values decreased by 23% and 17% in the FC and IC relative to baseline. No significant changes were observed in the AVT ($V=24$; $p=0.46$), LG ($V=24$; $p=0.46$), and SB ($V=28$; $p=0.195$).

The average alcohol consumed during free drinking was 1.3 ± 0.7 g/kg/day (see supplementary Table 1¹). The association analysis revealed no significant relationship of baseline BP_{ND} with alcohol self-administration, however, a trend of increased binding with increased alcohol intake was observed in the FC ($\rho_S=0.642$; $p=0.096$). A significant positive correlation of post BP_{ND} with alcohol self-administration was observed in the LG ($\rho_S=0.809$; $p=0.021$), tabulated in Figure 2C. No significant trends between change in BP_{ND} and alcohol self-administration were observed.

4. DISCUSSION

The design of the present study allowed for unique insight into individual neurochemical responses of the nAChR system to regulated chronic alcohol exposure via PET imaging. The results demonstrate, for the first time, evidence of reduced cortical $\alpha 4\beta 2^*$ nAChR availability in response to chronic alcohol administration. Furthermore, the average daily *ad libitum* alcohol consumed after scanning procedures was found to have a significant positive correlation with post BP_{ND} in the LG.

The finding of reduced binding in the FC and IC following alcohol exposure is in contrast to previous neuroimaging studies, which found no significant change in cortical binding levels following 18 weeks of free alcohol access in rhesus monkeys (Cosgrove et al., 2010), and no significant difference in cortical $\alpha 4\beta 2^*$ nAChR availabilities between heavy drinkers and controls in humans (Esterlis et al., 2010). All studies, however, found no significant change in thalamic binding following alcohol drinking, albeit under different conditions. The different findings of changes in cortical binding levels may be attributed to a variety of a

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factors: larger sample size in the present work, increased sensitivity of [^{18}F]nifene to changes in synaptic ACh concentrations (Hillmer et al., 2013), different administered alcohol dosages, different subject ages, or different histories of alcohol exposure. Modulations to $\alpha 4\beta 2^*$ nAChRs have also been observed in response to acute and chronic nicotine exposure and abstinence (Cosgrove et al., 2009; Brody et al., 2013). Considering the high comorbidity of alcohol and nicotine abuse, the nAChR system may therefore be a common underlying neurochemical target mediating the use and abuse of these substances.

A significant positive correlation between post BP_{ND} in LG and alcohol self-administration was observed, with a similar nonsignificant trend in AVT. These findings suggest that increased $\alpha 4\beta 2^*$ nAChR binding sites may be available in the thalamic nuclei of individuals who self-administer more alcohol. The relevance of this finding to functional mechanisms is unclear and merits further investigation. Additionally, baseline BP_{ND} in the FC positively trended with alcohol self-administration, suggesting that greater nAChR availability in an alcohol naïve state may be associated with alcohol self-administration. These diverse regional $\alpha 4\beta 2^*$ nAChR binding relationships could speculatively result from alcohol influenced relationships with the nAChR system in FC and IC that are different from AVT and LG. Interestingly, no significant correlations of change in BP_{ND} were observed with alcohol self-administration, in agreement with previous findings by Cosgrove and colleagues (2010).

Previous studies presented evidence of increased B_{max} in the thalamus following prolonged ethanol exposure (Yoshida et al., 1982; Booker and Collins, 1997; Robles and Sabria, 2006) while no differences in B_{max} were observed in the cortex (Booker and Collins, 1997). As increases in B_{max} would increase [^{18}F]nifene binding levels, these *in vitro* data would be inconsistent with the present findings unless the differences in cortical binding levels are dominated by a neurochemical process other than B_{max} changes. Extracellular ACh increases in cortex were observed in microdialysis experiments following acute ethanol exposure (Stancampiano et al., 2004). Although there is little experimental evidence concerning the effects of chronic ethanol on synaptic ACh concentrations, it is plausible given the observed acute effects that changes in synaptic ACh due to chronic alcohol exposure may occur. Additionally, existing evidence suggests that ligand affinity at the nAChR site may be modified by ethanol (Dopico and Lovinger, 2009). Either increases in synaptic ACh or decreases in affinity at the receptor site (or a combination of both) would be consistent with the presently observed decreases in [^{18}F]nifene BP_{ND} .

The protocol of alcohol administration is a major strength of the present work, as it provides tightly controlled administration of alcohol to alcohol naïve subjects of the same age, rearing conditions, and history of alcohol exposure (Vivian et al., 2001; Schneider et al., 2013), however, the different prenatal conditions may have introduced variability into the data. Individual variations in alcohol distribution and metabolism could have resulted in different cerebral alcohol concentrations despite identical administered doses, potentially limiting this study. Moreover, given the clouded body of *in vitro* work concerning the nAChR system and alcohol, interpretation of the underlying physiological alterations inducing BP_{ND} changes is limited since this composite parameter depends simultaneously on B_{max} , ACh concentration, and receptor affinity.

The findings presented herein identify decreases in cortical $\alpha 4\beta 2^*$ nAChR binding levels following chronic controlled alcohol exposure, emphasizing the importance of this receptor system in understanding both alcohol dependence and the comorbidity of alcohol and nicotine abuse. Additionally, regionally variant correlations of $\alpha 4\beta 2^*$ binding levels suggest a relationship between the nAChR system and alcohol self-administration, implying the possibility of these receptors as treatment targets to prevent or counteract chronic alcohol exposure. Further work is underway to analyze imaging data with other neurotransmitter systems from the same cohort.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The ethanol dose administration protocol used for the presented experiments, based on procedures established by Vivian and colleagues (2001). Time is shown in months along the x-axis. The y-axis is scaled by ethanol dosage in g/kg/day, shown above the x-axis. Periods of PET scanning procedures are shown below the x-axis.

Figure 2.

[¹⁸F]Nifene BP_{ND} values, representative of $\alpha 4\beta 2^*$ nAChR binding, are shown for eight subjects at an alcohol-naïve state (Baseline) and during controlled daily exposure to moderate doses of alcohol (Post) in the frontal cortex (**A**) and insular cortex (**B**). Each distinct symbol represents a different subject, connected by lines to aid in visualizing changes in BP_{ND}. * denotes significance at $p < 0.05$ of difference in BP_{ND} from baseline with Wilcoxon's signed rank test. **C** is a table showing Spearman's rank correlation rho values (ρ_s) with corresponding p-values of the relationship between post BP_{ND} and alcohol preference quantified by average alcohol consumed during the free drinking period. * indicates a significant association at $p < 0.05$ between the two metrics.