Positron Emission Tomography Shows Elevated Cannabinoid CB_1 Receptor Binding in Men with Alcohol Dependence

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Background: Several lines of evidence link cannabinoid (CB) type 1 (CB_1) receptor-mediated endogenous CB (eCB) signaling to the etiology of alcohol dependence (AD). However, to date, only peripheral measures of eCB function have been collected in living humans with AD and no human in vivo data on the potentially critical role of the brain CB_1 receptor in AD have been published. This is an important gap in the literature, because recent therapeutic developments suggest that these receptors could be targeted for the treatment for AD.

Methods: Medication-free participants were scanned during early abstinence 4 weeks after their last drink. Using positron emission tomography (PET) with a high-resolution research tomograph and the CB_1 receptor selective radiotracer [^{11}C]OMAR, we determined [^{11}C]OMAR volume of distribution (V_T) values, a measure of CB_1 receptor density, in a priori selected brain regions in men with AD (n = 8, age 37.4 ± 7.9 years; 5 smokers) and healthy control (HC) men (n = 8, age 32.5 ± 6.9 years; all nonsmokers). PET images reconstructed using the MOLAR algorithm with hardware motion correction were rigidly aligned to the subject-specific magnetic resonance (MR) image, which in turn was warped to an MR template. Time–activity curves (TACs) were extracted from the dynamic PET data using a priori selected regions of interest delineated in the MR template space.

Results: In AD relative to HC, [^{11}C]OMAR V_T values were elevated by approximately 20% (p = 0.023) in a circuit, including the amygdala, hippocampus, putamen, insula, anterior and posterior cingulate cortices, and orbitofrontal cortex. Age, body mass index, or smoking status did not influence the outcome.

Conclusions: These findings agree with preclinical evidence and provide the first, albeit still preliminary in vivo evidence suggesting a role for brain CB_1 receptors in AD. The current study design does not answer the important question of whether elevated CB_1 receptors are a preexisting vulnerability factor for AD or whether elevations develop as a consequence of AD.

Key Words: Alcohol Dependence, Brain Imaging, Positron Emission Tomography, Endogenous Cannabinoids, CB_1 Receptor.

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ALMOST 2 OF 3 alcoholics resume drinking after engaging in alcohol treatment (Brown et al., 1995; Hunt et al., 1971), highlighting the substantial need to improve the treatments for this patient population. At a neurobiological level, studies show impairments in decision making in patients with AD (Dom et al., 2006), which is associated with altered functions in cortico–limbic–striatal circuitry including the amygdala, hippocampus, anterior and posterior cingulate cortices, insula, and subregions of the striatum, that is, putamen (Goldstein et al., 2004; Makris et al., 2008). At least 3 sets of factors may contribute to high alcohol relapse rates. First, individual differences in the positive, reinforcing properties of alcohol may increase risk of alcoholism and possibly alcohol relapse (Schuckit and Smith, 1996). Second, stimuli previously associated with alcohol use and its physiological and subjective effects may become paired with alcohol and serve as "conditioned cues" that can increase alcohol craving and subsequent alcohol use (O'Brien

et al., 1998). Finally, differences in stress responsiveness may contribute to the development and maintenance of alcohol addiction (Brown et al., 1990; Miller et al., 1996; Sinha, 2001). All 3 factors can be linked to the endogenous cannabinoid (eCB) system and its attending CB_1 receptor (Serrano and Parsons, 2011), and a growing body of evidence points to an involvement of CB_1 receptors in the acquisition and maintenance of AD (Vinod and Hungund, 2006).

The behavioral effects of CB_1 receptor knockout (Hungund et al., 2003; Naassila et al., 2004; Poncelet et al., 2003; Thanos et al., 2005) and pharmacological manipulation of CB_1 receptor function (Femenia et al., 2010; Maccioni et al., 2008, 2010; Malinen and Hyytia, 2008) result in reduced voluntary alcohol intake. In contrast, administration of a CB_1 receptor agonist (Colombo et al., 2002; Gallate et al., 1999; Vinod et al., 2008) enhances alcohol consumption. These findings suggest that up-regulation of CB_1 receptor-mediated G-protein signaling in a circuit involving the amygdala, hippocampus, ventromedial prefrontal and orbitofrontal cortex, insula, and striatum (Sullivan and Pfefferbaum, 2005) might contribute to the increased alcohol consummatory behavior in patients with AD.

Positron emission tomography (PET) imaging is a direct, sensitive, and straightforward means of probing the functional neurochemistry of humans and assessing molecular targets in the brain in vivo. The recent development of a CB_1 receptor selective radiotracer, designated [^{11}C]OMAR (Horti et al., 2006; Wong et al., 2010), facilitates in vivo assessment of [^{11}C]OMAR volumes of distribution (V_T), a measure of total binding in the brain, and proportional to CB_1 receptor density. Given the higher rates of AD among men compared with women (Nolen-Hoeksema and Hilt, 2006) and important sex differences in the biological underpinnings of AD (Fox et al., 2009), we measured CB_1 receptors using PET and [^{11}C]OMAR in men with AD during early abstinence defined as 4 weeks after their last drink and in healthy control (HC) subjects. Based on preclinical data, we hypothesized that CB_1 receptor binding will be increased in men with AD compared to matched HC participants.

MATERIALS AND METHODS

Subjects

Eight men with AD, all medication-free in early abstinence 4 weeks after their last drink and 8 male age- and body mass index (BMI)-matched HC participants without a history of psychiatric or substance use disorder were recruited through public advertisement (Table 1). After giving informed consent, participants were evaluated and diagnosed using Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria and the Structured Clinical Interview for DSM-IV (SCID; First et al., 1996). Patients with AD did not have any additional Axis-I diagnosis and were treatment-engaged. They were participating in standard 3 times weekly substance abuse counseling and had been abstinent from alcohol for 4 weeks at the time of the PET scan. Abstinence was confirmed with regular breathalyzer and urine toxicology tests at each clinic visit to ensure abstinence before the PET scan. Signs and symptoms of alcohol withdrawal were quantified with the Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised (CIWA-Ar). Patients with AD were past the acute alcohol-withdrawal period at the time of the PET scan. All participants were evaluated by physical examination, electrocardiogram, standard laboratory tests, urine analysis, and toxicology and were free of significant medical or neurological conditions. Cannabis users were excluded given recent evidence showing reversible and regionally selective down-regulation of brain CB_1 receptors in human subjects who chronically smoke cannabis (Hirvonen et al., in press). No participants received medication for at least 4 weeks prior to PET scanning. The protocol was approved by the Yale University School of Medicine Human Investigation Committee, the Human Subjects Subcommittee of the Veterans Affairs Connecticut Healthcare System, the Magnetic Resonance Research Center, and the Yale New Haven Hospital Radiation Safety Committee.

Scanning and Imaging Procedures

[^{11}C]OMAR was prepared at high specific activity (212 ± 95 MBq/nmol at the end of synthesis) by methods previously described but adopted for synthesis using the TRACERlab FXC Pro automated synthesis module (GE Healthcare, Milwaukee, WI; Horti et al., 2006). The radiotracer (651 ± 69 MBq, 45 ± 27 ng/kg) was infused as a slow bolus over 1 minute through the antecubital vein. The radioactivity concentration of blood from the radial artery was measured continuously using an integrated peristaltic pump and calibrated radioactivity detector (PBS101; Veenstra Instruments, Joure, the Netherlands) for the first 7 minutes after radiotracer administration with discrete sample manually obtained at 3, 5, 15, 30, 60, and 90 minutes postinjection. Manual samples were measured on a gamma counter (Wizard 1480; Perkin-Elmer, Waltham, MA) to determine radioactivity concentration in whole blood and in plasma. The whole blood–plasma ratio was calculated and used to scale the continuous whole blood concentration data measured by the continuous blood counter used at the beginning of the scan. Selected discrete blood samples were analyzed for the fraction of unchanged [^{11}C]OMAR and its radiometabolites using a column-switching high-performance liquid chromatography assay (Hilton et al., 2000).

List-mode emission data were collected for 120 minutes using the HRRT PET scanner (Siemens Medical Systems, Knoxville, TN). Subject motion was measured using the Polaris Vicra optical tracking system (Northern Digital Inc., Waterloo, ON, Canada). Vicra recordings were used to correct list-mode data in the motion-compensation ordered subsets expectation maximization algorithm for resolution-recovery reconstruction (Carson et al., 2003), which produced dynamic images corrected for motion, photon scatter and attenuation, random coincidences, scanner dead-time, and detector inhomogeneity. The PET image sequences were registered to subject-specific T1-weighted MR images acquired on a 3 Tesla Trio imaging system (Siemens Medical Systems, Erlangen, Germany). Anatomical MR images were in turn nonlinearly warped to an MR template where regions of interest (ROIs) were defined (Tzourio-Mazoyer et al., 2002). These regions (Fig. 1, Table 2) constitute a neural circuit consistently implicated in the pathophysiology of AD and are known to contain high levels of CB_1 receptors. Regional time–activity curves (TACs) were extracted from the dynamic PET data and analyzed using multilinear analysis (Ichise et al., 2002) with metabolite-corrected arterial input functions and cutoff time of $t^* = 30$ minutes. This kinetic analysis yields regional estimates of total V_T, the equilibrium ratio of radioligand in tissue relative to arterial plasma (Innis et al., 2007), which reflects binding of [^{11}C]OMAR to CB_1 receptors plus nondisplaceable binding. The multilinear analysis method used here was found to yield V_T values in excellent agreement with full compartmental modeling (Normandin et al., 2010a). The cutoff time of $t^* = 30$ minutes was

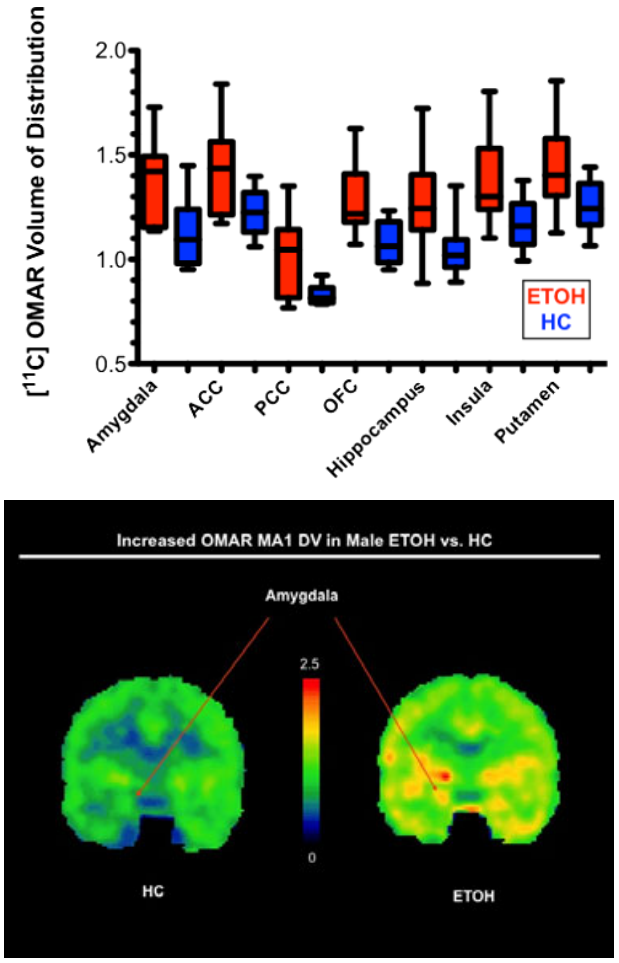

Fig. 1. Upper panel: box plots showing significantly different [^{11}C] OMAR volume of distribution (V_T) values between alcohol-dependent patients (ethanol [EtOH]) and healthy control subjects (HC) in individual regions of interest constituting a circuit that has been implicated in the etiology of alcohol dependence. ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; OFC, orbitofrontal cortex. Lower panel: [^{11}C]OMAR positron emission tomography images (coronal view) illustrate elevated amygdala [^{11}C]OMAR binding in a single patient with alcohol dependence (right) relative to an HC subject (left).

the shortest that adequately fit data from all regions and exhibited the best test–retest reproducibility in an initial cohort of healthy subjects (Normandin et al., 2010b). Because no region without specific binding has been found for [^{11}C]OMAR, binding potential cannot be determined.

Statistical Analysis

Independent sample *t*-tests were used to compare continuous clinical and demographic variables between AD and HC. Data were normally distributed as determined by visual inspection and the Kolmogorov–Smirnov *D* test. Chi-square test was used in the case of dichotomous variables. [^{11}C]OMAR V_T values were subjected to a repeated measures analysis of variance (rmANOVA) with group as a between-subject factor and brain region as a within-subject factor. Significant results for the omnibus test were followed up with post hoc analyses using ANOVA with regional [^{11}C]OMAR V_T as dependent variable and group as predictor variable to determine significant differences between groups in individual ROIs. Tests of association between continuous variables were performed using Pearson's product–moment correlations. All tests were performed 2-tailed, with results considered significant at $p < 0.05$. Means and standard deviations are reported. All statistical analyses were conducted using SPSS version 16.0 (SPSS Inc., Chicago, IL).

Table 1. Study Participant Demographic, Clinical, and Positron Emission Tomography Procedural Characteristics

	Alcohol-dependent ($N = 8$)	Healthy control ($N = 8$)	*p*-Value
Demographics			
Age, years	37.4 ± 7.9	32.5 ± 6.9	0.20
Age range, years	25 to 49	24 to 45	–
Race/ethnicity	3C, 5AA, 0H	4C, 2AA, 2H	–
BMI	26.0 ± 4.0	26.4 ± 2.7	0.83
Smoking status (Y/N)	5/3	0/8	0.007
Age at first drink, years	15.8 ± 2.4	21.9 ± 3.7	0.002
Injection parameters			
Injected dose (MBQ)	669 ± 15.4	633 ± 96	0.33
Specific activity (MBQ/nmol)	107 ± 44.3	125 ± 59.3	0.52
Injected mass (μg)	3.82 ± 2.40	3.27 ± 2.22	0.64

Data presented in mean ± standard deviation, unless otherwise indicated. *p*-Values determined by independent sample *t*-tests for continuous variables or by chi-square test for dichotomous variables.

AA, African–American; BMI, body mass index; C, Caucasian; H, Hispanic.

RESULTS

Demographics and Clinical Characteristics

Participants in the AD and HC groups were matched for age and BMI; smoking frequency was greater in the AD compared to HC group. As expected, patients with AD relative to HC had a significantly earlier age at their first drink (15.8 ± 2.4 vs. 21.9 ± 3.7 years) and had a mean illness duration of 16.6 ± 9.0 years. Participants in the AD group experienced low levels of alcohol withdrawal at the time of the PET scan as indicated by a mean CIWA-Ar score of 4.6 ± 4.2 (Table 1).

Neuroreceptor Imaging

[^{11}C]OMAR V_T values within the circuit were significantly higher in the AD group compared to the HC group (main effect of group, $F = 6.56$; df = 1,14; $p = 0.023$). Post hoc testing revealed elevated [^{11}C]OMAR V_T values in the a priori selected ROIs in patients with AD compared to the HC group (Fig. 1, upper panel; Table 2), which is evident also in an example comparing amygdala [^{11}C]OMAR V_T in a patient with AD relative to an HC subject (Fig. 1, lower panel). Including smoking status as a covariate in the rmANOVA did not influence the outcome. Exploratory analyses in other regions outside of the circuit including caudate, pallidum, parietal cortex, or thalamus did not yield any significant between-group differences.

Table 2. Regional [^{11}C]OMAR Volumes of Distribution (V_T) Values in Men with Alcohol Dependence and Healthy Control Subjects

Brain region	Alcohol dependence (n = 8)	Healthy control (n = 8)	Mean difference	Percentage of difference	Post hoc analysis of variance (ANOVA)
Amygdala	1.37 ± 0.21	1.13 ± 0.17	0.24	21.2	$F(1, 14) = 6.78$, $p = 0.021$
ACC	1.44 ± 0.23	1.23 ± 0.12	0.21	17.1	$F(1, 14) = 5.50$, $p = 0.034$
PCC	1.02 ± 0.20	0.83 ± 0.05	0.19	22.9	$F(1, 14) = 6.95$, $p = 0.020$
OFC	1.28 ± 0.18	1.07 ± 0.11	0.21	19.6	$F(1, 14) = 7.66$, $p = 0.015$
Insula	1.27 ± 0.24	1.05 ± 0.14	0.22	20.1	$F(1, 14) = 5.40$, $p = 0.036$
Hippocampus	1.38 ± 0.22	1.17 ± 0.13	0.21	17.9	$F(1, 14) = 5.07$, $p = 0.041$
Putamen	1.45 ± 0.22	1.25 ± 0.13	0.20	16	$F(1, 14) = 4.71$, $p = 0.048$

Mean ± standard deviation [^{11}C]OMAR V_T values in 7 a priori regions of interest in alcohol-dependent compared to healthy control subjects. Statistics in table represents between-subject post hoc ANOVA results with regional [^{11}C]OMAR V_T as dependent variable and group as predictor variable.
ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; OFC, orbitofrontal cortex.

There were no associations between [^{11}C]OMAR V_T values and age or BMI in either group. There were no associations between [^{11}C]OMAR V_T values and CIWA-Ar score, age at first drink, or illness duration in the AD group.

DISCUSSION

Herein, we demonstrate for the first time in vivo elevated V_T values of [^{11}C]OMAR in chronic patients with AD during early abstinence relative to matched HC subjects. Age at onset of drinking, number of drinks, or illness duration did not correlate with [^{11}C]OMAR V_T. We interpret these findings as reflecting elevations in CB_1 receptor density in patients with AD.

Regional V_T values showed good agreement with the known density and distribution of CB_1 receptors in human autoradiography studies (Glass et al., 1997; Herkenham et al., 1990). Relatively lower hippocampus [^{11}C]OMAR V_T values despite highest [^{3}H]CP55,940 binding shown in the autoradiography studies were also reported by other groups in their in vivo PET studies using different CB_1 receptor-specific radioligands (Burns et al., 2007; Terry et al., 2010, 2009; Van Laere et al., 2008; Wong et al., 2010) and could be explained by the limited availability of the hippocampus CB_1 receptor for in vivo binding as suggested by data showing that approximately 85% of these receptors are located in intracellular vesicles (Burns et al., 2007; Coutts et al., 2001).

AD is characterized by the episodes of alcohol deprivation alternating with the episodes of increased alcohol intake. Therefore, the timing of the PET studies in relation to alcohol consumption and deprivation is important to consider. Consistent with the alcohol deprivation phase in animals exposed to chronic alcohol intake, which is associated with an up-regulation of CB_1 receptor-mediated G-protein signaling in response to reduced eCB levels (Vinod et al., 2006), we found elevated [^{11}C]OMAR V_T values in AD subjects, indicating a possible up-regulation of CB_1 receptors. Similarly to animal studies, CB_1 receptor up-regulation could be associated with enhanced CB_1 receptor-mediated G-protein signaling. We recently showed decreased serum anandamide levels in patients with AD (Mangieri et al., 2009), and although no significant correlation exists between anandamide concentrations in cerebrospinal fluid and serum (Koethe et al., 2009), it can be speculated that the CB_1 receptor up-regulation in AD is a compensatory mechanism for a decrease in eCB tone during early abstinence. This change may represent a vulnerability factor for the development of craving and relapse. It is unclear, however, whether these alterations are a state marker of early abstinence or represent a trait characteristic for AD. Animal models would suggest that we describe herein a state marker for early abstinence because CB_1 receptor function (Ortiz et al., 2004) as well as expression (Basavarajappa and Hungund, 2005; Basavarajappa et al., 1998) were reduced immediately after chronic alcohol intake. Future PET studies in actively drinking patients with AD will answer this important question.

Our study does not answer if elevated CB_1 receptors are a preexisting vulnerability factor for AD per se or result from alterations in other neurochemical systems that have been linked to the etiology of AD. For example, interactive effects between reduced striatal dopamine 2 and elevated CB_1 receptor availability were reported recently in animal models of AD (Thanos et al., 2011) and were substantiated with our human in vivo data showing elevated binding in the putamen suggesting complex system interactions moderating the reinforcing and addictive effects of alcohol in AD. Future longitudinal PET studies need to validate these animal models clarifying whether the changes of CB_1 receptor-mediated G-protein signaling change in different phases of alcohol intake and abstinence as well as whether altered CB_1 receptor expression in AD represents a change per se in the presence and absence of alterations in other neurochemical systems linked to AD.

In addition, it needs to be determined whether women with AD show similar changes in CB_1 receptor binding, importantly because dopamine and CB_1 receptor interactions have only been studied in male mice to date (Thanos et al., 2011) and sex-dependent differences in eCB and dopaminergic functions do not allow to necessarily generalize our results for both sexes.

In conclusion, we report for the first time elevated CB_1 receptor binding in AD. Chronic alcohol use is seen as persistent challenges to the organism, leading to an altered set point across multiple systems. This hypothesis is consis-

tent with the evidence of adaptations in brain reward and stress circuits that could contribute to important aspects of addictive processes like craving or urges, decreases in self-control, and a compulsive engagement in unhealthy behaviors, all of which characterize patients with AD. Our study directly implicates the CB_1 receptor in AD and suggests a mechanism for novel treatment development for AD (Bailey and Neumeister, 2011).

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