Reduced cannabinoid CB₁ receptor binding in alcohol dependence measured with positron emission tomography

J Hirvonen¹, P Zanotti-Fregonara¹,²,³, JC Umhau²,³, DT George², D Rallis-Frutos¹, CH Lyoo¹, C-T Li¹, CS Hines¹, H Sun², GE Terry¹, C Morse¹, SS Zoghbi¹, VW Pike¹, RB Innis¹,³ and M Heilig²,³

Brain cannabinoid CB₁ receptors contribute to alcohol-related behaviors in experimental animals, but their potential role in humans with alcohol dependence is poorly understood. We measured CB₁ receptors in alcohol dependent patients in early and protracted abstinence, and in comparison with control subjects without alcohol use disorders, using positron emission tomography and [¹⁸F]FMPEP-d₂, a radioligand for CB₁ receptors. We scanned 18 male in-patients with alcohol dependence twice, within 3–7 days of admission from ongoing drinking, and after 2–4 weeks of supervised abstinence. Imaging data were compared with those from 19 age-matched healthy male control subjects. Data were also analyzed for potential influence of a common functional variation (rs2023239) in the CB₁ receptor gene (CNR1) that may moderate CB₁ receptor density. On the first scan, CB₁ receptor binding was 20–30% lower in patients with alcohol dependence than in control subjects in all brain regions and was negatively correlated with years of alcohol abuse. After 2–4 weeks of abstinence, CB₁ receptor binding remained similarly reduced in these patients. Irrespective of the diagnostic status, C allele carriers at rs2023239 had higher CB₁ receptor binding compared with non-carriers. Alcohol dependence is associated with a widespread reduction of cannabinoid CB₁ receptors, binding in the human brain and this reduction persists at least 2–4 weeks into abstinence. The correlation of reduced binding with years of alcohol abuse suggests an involvement of CB₁ receptors in alcohol dependence in humans.

Molecular Psychiatry (2013) 18, 916–921; doi:10.1038/mp.2012.100; published online 10 July 2012

Keywords: alcohol dependence; cannabinoid CB₁ receptor; endocannabinoid; imaging; positron emission tomography

INTRODUCTION

Animal studies suggest that the brain cannabinoid system contributes to alcohol-related behaviors.¹–³ This system consists of endogenous cannabinoids, such as anandamide and 2-arachidonoylglycerol, enzymes responsible for their synthesis and catabolism and cannabinoid receptors of the CB₁ and CB₂ subtypes. The CB₁ receptor is widely distributed in the human brain, with highest densities in the basal ganglia, hippocampus, cingulate cortex and molecular layer of cerebellum.⁴,⁵ Most CB₁ receptors are pre-synaptic, and inhibit release of other neurotransmitters, such as γ-aminobutyric acid and glutamate.⁶,⁷

Animal studies suggest that reinforcing properties of alcohol are in part mediated through the endocannabinoid system. Stimulation of CB₁ receptors increases alcohol intake across a variety of rodent models,⁸ whereas the opposite is observed with genetic⁹–¹¹ or pharmacological¹²–¹⁴ CB₁ receptor blockade. Chronic alcohol exposure increases the concentration of endogenous cannabinoids in most brain regions⁵ and decreases the density of CB₁ receptors,¹⁵–¹⁷ a change that is reversible upon abstinence.¹⁶,¹⁷ Accordingly, a postmortem study recently found decreased CB₁ receptor density in the ventral striatum of patients with alcohol dependence.¹⁸ Whether cannabinoid CB₁ receptors are decreased in vivo in human subjects with alcohol dependence is unknown.

Functional genetic variation that moderates endocannabinoid signaling in response to alcohol might moderate susceptibility for excessive alcohol consumption. Accordingly, a common single-nucleotide polymorphism (SNP), rs2023239, in the gene encoding CB₁ receptors (CNR1) tags a haplotype associated with substance use disorders including alcohol dependence.¹⁹ A recent study of patients with alcohol dependence linked the rs2023239 C allele with greater subjective reward from alcohol, greater midbrain and prefrontal cortex activation in response to alcohol cues, and higher density of CB₁ receptors in postmortem samples of prefrontal cortex.²⁰ Whether this SNP also moderates CB₁ receptor density in vivo is unknown.

Here, we evaluated CB₁ receptor binding in patients with alcohol dependence and controls without alcohol use disorders. We used positron emission tomography (PET) and a recently developed inverse agonist radioligand for CB₁ receptors, [¹⁸F]FMPEP-d₂,²¹,²² with high affinity and selectivity in the human brain.²¹ Patients were scanned twice: within 3–7 days of admission from ongoing alcohol use, and after 2–4 weeks of abstinence on a monitored unit. Based on animal experiments,⁸ we hypothesized that CB₁ receptor binding would be decreased in patients with alcohol dependence immediately after ongoing alcohol use. Based on human postmortem data,²⁰ we predicted that the C allele of rs2023239 would be associated with higher CB₁ receptor binding. Finally, we asked whether a hypothesized decrease in CB₁ receptor binding in patients with alcohol dependence recovers after abstinence.

SUBJECTS AND METHODS

The NIH CNS Institutional Review Board approved the protocol and the consent forms. Written informed consent was obtained from all subjects.

¹Molecular Imaging Branch, National Institute of Mental Health, NIH, Bethesda, MD, USA and ²Laboratory of Clinical and Translational Studies, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD, USA. Correspondence: Dr M Heilig, Laboratory of Clinical and Translational Studies, National Institute on Alcohol Abuse and Alcoholism, Building 10, Room 1E-5334, 10 Center Drive, Bethesda, MD 20892-1108, USA. E-mail: markus.heilig@mail.nih.gov or markus.heilig@nih.gov
²These authors contributed equally to this work.
Subjects
Detailed eligibility criteria can be found at clinicaltrials.gov (NCT00816439). Patients (N = 18) were males with alcohol dependence according to DSM-IV, established by structured diagnostic interviews (SCID), and alcohol consumption within the last month (Table 1). Subjects did not have other substance use disorders except nicotine dependence, as determined by the SCID. Urine samples on admission were negative for cannabinoids, opiates, amphetamines, cocaine metabolites and benzodiazepines. Patients were admitted to an in-patient unit at the NIH Clinical Center, and assessed for severity of alcohol dependence (Alcohol Dependence Scale), alcohol use in the previous 90 days (Time-Line Follow Back), alcohol craving (Penn Alcohol Craving Scale) and symptoms of depression and anxiety (Comprehensive Psychopathology Rating Scale). Withdrawal intensity was assessed with the Clinical Institute Withdrawal Assessment Scale. A total of 10 (56%) patients received oxazepam to relieve withdrawal symptoms. The cumulative oxazepam dose (mean: 273 mg, range: 30–540 mg) was used to control for possible medication effects. In all, 13 of the patients (72%) smoked cigarettes. Throughout the study, all subjects participated in a standard behavioral in-patient alcohol rehabilitation program, but did not receive any prescription medications other than benzodiazepines as described above and vitamin B1 (thiamine) supplementation according to clinical guidelines.

Healthy male controls (N = 19) were free of somatic and psychiatric illness as confirmed by history, physical examination, structured diagnostic interviews (SCID, full version) electrocardiogram and blood and urine tests. Control subjects did not have current or lifetime history of alcohol use or other substance use disorders, and had urine samples negative for cannabinoids, opiates, amphetamines, cocaine metabolites and benzodiazepines. Control subjects completed assessments of alcohol use and severity of alcohol dependence as described for the patients. None of the controls were total abstainers, but 7 out of 19 (37%) reported no alcohol use in the past 90 days. Two control subjects (11%) smoked cigarettes (Table 1).

Genotyping
Genotyping for CNR1 rs202039 was done largely as described previously and in detail in Supplementary Methods online.

Positron emission tomography and measurement of parent radioligand in arterial plasma
\[^{18}\text{F}\]FMPEP-d\(_2\), was prepared as described previously and in detail in our Investigational New Drug Application 105198, submitted to the US Food and Drug Administration (available at http://pdsp.med.unc.edu/snidd/). The radioligand was obtained in high radiochemical purity (>-99%) and had a specific radioactivity of 110 ± 43 MBq mmol\(^{-1}\) at time of injection. After intravenous injection of \[^{18}\text{F}\]FMPEP-d\(_2\) (Table 1), images were acquired for 120 min using an Advance camera (GE Healthcare, Milwaukee, WI, USA) as described previously. Arterial blood samples were drawn as previously described. Plasma time-activity curve was corrected for the fraction of unchanged radioligand by radio-high-performance liquid chromatography separation, and the plasma-free fraction was measured using ultrafiltration.

PET images were analyzed by applying a template of volumes of interest as implemented in PMOD, version 3.0 (PMOD Technologies, Zurich, Switzerland), in the standard stereotaxic space as previously described. Distribution volume (\(V_T\)) was estimated according to the two-tissue compartmental model with concentration of parent radioligand in plasma as input function, using PMOD, as described previously. To assess the potential confound of brain atrophy, PET images were corrected for partial volume effects using the Rousset method implemented in the PVElab software. Statistical parametric mapping of \(V_T\) values at voxel level was done using SPM8 as described previously.

Statistical analysis of \(V_T\) data
Data were analyzed using the SPSS Statistics 17.0, for Windows (Chicago, IL, USA; Release 17.0.0, copyright SPSS, 1993–2007). \(V_T\) values were normally distributed except for 4 out of 18 brain regions in the patient group (Shapiro–Wilk test). Therefore, results were confirmed with non-parametric Mann–Whitney U tests. Variance was homogeneous across groups (Levene’s test). To test whether CB1 receptors were decreased in patients with alcohol dependence at baseline, we first applied a mixed model two-way analysis of variance, with group status (alcohol dependence vs control) as between-subject factor and brain region as within-subject factor. Body mass index entered the model as a covariate as it affects \(V_T\). To assess the contribution of the CNR1 rs2022339 SNP, this factor was then entered into the model. Correlations with clinical variables were assessed with Pearson’s correlation coefficient. To test whether CB1 receptors increased after abstinence, we applied a two-way analysis of variance with time point (early vs protracted abstinence) and brain region as within-subject factors. Potential effects of genotype were also evaluated with time × genotype interaction. P-values <0.05 were considered statistically significant.

RESULTS
Overall, \(V_T\) of \[^{18}\text{F}\]FMPEP-d\(_2\) was lower in patients with alcohol dependence than in control subjects (main effect of group: \(F(1,34) = 10.1, P = 0.003\)). A decrease was seen in all brain regions examined, but its magnitude varied significantly between regions (group × region interaction: \(F(3,103) = 4.21, P = 0.007\); Figure 1); decrease in body mass index-adjusted \(V_T\) ranged from 21% in hippocampus to 35% in white matter. This finding was confirmed by an independent statistical parametric mapping analysis of voxel-wise \(V_T\) values (Figure 2) and a non-parametric Mann–Whitney U test (all \(P<0.019\)). The main effect of diagnostic status was also significant when analyzed without correcting for body mass index \((F(1,35) = 9.36, P = 0.004\)). Among patients with alcohol dependence, years of alcohol use correlated negatively with \(V_T\) in midbrain \((R^2 = 0.28, P = 0.023\), posterior cingulate cortex \((R^2 = 0.37, P = 0.008\); Supplementary Figure 1) and putamen \((R^2 = 0.26, P = 0.030\); patients who had been drinking longer had lower \(V_T\) than those who had been drinking for a shorter period. This analysis was not confounded by age, as age did not correlate with \(V_T\) in control subjects. Whole-brain \(V_T\) did not correlate with alcohol dependence severity (Alcohol Dependence Severity \((R^2 = 0.02, P = 0.56\), measures of craving \((R^2 = 0.08, P = 0.26\), average drinks per day \((R^2 = 0.01, P = 0.97\), peak withdrawal score \((R^2 = 0.16, P = 0.10\), benzodiazepine dose received \((R^2 = 0.05, P = 0.38\) or ratings of depression \((R^2 = 0.01, P = 0.92\) or anxiety \((R^2 = 0.01, P = 0.90\).

Table 1. Demographic, clinical and radiochemical information of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Alcohol</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>18</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 ± 10</td>
<td>40 ± 8</td>
<td>0.251</td>
</tr>
<tr>
<td>Body mass index (kg m(^{-2}))</td>
<td>27 ± 4</td>
<td>28 ± 5</td>
<td>0.738</td>
</tr>
<tr>
<td>Tobacco smokers/non-smokers (N)</td>
<td>11/7</td>
<td>2/17</td>
<td>0.001</td>
</tr>
<tr>
<td>Amount of alcohol use (drinks per day, TLFB)</td>
<td>18 ± 6</td>
<td>&lt;1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol Dependence Severity (ADS)</td>
<td>20 ± 6</td>
<td>&lt;1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline craving (PACS)</td>
<td>12 ± 8</td>
<td>1 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline peak withdrawal (CIWA)</td>
<td>9 ± 5</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>Injected activity of [^{18}\text{F}]FMPEP-d(_2)</td>
<td>187 ± 10</td>
<td>181 ± 12</td>
<td>0.103</td>
</tr>
<tr>
<td>Injected amount of [^{18}\text{F}]FMPEP-d(_2) (MBq)</td>
<td>1.7 ± 0.9</td>
<td>1.9 ± 0.8</td>
<td>0.547</td>
</tr>
<tr>
<td>Fraction of free [^{18}\text{F}]FMPEP-d(_2) in plasma (%)</td>
<td>0.38 ± 0.1</td>
<td>0.39 ± 0.2</td>
<td>0.886</td>
</tr>
</tbody>
</table>

Abbreviations: ADS, Alcohol Dependence Scale; CIWA, Clinical Institute Withdrawal Assessment Scale; N.A., not applicable; PACS, Penn Alcohol Craving Scale; TLFB, Time-Line Follow Back.

Values are number or mean ± s.d.
CB₁ receptor downregulation in alcohol dependence
J Hirvonen et al

Among control subjects and patients, 28 and 39% carried the rs2023239 C allele, respectively ($\chi^2 = 0.50; P = 0.48$). When we included genotype as a factor in the analysis, the main effect of group remained ($F(1, 31) = 7.72, P = 0.009$), and a main genotype effect was found ($F(1, 31) = 4.44, P = 0.043$). There was no significant group × genotype ($F(1, 31) = 1.47, P = 0.24$) or group × genotype × region ($F(3, 96) = 2.00, P = 0.12$) interaction, suggesting that genotype affected CB₁ binding similarly in both groups. Carriers of the C allele had 31% higher $V_T$ than non-carriers among patients with alcohol dependence and 19% among control subjects (Figure 3). There were no statistically significant differences between carriers and non-carriers of the C allele in any of the demographic, or in the clinical variables among patients with alcohol dependence.

More patients with alcohol dependence (61%) than control subjects (11%) smoked cigarettes. However, smoking had no effect on $V_T$ among patients with alcohol dependence (main effect: $F(1, 33) = 0.01, P = 0.93$; smoking × region interaction: $F(3, 99) = 0.34, P = 0.80$). Furthermore, lower $V_T$ in patients with alcohol dependence than in control subjects remained significant even after smoking was included in the statistical model (main effect of group: $F(1, 33) = 7.32, P = 0.011$). Therefore, cigarette smoking was unlikely to significantly confound the main finding.

To control for a potential confound of brain atrophy in the patient group, we corrected the PET data for partial volume effects. After correction, group difference in $V_T$ remained significant (main effect of group: $F(1, 34) = 8.42, P = 0.006$; group × region interaction: $F(3, 99) = 3.79, P = 0.011$).

Finally, to determine whether decreased $V_T$ in patients with alcohol dependence is reversible, we repeated PET measurements after 19 ± 4 days of abstinence (range 14–28 days). After protracted abstinence, $V_T$ did not significantly change in any brain region (main effect of time: $F(1, 17) = 1.18, P = 0.29$; time × region interaction: $F(3, 49) = 0.71, P = 0.55$), and thus, remained significantly decreased (Figure 4). The average value of whole-brain $V_T$ increased by only $+5\%$. Change after abstinence remained not significant after controlling for rs2023239 genotype (main effect of time: $F(1, 16) = 0.83, P = 0.38$; time × genotype interaction: $F(1, 16) = 0.34, P = 0.57$). Change in whole-brain $V_T$ correlated positively with baseline Alcohol Dependence Score ($R^2 = 0.27, P = 0.027$; Supplementary Figure 2), but not with other clinical variables at baseline, with change in clinical variables after abstinence, or with interval between two PET scans. Fraction of free radioligand in arterial plasma did not change after abstinence ($P = 0.75$).
DISCUSSION

When evaluated within 1 week of admission from ongoing alcohol use, patients with alcohol dependence had 20–30% lower $V_1$ of $\text{[18F]FMPEP-d}_2$ in all brain regions compared with control subjects without substance use or other psychiatric disorders, consistent with a widespread downregulation of CB$_1$ receptors. This downregulation was likely related to long-term alcohol exposure, because it was more pronounced in patients who had used alcohol longer than in those who had done so for a shorter time, while no effect of age per se was observed in controls. $V_1$ of $\text{[18F]FMPEP-d}_2$ remained similarly lowered in the alcohol dependent patients after 2–4 weeks of abstinence. This lack of reversibility is in contrast with the reversible downregulation of cortical CB$_1$ receptors recently found in subjects who chronically abuse cannabis, and suggests a more persistent neuroadaptive change in CB$_1$ receptors in alcohol dependence. We also demonstrated a significant effect of genetic variation at rs2023239, a CNR1 SNP previously associated with risk for substance use disorders including alcohol dependence, with brain responses to alcohol associated cues in functional imaging, and with cortical CB$_1$ receptor density on postmortem analysis.

Consistent with the postmortem binding data, carriers of the C allele in our study had higher $V_1$ of $\text{[18F]FMPEP-d}_2$ than non-carriers in vivo, providing additional support for the validity of our data.

Decreased $V_1$ of $\text{[18F]FMPEP-d}_2$ in patients with alcohol dependence was unlikely caused by methodological confounds. First, potential alterations in peripheral distribution or metabolism of the radioligand in patients with alcohol dependence did not affect our results, because the outcome measure $V_1$ corrects for these effects. Second, only free radioligand (not bound to plasma proteins) is able to enter the brain, while the fraction of free radioligand was similar between the groups (Table 1). Third, about 90% of $\text{[18F]FMPEP-d}_2$ $V_1$ in monkey brain represents specific binding to CB$_1$ receptors, therefore, changes in nonspecific binding (for example, to brain lipids) would be unlikely to confound the main finding. Fourth, brain atrophy in patients with alcohol dependence may have ‘diluted’ the PET signal via partial volume effects. However, the group differences remained similar even after correction for partial volume effect based on structural images. Fifth, cigarette smoking was more prevalent among patients with alcohol dependence than among control subjects. Yet, the main finding persisted after controlling for smoking in the statistical model, and $V_1$ was not different between smoking and nonsmoking patients. This is in agreement with our recent observation that cigarette smoking did not influence $\text{[18F]FMPEP-d}_2$ $V_1$ in people who chronically abuse cannabis. Sixth, a lack of correlation between withdrawal intensity, benzodiazepine dose and $V_1$ of $\text{[18F]FMPEP-d}_2$, as well as the persistence of the findings 2–4 weeks after admission makes a confound by these factors unlikely.

Finally, we considered whether differential displacement of the radioligand $\text{[18F]FMPEP-d}_2$ by endogenous cannabinoids in patients compared with control subjects could confound our results. As some PET radioligands can be displaced by endogenous neurotransmitters, we have previously tested the effects of cannabinoid agonists on the binding of $\text{[11C]MePPEP}$, a closely related analog, in rat brain. In that study, we used high doses of cannabinoid agonists (anandamide, methanandamide or CP 55,940) or an inhibitor of fatty acid amide hydrolase, the main enzyme that breaks down endocannabinoids. Although rimonabant, an inverse CB$_1$ agonist, potently displaced $\text{[11C]MePPEP}$, none of the direct agonists did so. Importantly, potentiation of endogenous cannabinoid transmission by fatty acid amide hydrolase was also without effect. These findings suggest that $\text{[11C]MePPEP}$, a close structural analog of $\text{[18F]FMPEP-d}_2$ used in this study, is not displaced by direct CB$_1$ agonists, including the endogenous ligand anandamide. Based on these observations, we believe it unlikely that differential displacement of our PET ligand by endocannabinoids accounts for the differences observed between patients and controls in this study. Nevertheless, additional studies would be required to directly determine whether $\text{[18F]FMPEP-d}_2$ can be displaced by endogenous (and exogenous) CB$_1$ receptor agonists in humans.

Animal studies utilizing genetic inactivation as well as pharmacological blockade of CB$_1$ receptors have consistently shown that reinforcement of alcohol consumption is in part mediated by endocannabinoids acting at this receptor. Accordingly, chronic alcohol exposure has been shown to result in elevated tissue levels of endocannabinoids, while more recent microdialysis experiments have directly shown that self-administration of alcohol is associated with increased central levels of extracellular endocannabinoids, and that this increase is correlated with the amount of alcohol consumed. Consistent with increased endocannabinoid activity resulting from alcohol consumption, most animal studies have found downregulation of CB$_1$ receptors in several brain regions after chronic alcohol exposure (although see Gonzalez et al. for a negative finding). One postmortem human study also found decreased CB$_1$ receptor protein in the ventral striatum of alcoholics. Our present finding that long-term alcohol exposure in alcohol dependence is associated with downregulation of CB$_1$ receptors in vivo thus represents a direct translation of the animal data. Our findings
dive from the animal studies in that those found evidence of reversibility after 1 \textsuperscript{16} and 40 days. A likely reason for this divergence is the difference in duration of alcohol exposure, which was 3–60 days in the animal studies and 5–50 years in our patients. Our finding of downregulated CB\textsubscript{1} receptors in alcohol dependence is consistent with a conceptualization of this condition as an allostatic process.\textsuperscript{41,42} According to this framework, alcohol is initially consumed for its pleasurable, positively reinforcing properties, but neuroadaptions to continued heavy alcohol use gradually reduce these alcohol actions. Because of the established role of CB\textsubscript{1} receptors in positive reinforcement from alcohol, their downregulation with prolonged heavy alcohol use is a plausible candidate mechanism that might contribute to this process. Although the positively reinforcing effects of alcohol are gradually lost with time, brain stress systems become overactive and alcohol use becomes pursued in large part to ameliorate negative emotionality and promote stress coping.\textsuperscript{43} Animal studies have shown that CB\textsubscript{1} receptors have a critical role in modulating stress responses through actions in basolateral amygdala and hippocampus.\textsuperscript{33,44} Downregulation of CB\textsubscript{1} receptors in these structures in later stages of alcohol dependence can therefore be expected to contribute to enhanced negative emotionality and impaired stress coping. This in turn would provide an incentive for resumption of alcohol intake to restore endocannabinoid signaling and its ability to promote stress coping and normal stress response. In this context, we note that the CB\textsubscript{1} receptor inverse agonist rimonabant was not effective when evaluated in chronic alcohol dependence.\textsuperscript{45,46} Several factors may have contributed to these negative results, but one possibility is that a blockade by rimonabant of positively reinforcing properties of alcohol was in part offset by an increased incentive for consumption to restore affective homeostasis. If this interpretation is correct, augmenting rather than inhibiting endogenous cannabinoid function (for example, by inhibiting their metabolism\textsuperscript{47}) might be of interest for treatment of alcohol dependence. First, augmented endocannabinoid function might restore affective homeostasis in the absence of alcohol, thereby reducing or eliminating the incentive to consume alcohol for its negatively reinforcing properties. Second, and more generally, CB\textsubscript{1} receptor stimulation reduces glutamate release, and is therefore neuroprotective.\textsuperscript{48} Chronic alcohol dependence is characterized by a hyperglutamatergic state\textsuperscript{48} and CB\textsubscript{1} receptor stimulation protects against glutamatergic excitotoxic cell death during alcohol withdrawal.\textsuperscript{49}

In a direct \textit{in vivo} correlate of postmortem ligand-binding data,\textsuperscript{20} we found higher CB\textsubscript{1} ligand binding in carriers of the C allele at the rs2022329 CNR1 SNP, irrespective of diagnostic group. Genetic association data suggest a small but significant contribution of this allele to the risk for substance use disorders, including alcohol dependence.\textsuperscript{13} This risk increase may be mechanistically accounted for by the observations that carriers of the C allele exhibit greater brain responses to alcohol associated cues and greater alcohol-induced reward, presumably in part via higher CB\textsubscript{1} receptor density.\textsuperscript{45} As indicated above, the positive reinforcing properties of alcohol are likely most important in early stages of alcohol dependence, and enhanced reward from alcohol due to a gain-of-function mutation at the CNR1 locus would be expected to increase alcoholism risk. In later stages of alcohol dependence, alcohol reward becomes less important, stress systems are activated and restored CB\textsubscript{1} receptor function may be beneficial by counteracting negative emotionality and glutamate excitotoxicity.

In conclusion, we found that CB\textsubscript{1} receptor binding is decreased in patients with alcohol dependence and that this downregulation persists several weeks into abstinence. We also found that the C allele of the rs2022329 locus is associated with higher CB\textsubscript{1} receptor binding in vivo, similar to what has been found on postmortem analysis. Our findings suggest that CB\textsubscript{1} receptor has a different role in early vs late phases of alcohol dependence. A potential implication of our findings is that enhanced, rather than blocked CB\textsubscript{1} signaling may be beneficial in late stage, treatment seeking alcohol dependent patients.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

We thank Kimberly Jenko, Kacey Anderson and David Clark for assisting in the measurements of radioisotopic plasma; Maria D Ferraris Araneta, Gerald Hodges, William C Kreisel and Barbara Scupera as well as Chris Geyer and the NIAAA nursing staff for subject recruitment and care; the NIH PET Department for imaging; and the PMOD Technologies for providing its image analysis and modeling software. This research was supported by the Intramural Programs of NIMH and NIAAA. Jussi Hirvonen was supported by personal grants from The Academy of Finland; The Finnish Cultural Foundation; The Finnish Foundation for Alcohol Studies; The Finnish Medical Foundation; The Instrumentarium Foundation; The Jalmari and Rauha Ahokas Foundation; The Paulo Foundation; The Research Foundation of Orion Corporation; and The Yrjö Jahnsson Foundation.

**REFERENCES**


CB1 receptor downregulation in alcohol dependence
J Hirvonen et al

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)