Published in final edited form as:

Biol Psychiatry. 2010 May 1; 67(9): 800–803. doi:10.1016/j.biopsych.2009.12.028.

Serotonin 1B Receptor Imaging in Alcohol Dependence

Jian Hu^{1,2}, Shannan Henry^{1,2}, Jean-Dominique Gallezot³, Jim Ropchan³, John F. Neumaier⁴, Marc N. Potenza^{2,5}, Rajita Sinha², John H. Krystal², Yiyun Huang³, Yu-Shin Ding³, Richard E. Carson³, and Alexander Neumeister^{1,2}

¹Molecular Imaging Program, Clinical Neurosciences Division, VA National Center for PTSD, VA Connecticut Healthcare System, West Haven, CT

²Department of Psychiatry, Yale University School of Medicine, New Haven, CT

³Positron Emission Tomography Center, Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, CT

⁴Department of Psychiatry, University of Washington, Seattle, WA

⁵Child Study Center, Yale University School of Medicine, New Haven, CT

Abstract

Background—Although animal models suggest that alcohol dependence (AD) is associated with elevations in the number of serotonin-1B receptors (5HT_{1B}R), 5HT_{1B}R levels have not been investigated in people with AD. The selective 5HT_{1B}R antagonist radioligand, [¹¹C]P943, permits in vivo assessment of central 5HT_{1B}R binding potential (BP_{ND}) using positron emission tomography (PET). Because of its central role in AD, we were particularly interested in ventral striatal 5HT_{1B}R BP_{ND} values.

Methods—Twelve medication-free, recently abstinent (at least 4 weeks) patients with AD (mean age 35.2±10.1 years, 5 women) and 12 healthy control subjects (HC) (mean age 30.6±9.2 years, 5 women) completed [¹¹C]P943 PET on a high resolution research tomograph (HRRT). Individual MRI scans were collected to exclude individuals with anatomical abnormalities and for co-registration. Imaging data were analyzed using a multilinear reference tissue model.

Results—Ventral striatal 5-HT_{1B}R BP_{ND} values (2.01±0.57 and 1.55±0.09, 29% between-group difference, p=.006) were increased in AD compared to HC subjects. No influence of demographic or clinical variables or amount of injected radiotracer was observed.

Conclusions—This study provides the first evidence that AD in humans, like in rodent models, is associated with increased levels of ventral striatal 5HT_{1B}Rs.

Keywords

alcohol dependence; human subjects; brain imaging; serotonin 1 B receptor; positron emission tomography

Corresponding Author: Alexander Neumeister, M.D., 950 Campbell Avenue, West Haven, Connecticut 06516, Tel: 203-932-5711 ext. 2428, Fax: 203-937-3481, alexander.neumeister@yale.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Disturbances in the regulation of brain serotonin (5HT) systems have been implicated diminished inhibitory control of behavior, including pathological alcohol use (1-3). However, the specific mechanisms through which 5HT systems are dysregulated in alcohol dependence (AD) remain unclear. For example, recent studies have yielded conflicting results regarding the regulation of 5HT transporter (4-6) and 5HT_{1A} receptor function (4) in AD. Growing evidence suggests that serotonin-1B receptors (5HT_{1B}Rs) modify the reinforcing, intoxicating, and discriminative stimulus effects of alcohol, and regulate its voluntary intake (7). The ventral striatum (VST) including globus pallidus and the nucleus accumbens area may be particularly important regions for the modulatory role of 5HT_{1B}Rs on alcohol-related behaviors (8). In rodents, increased expression of 5HT_{1B}R in the VST modulates drinking behavior, i.e. increases ethanol consumption and shifts the animal's preference for a stronger concentration of ethanol (7). In humans, polymorphisms in the 5HT_{1B}R gene have been linked to antisocial AD (9), although not unequivocally (10).

The purpose of the current study was to determine whether, as predicted by animal models, human AD would be associated with increased 5HT_{1B}R levels in the globus pallidus and the nucleus accumbens area. The development of the selective 5-HT_{1B}R radioligand, [¹¹C]P943, permitted *in vivo* assessment of central 5-HT_{1B}R binding during positron emission tomography (PET) imaging.

Methods

Subjects

Twelve alcohol dependent subjects (5F, mean age 35.2±10.1 years, range 22-48) meeting DSM-IV criteria for current AD, and 12 healthy control subjects (5F, mean age 30.6±9.2 years, range 19-44) were recruited through public advertisement (Table 1.). Individuals reporting abuse or dependence of any other substance other than alcohol or nicotine were excluded. 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. Written informed consent was obtained from all participants after full explanation of study procedures. All participants were evaluated by physical examination, electrocardiogram, standard laboratory tests, urine analysis and toxicology. Subjects with significant medical or neurological conditions, and with history of head injury with loss of consciousness were excluded from the study. AD subjects were admitted to the Clinical Neuroscience Research Unit (CNRU) at Connecticut Mental Health Center (CMHC) for detoxification and to ensure their medication-free status for at least 4 weeks before imaging. Urine toxicology and breathalyzer were collected repeatedly during the inpatient admission to the CNRU preceding the imaging studies, and on the days of the MRI and PET scans.

Scanning and Imaging Procedures

Subject preparation for the PET scan consisted of indwelling venous catheter placement. A transmission scan using a ¹³⁷Cs point source was obtained before the emission scan. The PET scans were acquired for 120 minutes at rest using a single intravenous injection of high-specific activity [¹¹C]P943, a selective 5HT_{1B} receptor antagonist radiotracer (11), on an HRRT PET scanner (207 slices, resolution less than 3 mm full-width at half-maximum in 3D acquisition mode). Dynamic scan data were reconstructed with corrections (attenuation, normalization, scatter, randoms, and deadtime). Motion correction of PET data was performed by coregistering each reconstructed frame to an early summed image (0-10 min

postinjection) using a 6-parameter mutual information algorithm and FMRIB's Linear Image Registration Tool (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK).

Magnetic resonance (MR) images were obtained for each subject on a Siemens 3 T Trio system to exclude individuals with anatomical abnormalities and for co-registration. A second summed image (0-10 min postinjection) was created from the motion-corrected PET data and registered to the subject's MR image, which, in turn, was registered (12-parameter affine transformation) to an MR template (MNI space). The globus pallidus/nucleus accumbens region of interest was taken from the template (Anatomical Automatic Labeling (AAL, (12) for SPM2) and applied to the PET data to produce time-activity curves for cerebellum. Pixel by pixel analysis was performed using the multilinear reference tissue model, MRTM2 (13) to produce images of binding potential (BP_{ND}) (14). The interpretation of BP_{ND} is $f_{ND} \cdot B_{avail}/K_d$ where f_{ND} is the tracer free fraction in a region without specific binding, B_{avail} is the unoccupied receptor concentration, and K_d is dissociation equilibrium constant of the tracer. Cerebellum was used as the reference region since it is practically devoid of 5HT_{1B} receptors (15). Assuming that there is no change in affinity or non-specific binding between subject groups, changes in BP_{ND} were interpreted as changes in receptor concentration. BP_{ND} values from MRTM2 have provided highly comparable results to those obtained with arterial input functions (16).

Statistical Analysis

Unpaired t-tests were used to compare clinical and demographic variables and a Mann-Whitney U test was used to compare 5HT_{1B} BP_{ND} values in the VST between AD and control groups. All tests were performed two-tailed, with results considered significant at $p < 0.05$. Data are presented as means $\pm$ SD.

Results

5HT_{1B} BP_{ND} was significantly elevated in the AD group relative to the healthy control group in the globus pallidus/nucleus accumbens area (2.01 ± 0.57 and 1.55 ± 0.09 , respectively; 29% between-group difference; $U = 119.0$, $z = 2.71$, $p = .006$) (Fig. 1), with effects found bilaterally (left hemisphere 35% between-group difference and right 24% between-group difference). None of the clinical measures or injection parameters correlated with 5HT_{1B} BP_{ND} in either group.

Discussion

The principal observation of this study was that ventral striatal 5HT_{1B}R BP_{ND} was increased in AD subjects who were scanned during early abstinence relative to a group of HC subjects. This increase was not directly related to features of the history of alcohol consumption among patients in this study. Our data do not clarify whether these differences are a pre-existing condition in AD patients or alternatively a consequence of the disorder.

The mechanism underlying the increase in ligand binding to 5HT_{1B}Rs is not evident from the current data. The increases in 5HT_{1B}R BP_{ND} might reflect an overexpression of 5HT_{1B}Rs or a higher affinity of these receptors. Alternatively, it is possible that low synaptic 5HT concentrations could leave more unoccupied 5HT_{1B}Rs for the radioligand, [¹¹C]P943 binding and ligands that are sensitive to endogenous 5HT may be developed in the future. In either case, alterations in 5HT_{1B}R function might contribute to AD by influencing 5HT input to the VST via their role as 5HT terminal autoreceptors, dopaminergic input to the striatum via the role of these receptors as heteroreceptors on GABA terminals within the ventral tegmental area, and glutamatergic activity within the VST via heteroreceptors on corticofugal projections (17). We acknowledge that PET

imaging cannot discriminate between pre- and postsynaptic receptors, and auto- and heteroreceptors and only provides a measure of the total number of receptors in a ROI. Emerging evidence, however, suggests that 5HT_{1B}Rs in the nucleus accumbens shell neurons specifically are involved in the drug rewarding processes of ethanol (7) and other drugs of abuse (18).

A limitation of the present study is the difference in nicotine use between groups. Given the high prevalence of nicotine use among AD patients, and in order to study a representative cohort of AD, we decided to accept smokers into the study. We acknowledge, however, that our sample size is currently too small to specifically address the issue of co-morbidity between smoking and AD, and therefore do not know whether it affects the outcome of the study.

Altogether, the presented preliminary data suggest an important role for ventral striatal 5HT_{1B}Rs in AD. Our data do not clarify, however, whether an overexpression of 5HT_{1B}Rs is a biomarker for AD specifically, or is associated with drug reward responses as well as increased vulnerability to substance use disorders in general (19). Future research will be needed to determine whether these alterations are pre-existing or a consequence of AD and whether 5HT_{1B}Rs might be productively targeted to treat AD.

Acknowledgments

The authors acknowledge the excellent work of the staff of the Yale PET Center and the nursing support from Sue Kasserman, R.N. for help in recruitment and patient care and Brenda Breault, R.N., B.S.N. for her contributions with patient care during the PET scans. In addition, we acknowledge the Yale-Pfizer Bioimaging Alliance for support in the development of [¹¹C]P943.

References

1. Nishikawa M, Diksic M, Sakai Y, Kumano H, Charney D, Palacios-Boix J, et al. Alterations in brain serotonin synthesis in male alcoholics measured using positron emission tomography. *Alcohol Clin Exp Res*. 2009; 33:233–239. [PubMed: 18976348]
2. Buydens-Branchey L, Branchey MH, Noumair D, Lieber CS. Age of alcoholism onset. II. Relationship to susceptibility to serotonin precursor availability. *Arch Gen Psychiatry*. 1989; 46:231–236. [PubMed: 2919952]
3. Virkkunen M, Linnoila M. Serotonin in early-onset alcoholism. *Recent Dev Alcohol*. 1997; 13:173–189. [PubMed: 9122495]
4. Martinez D, Slifstein M, Gil R, Hwang DR, Huang Y, Perez A, et al. Positron emission tomography imaging of the serotonin transporter and 5-HT(1A) receptor in alcohol dependence. *Biol Psychiatry*. 2009; 65:175–180. [PubMed: 18962444]
5. Brown AK, George DT, Fujita M, Liow JS, Ichise M, Hibbeln J, et al. PET [¹¹C]DASB imaging of serotonin transporters in patients with alcoholism. *Alcohol Clin Exp Res*. 2007; 31:28–32. [PubMed: 17207098]
6. Heinz A, Ragan P, Jones DW, Hommer D, Williams W, Knable MB, et al. Reduced central serotonin transporters in alcoholism. *Am J Psychiatry*. 1998; 155:1544–1549. [PubMed: 9812115]
7. Hoplight BJ, Sandygren NA, Neumaier JF. Increased expression of 5-HT1B receptors in rat nucleus accumbens via virally mediated gene transfer increases voluntary alcohol consumption. *Alcohol*. 2006; 38:73–79. [PubMed: 16839853]
8. Koob GF. Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. *Pharmacopsychiatry*. 2009; 42 1:S32–41. [PubMed: 19434554]
9. Lappalainen J, Long JC, Eggert M, Ozaki N, Robin RW, Brown GL, et al. Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. *Arch Gen Psychiatry*. 1998; 55:989–994. [PubMed: 9819067]

10. Kranzler HR, Hernandez-Avila CA, Gelernter J. Polymorphism of the 5-HT1B receptor gene (HTR1B): strong within-locus linkage disequilibrium without association to antisocial substance dependence. *Neuropsychopharmacology*. 2002; 26:115–122. [PubMed: 11751038]
11. Nabulsi N, Huang Y, Weinzimmer D, Ropchan J, Frost JJ, Neumeister A, et al. High resolution imaging of brain 5-HT1B receptors in the rhesus monkey using [11C]P943. *Nucl Med Biol.* in press.
12. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002; 15:273–289. [PubMed: 11771995]
13. Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, et al. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab*. 2003; 23:1096–1112. [PubMed: 12973026]
14. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab*. 2007; 27:1533–1539. [PubMed: 17519979]
15. Varnas K, Hurd YL, Hall H. Regional expression of 5-HT1B receptor mRNA in the human brain. *Synapse*. 2005; 56:21–28. [PubMed: 15700286]
16. Gallezot JD, Nabulsi N, Neumeister A, Planeta-Wilson B, Williams JW, Singhal T, et al. Kinetic modeling of the serotonin (5-HT1B) receptor radioligand [11C]P943 in humans. *Journal of Cerebral Blood Flow & Metabolism*. in press.
17. Morikawa H, Manzoni OJ, Crabbe JC, Williams JT. Regulation of central synaptic transmission by 5-HT(1B) auto- and heteroreceptors. *Mol Pharmacol*. 2000; 58:1271–1278. [PubMed: 11093763]
18. Barot SK, Ferguson SM, Neumaier JF. 5-HT(1B) receptors in nucleus accumbens efferents enhance both rewarding and aversive effects of cocaine. *Eur J Neurosci*. 2007; 25:3125–3131. [PubMed: 17509084]
19. Neumaier JF, Vincow ES, Arvanitogiannis A, Wise RA, Carlezon WA Jr. Elevated expression of 5-HT1B receptors in nucleus accumbens efferents sensitizes animals to cocaine. *J Neurosci*. 2002; 22:10856–10863. [PubMed: 12486179]

Figure 1.

In the upper row, the boxplot showing significant differences in ventral striatum (VST), including the globus pallidus and nucleus accumbens area $[^{11}\text{C}]\text{P943}$ binding potential (BP_{ND}) between patients with alcohol dependence (AD) and healthy control subjects (HC) is presented. In the lower row, the average $[^{11}\text{C}]\text{P943 } BP_{ND}$ co-registered positron emission tomography (PET) images illustrate increased VST $[^{11}\text{C}]\text{P943 } BP_{ND}$ in AD (right) relative to HC (left).

Table 1

Demographics and Clinical Characteristics

Group	Healthy Control (N = 12)	Alcohol Dependence (N = 12)	P
Age (Years)	30.6 ± 9.2	35.2 ± 10.1	0.25
	range: 18 – 49 years	range: 22 – 51 years	-
Gender	5F; 7M	5F; 7M	1
Race	1Mix/1AS/10C	1Mix/4AA/7C	-
BMI	25.2 ± 4.0	25.5 ± 5.0	0.87
Smoking status	10 N; 2 S	3 N; 9 S	0.01*
Age at First Drinking (years)	-	13.3 ± 4.3	-
Total Years of Drinking	-	15.3 ± 9.9	-
Injected Dose (MBQ)	668.4 ± 30.2	695.3 ± 51.4	0.17
Specific Activity (MBQ/nmol)	9.8 ± 3.4	8.7 ± 2.6	0.39
Injected Mass (µg)	1.6 ± 0.8	1.8 ± 0.6	0.32

- AA, African-American; AS, Asian-American; BMI, body mass index; C, Caucasian; F, female; M, male; Mix, mixed; N, nonsmoker; S, smoker.

- Data represent mean ± SD. *p* Values by independent unpaired *T*-tests, except Gender and smoking status by Fisher's Chi-square tests.