Higher Serotonin 1A Binding in a Second Major Depression Cohort: Modeling and Reference Region Considerations

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Background: Serotonin 1A receptors (5-HT_{1A}) are implicated in major depressive disorder (MDD). We previously reported higher 5-HT_{1A} binding potential (BP_F) in antidepressant naive MDD subjects compared with control subjects, while other studies report lower BP_{ND}. Discrepancies can be related to differences in study population or methodology. We sought to replicate our findings in a novel cohort and determine whether choice of reference region and outcome measure could explain discrepancies.

Methods: Nine new control subjects and 22 new not recently medicated (NRM) MDD subjects underwent positron emission tomography. BP_F and BP_{ND} were determined using a metabolite and free fraction corrected arterial input function. BP_{ND} was also determined using cerebellar gray matter (CGM) and cerebellar white matter (CWM) reference regions as input functions.

Results: BP_F was higher in the new NRM cohort ($p = .037$) compared with new control subjects, comparable to the previous cohort ($p = .04$). Cohorts were combined to examine the reference region and outcome measure. BP_F was higher in the NRM compared with control subjects ($p = .0001$). Neither BP_{ND} using CWM ($p = .86$) nor volume of distribution (V_T) ($p = .374$) differed between groups. When CGM was used, the NRM group had lower 5-HT_{1A} BP_{ND} compared with control subjects ($p = .03$); CGM V_T was higher in NRM compared with control subjects ($p = .007$).

Conclusions: Choice of reference region and outcome measure can produce different 5-HT_{1A} findings. Higher 5-HT_{1A} BP_F in MDD was found with the method with fewest assumptions about nonspecific binding and a reference region without receptors.

Key Words: Antidepressants, genotype, medication, modeling, polymorphism, unipolar

[^{11}C]WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide) is a serotonin 1A receptor (5-HT_{1A}) antagonist positron-emission tomography (PET) ligand (1–8). We first reported higher 5-HT_{1A} binding potential ($BP_F = B_{avail}/K_D$ where B_{avail} is the total number of available receptors and $1/K_D$ is the affinity of the tracer for the receptor) in antidepressant-naïve (AN) subjects with major depressive disorder (MDD) compared with antidepressant-exposed (AE) MDD subjects and control subjects (9). Also, raphe nuclei (RN) BP_F increases with G allele load of the functional 5-HT_{1A} G(−1019)C promoter polymorphism (9), elevated BP_F predicts nonresponse to antidepressants (10), and remitted depressed have higher BP_F compared with control subjects (11). Other PET studies have reported lower 5-HT_{1A} $BP_{ND} = [f_{ND}]B_{avail}/K_D$ (where f_{ND} is the fraction of radioligand free and nonspecifically bound) in MDD (8,12).

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Two explanations are differences in study population or in methodology. We sought to replicate our findings and reconcile the seemingly disparate findings. For the former, we studied a new cohort of 9 control subjects and 22 MDD. For the latter, we compared two different reference regions (RR) and two different outcome measures (BP_F and BP_{ND}) in the combined new and previous cohorts of 51 control subjects and 30 not recently medicated (NRM). Previously, we found a small number of 5-HT_{1A} receptors in the cerebellar vermis and cerebellar gray matter (CGM) compared with cerebellar white matter (CWM) (13). We concluded that CWM is the best RR for [^{11}C]WAY-100635 studies because it appears devoid of receptors, is best fit with a one-tissue compartment model, has the lowest binding, better test-retest reliability, identifiability, and time stability. Perhaps unique to [^{11}C]WAY-100635, CWM has the same nonspecific binding as CGM ex vivo (14) and therefore can be used to estimate free and nonspecific binding in gray matter regions. To determine in vivo extensibility, we have reanalyzed previous 5-HT_{1A} blocking studies (15). We hypothesized that [^{11}C]WAY-100635 binding in CWM would not change following pindolol but decrease in CGM. Additionally, we calculated BP_{ND} using compartmental modeling and the simplified reference tissue method (SRTM) (16) using both CGM and CWM.

Methods and Materials

Subjects

Thirty subjects who met DSM-IV (17) criteria for a current major depressive episode and 51 control subjects were included. Inclusion criteria were assessed through history, chart review, Structured Clinical Interview for DSM-IV Axis I Disorders (18), review of systems, physical examination, routine blood tests, pregnancy test, urine toxicology, and electrocardiogram. The

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Table 1. Clinical and Demographic Data

	Control Subjects (n = 51)	Not Recently Medicated (n = 30)	p Value (Control Subjects vs. NRM)
Age	37.35 ± 14.44	40.59 ± 13.05	.316
Hamilton Depression Rating Scale	.73 ± .98	26.20 ± 6.98	<.001
Years of Education	16.56 ± 2.89	14.36 ± 3.67	.004
Beck Depression Inventory	1.59 ± 2.53	26.43 ± 9.68	<.001
Lifetime Aggression	13.89 ± 3.72	17.38 ± 4.90	.001
Global Assessment Scale	90.25 ± 4.76	54.53 ± 11.11	<.001
Hopelessness	1.61 ± 2.33	9.60 ± 6.33	<.001
Age of Onset		25.04 ± 13.73	
Number of MDEs		13.04 ± 27.41	
Length of Current MDE (weeks)		56.32 ± 125.66	
% Female	56.86%	73.33%	.16
Family History of MDD		56.67%	
Melancholic Depression		33.33%	
History of Posttraumatic Stress Disorder		26.7%	
History of Suicide Attempt		23.3%	
History of Panic Disorder		20.0%	
History of Dysthymia		13.3%	
History of Social Phobia		13.3%	

MDD, major depressive disorder; MDE, major depressive episode.

Beck Depression Inventory (19), Hamilton Depression Rating Scale (20), and Global Assessment Scale (21) assessed subjective and objective depression severity and functional impairment, respectively (Table 1). Criteria for depressed subjects included: 1) age 18 to 65 years; 2) DSM-IV criteria for current major depressive episode and MDD; 3) capacity to provide informed consent; and absence of: 4) psychotropic medications for at least 2 weeks (6 weeks for fluoxetine, 4 weeks for neuroleptics); 5) lifetime history of alcohol or substance abuse or dependence; 6) lifetime exposure to 3,4-methylenedioxymethamphetamine; 7) significant medical conditions; 8) pregnancy; and 9) psychosis, bipolar disorder, or schizophrenia. Criteria for control subjects were similar except for the required absence of medical, neurological, and psychiatric history or a history of a mood or psychotic disorder in a first-degree relative. The Institutional Review Board of the New York State Psychiatric Institute approved the protocol. Subjects gave written informed consent after an explanation of the study.

Pindolol data came from a separate sample of nine control subjects, of which 6 had usable data (15). Briefly, subjects underwent a baseline [^{11}C]WAY-100635 scan, followed by titrated doses of pindolol CR (7.5 mg/day for 7 days, 22.5 mg/day on day 8, and then 30 mg/day on day 9); a [^{11}C]WAY-100635 scan occurred 4 hours after the 30-mg dose on day 9.

Prior Medication History

We have shown that AE, medication-free MDD have lower BP_F values than medication-free AN MDD. We defined AE as those being on an adequate dose of antidepressant for at least 4 weeks. Subjects who could not recall the name, dose, or duration of medication were classified as indeterminate (n = 13) and were not used in imaging data analysis; they were used for genetic analyses. Because we used more stringent criteria for classification, five subjects from the previous cohort were reclassified as indeterminate. Since the subjects off medications for greater than 4 years (n = 7) did not differ from the AN subjects in their composite binding data (df = 28, t = 1.39, p = .175), we combined these groups and called them NRM. There was no significant difference in demographic or clinical variables between the new and old cohorts (data not shown).

Radiochemistry and Input Function Measurement

Preparation of [^{11}C]WAY-100635 and measurement of the arterial input function, metabolites, and plasma free fraction (f_P) were conducted as described previously (6,13). Metabolite data were collected during the first 60 minutes; we were unable to quantify metabolites beyond this time point. Injected dose (ID), injected mass (IM), clearance, and f_P are presented in Table S1 in Supplement 1. The NRM had a significantly lower IM (df = 79; t = 3.82; p < .001) and ID (df = 79; t = 2.43; p = .02) than control subjects. Later studies done after our human dosimetry study (22) determined the ID (and consequently IM) needed to be lowered. No correlation was found within groups between IM or ID and BP_F in any region (data not shown). Analyses were done covarying for IM and ID.

Image Acquisition and Analysis

PET imaging was performed on an ECAT EXACT HR+ (Siemens/CTI, Knoxville, Tennessee). Emission data were collected for 110 minutes as 20 successive frames of increasing duration. Image analysis was performed using MATLAB 2006b (The Mathworks, Natick, Massachusetts) with extensions to the following: Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool (FLIRT) v5.2. (23), Brain Extraction Tool v1.2 (24), Statistical Parametric Mapping (SPM5) normalization (25), and segmentation routines (26). To correct for subject motion during the PET scan, de-noising filter techniques were applied to all PET images starting at frame 5. All frames were aligned using rigid body FLIRT to the eighth frame. A mean of motion corrected frames 8 through 18 was registered to the magnetic resonance image (MRI) using FLIRT.

Acquisition of T1-weighted MRI for co-registration of PET images and identification of regions of interest (ROIs) was performed as previously described using a 1.5 T Signa Advantage or a 3 T Signa HDx system (General Electric, Milwaukee, Wisconsin) (6). Regions of interest were hand drawn on the MRI by experienced technicians trained to reliably approximate these regions using brain atlases (27,28) and published reports (29,30). Regions of interest included the amygdala, hippocampus, parahippocampal gyrus, temporal cortex, anterior cingulate, cingulate cortex, dorsolateral prefrontal cortex, medial prefrontal cortex, ventral prefrontal cortex, insular cortex, occipital cortex, and parietal cortex. A fixed volume elliptical ROI (2 cm^3) was placed on the RN in the dorsal midbrain on a mean PET image, as described in Parsey *et al.* (10). Cylindrical ROIs were drawn in the CWM (13) and CGM, as far as possible from the occipital cortex. The ROI contours were refined using the segmented MRI to reflect the gyral pattern and differences between the PET and MRI fields of view.

Derivation of Regional Outcome Measures

The outcome measure of choice in PET studies is B_{avail}, the total number of available receptors. Without multiple injections and occupancy of the receptors, current technology permits measurement of $BP_F = B_{avail}/K_D$. Fortunately, there is no evidence for alterations in 5-HT_{1A} K_D in depression (31). BP_{ND} =