Decreased Hippocampal 5-HT_{2A} Receptor Binding in Major Depressive Disorder: In Vivo Measurement with [^{18}F]Altanserin Positron Emission Tomography

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Background: *Serotonin 5-HT_{2A} receptors play an important role in the regulation of many functions that are disturbed in patients with major depressive disorder. Postmortem and positron emission tomography studies have reported both increased and decreased 5-HT_{2A} receptor binding in different limbic and paralimbic regions.*

Methods: *We conducted a quantitative 5-HT_{2A} receptor binding study using positron emission tomography and [^{18}F]altanserin of four regions hypothesized to have altered levels of 5-HT_{2A} receptors in major depressive disorder. Using a four-compartment model, the 5-HT_{2A} receptor distribution was estimated by calculating the regional [^{18}F]altanserin k_3/k_4 ratio in which k_3 is the rate of binding to the receptor and k_4 is the rate of dissociation from the receptor. Forty-six antidepressant-free patients with major depressive disorder and 29 healthy control subjects were enrolled.*

Results: *5-HT_{2A} receptor binding in the hippocampus was reduced by 29% in depressed subjects ($p = .004$). In other regions, 5-HT_{2A} receptor binding was decreased (averaging 15%) but not significantly. Both groups had similar age-dependent decreases in 5-HT_{2A} receptors throughout all brain regions.*

Conclusions: *Altered serotoninergic function in the hippocampus is likely involved in the disturbances of mood regulation in major depressive disorder, although the specific role of the 5-HT_{2A} receptor changes is still unclear.*

Key Words: Positron emission tomography, hippocampus, depression, serotonin, 5-HT_{2A} receptors

Serotonin (5-HT) has been extensively implicated in the pathophysiology of major depressive disorder (MDD) and in the mechanism of antidepressant action (Maes and Meltzer 1995). Serotonin systems play a role in the regulation of many functions that are disturbed in patients with MDD (e.g., mood, anxiety, appetite, sleep, circadian rhythms, neuroendocrine regulation, body temperature, and sexual behavior) (Deakin 1991; Cowen 1991). Although numerous 5-HT receptor subtypes have been characterized, evidence for a role in MDD is most extensive for the 5-HT_{1A} and 5-HT_{2A} receptors, especially those located in limbic and paralimbic regions. For example, postmortem studies have shown altered 5-HT_2 receptor binding both in the frontal cortex (Stanley and Mann 1983; Mann et al 1986; Arora and Meltzer 1989; Yates et al 1990; Arango et al 1992; Hrdina et al 1993) and in hippocampal regions (Cheetham et al 1988; Rosel et al 1998, 2000) of depressed suicide completers.

In vivo assessment of human brain receptors is possible with positron emission tomography (PET) and the appropriate radioligand. [^{18}F]altanserin has been reported to be a high affinity, selective agent for imaging 5-HT_{2A} receptors (K_i = .13 nM) (Lemaire et al 1991), with binding in rat brain comparable to in vitro mapping of 5-HT_{2A} receptors (Biver et al 1997a), good test-retest reliability in vivo in human brain using PET (Smith et al 1998), and an established literature for use in humans (Biver et al 1997b; Smith et al 1998; Meltzer et al 1999; Larisch et al 2001). Altanserin has some, but likely insignificant, affinity for 5-HT_{2C} (K_i = 6 nM) (Tan et al 1999) and D_2 receptors (K_i = 62 nM) (Lemaire et al 1991), both of which are expressed in cortex to only a small degree compared to 5-HT_{2A} receptors.

The goal of the present study was to compare regional 5-HT_{2A} receptor binding in a large sample of depressed unmedicated patients with binding measured in healthy age-matched control subjects. Specifically, four regions with putative involvement in emotional regulation, evidence for functional or structural changes in major depression, and adequate number of 5-HT_{2A} receptors for PET imaging were selected. One region, the hippocampus, has been found in some structural magnetic resonance imaging (MRI) studies (Sheline et al 1999; MacQueen et al 2003) to have decreased volume in MDD which correlated with depression duration. A region heavily interconnected to the amygdaloid-hippocampal complex (Ongur and Price 2000), the gyrus rectus, has also been implicated in MDD. It has been shown to have decreased cell numbers in postmortem studies of MDD (Rajkowska et al 1999) and reduced total volume in imaging studies of MDD (Bremner et al 2002). Other paralimbic regions, the anterior and subgenual cingulate (Mayberg et al 1997; Drevets et al 1997; Bush et al 2000) have been found in patients with MDD to have volumetric loss, metabolic abnormalities at rest, and altered function during changes in mood state. Thus, these regions of interest (hippocampus, gyrus rectus, pregenual anterior cingulate, and subgenual anterior cingulate) were selected for analysis, given the existing literature indicating structural and functional changes in MDD. The number of regions analyzed was limited to minimize type II statistical errors. Finally, to provide sampling of additional brain areas, comparison regions of interest were used in our study and consisted of sections of the superior parietal cortex, dorsolateral prefrontal cortex, occipital cortex, and lateral temporal cortex. Differences in 5-HT_{2A} receptors for these regions were not hypothesized.

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Methods and Materials

Participants

Forty-six patients, 16 male patients and 30 female patients (mean age 49.6 ± 15.6 years, range 20–85 years), meeting DSM-IV criteria for MDD were recruited to the outpatient psychiatry service from referrals from other psychiatrists and also from the community by advertisement. Inclusion criteria were a current untreated episode meeting criteria for MDD, right-handedness, and no other medical illness potentially affecting the central nervous system (CNS). Clinical assessment was conducted by a psychiatrist experienced in the use of the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al 1994). If the patient's first episode of MDD was after the age of 50, they were subgrouped as late-onset depression (LOD), since different etiologies, pathophysiology, and treatment outcomes have been proposed (Alexopoulos et al 1988; Krishnan et al 1997). Exclusion criteria comprised a current or past neurologic disorder, head trauma, recent history of using any antidepressant or psychotropic medication within 4 weeks of the recruitment (or within 6 weeks for fluoxetine), uncontrolled hypertension, myocardial infarction or ischemia, diabetes, Cushing disease, steroid use, and drug/alcohol abuse. All subjects were interviewed to determine the duration of time in weeks since they were last treated with antidepressants. The control group included 29 healthy volunteers, 9 male subjects and 20 female subjects (mean age 45.8 ± 15.3 years, range 22–79 years) recruited primarily by advertisement and screened to exclude acute physical illness by physical exam, medical records, and laboratory testing. Also excluded were control subjects with alcohol or substance abuse; a family history of first-degree relatives with mood disorders; and those who used psychotropic medications, including sedative-hypnotics, during the past 3 months before recruitment.

Written informed consent was obtained from all subjects after the procedures had been fully explained. All patients and controls were assessed using the 17-item Hamilton Rating Scale for Depression (HAMD) (Hamilton 1960) on the day of the PET study. The study was approved by the Human Studies Committee and Radioactive Drug Research Committee of Washington University School of Medicine.

Radiopharmaceutical Preparation

[^{18}F]altanserin was prepared using modifications of methods previously reported (Lemaire et al 1991; Tan et al 1999; Sheline et al 2002). [^{18}F]fluoride was produced by bombardment of [^{18}O]water using a CS-15 medical cyclotron (Cyclotron Corporation, Knoxville, Tennessee) and purified using Dowex AG1 × 8 (carbonate form) resin (BioRed Laboratories, Richmond, California) (Schlyer et al 1990). The radioligand was synthesized by [^{18}F]fluorodenitration of nitroaltanserin (Monclus and Luxen 1992) and purified using semipreparative high-pressure liquid chromatography.

Imaging

Positron emission tomography images were obtained using the Siemens 961HR PET scanner (Siemens/CTI, Inc., Knoxville, Kentucky). Head position was stabilized by use of a softened thermoplastic mold. The subject was placed such that the lowest imaging plane was approximately 1 cm above and parallel to the canthomeatal line, and a transmission scan was obtained. An intravenous catheter was placed in an antecubital vein for radiopharmaceutical injection, and a 21-gauge catheter was placed under local anesthesia in the radial artery for blood sampling.

Positron emission tomography imaging of the 5-HT$_{2A}$ receptor was begun using a 20-mCi intravenous injection of [^{18}F]altanserin and initiating a 90-minute dynamic PET image collection (12 × 10 seconds, 8 × 60 seconds, and 16 × 300 seconds). At onset of PET scanning, rapid hand-drawn .6-mL arterial blood samples were obtained at a rate of every 5 seconds for 1 minute, every 15 seconds for 2 minutes, and then at decreasing frequency until end of the PET scan. Samples were centrifuged with the plasma weighed and counted. Five additional 3-mL blood samples were obtained periodically to allow determination of labeled metabolites. Each of these larger samples was extracted with acetone and applied to a stationary phase of a silica gel G (Fisher Scientific, Pittsburgh, Pennsylvania). Plates were developed with a mobile phase of CH_3OH/CH_2C_{l2} = 1/20 (vol/vol) and were scanned with an AR-2000 radio-TLC scanner (Bioscan Inc., Washington, DC).

After completion of the PET study, an MRI was obtained using a standard protocol on a 1.5T Vision system (Siemens Medical Systems, Erlanger, Germany). A three-dimensional (3-D) gradient echo magnetic resonance (MR) acquisition, magnetization-prepared rapid gradient echo (MPRAGE), was used to generate anatomic images.

Volume of Interest Analysis

Nine regions of interest (ROIs) were created. Four primary limbic/paralimbic ROIs (hippocampus, subgenual anterior cingulate, pregenual anterior cingulate, and gyrus rectus) and four additional (nonlimbic) ROIs (occipital cortex, dorsal lateral prefrontal cortex, lateral temporal cortex, and superior parietal cortex) were selected. Also, the cerebellum was chosen to provide a brain area that is very low in 5-HT$_{2A}$ receptors (Pazos et al 1987), similar to other [^{18}F]altanserin studies (Smith et al 1998; Meltzer et al 1998, 1999; Sheline et al 2002). Regions of interest were determined using a summed MPRAGE anatomic image. To create this image, all MPRAGE images in the study were first moved into the Talairach atlas coordinate system (Talairach and Tournoux 1988) using linear affine transformation (Woods et al 1992, 1993). Then, a single average MPRAGE image of all 75 subjects was created in atlas coordinates. This single image was used to define all ROIs. Anatomic boundaries were defined by specific rules (Duvernoy 1988; Bartzokis et al 1993; Carmichael and Price 1994; Sheline et al 1999), with the details of how these rules are adapted to the MRI reported previously (Sheline et al 2002). Due to the lower resolution of PET, the ROIs were constructed such that both gray and white matter were included (Figure 1).

Hippocampal Volume

Hippocampal gray matter volumes were determined using an identical protocol and identical measurement techniques to those previously described (Sheline et al 1999), with hippocampal anatomical boundaries defined by specific rules (Sheline et al 1999; Bartzokis et al 1993) using stereological estimation methods. Mean values for gray matter volumes were determined from an average of two independent measures by the same rater, who was blind to subject identity. Intrarater reliabilities were determined using the intraclass R (>.9).

Image Processing

For each subject, PET [^{18}F]altanserin images were corrected for head motion during the scan (Snyder 1996) and then coreg-

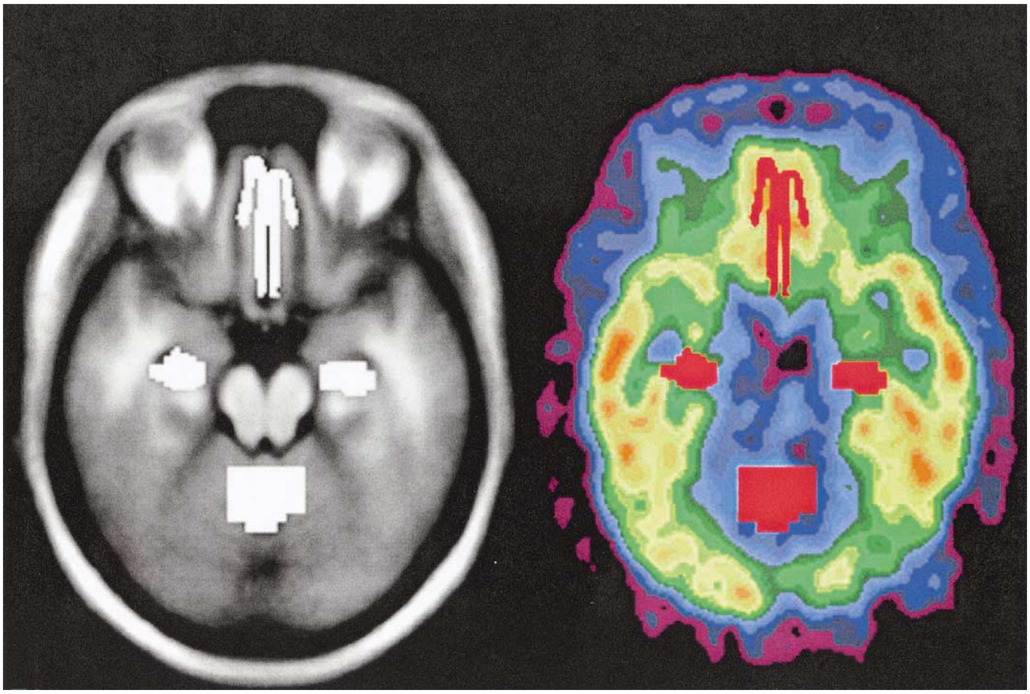

Figure 1. Selected slice from a PET scan of [^{18}F]altanserin (right) and coregistered averaged MRI image (left). The PET image represents the summed activity from 30 to 90 minutes after [^{18}F]altanserin injection and reconstructed at approximately 12 mm full-width half-maximum resolution. This transverse slice intersects three regions of interest: gyrus rectus (top), left and right hippocampus (mid level), and cerebellum (bottom). All ROIs are shown in white on the MRI image and in red on the PET image. Tissue time-activity curves for mathematical modeling analysis are generated by applying the ROIs to the dynamic PET frames reconstructed at approximately 5 mm full-width half-maximum resolution (FWHM). PET, positron emission tomography; MRI, magnetic resonance imaging; ROI, region of interest; FWHM, full-width half-maximum.

istered to the MR image (Black et al 2001). Each original high-resolution PET image frame was resliced to match the MPRAGE image, and the individual [^{18}F]altanserin ROI tissue-activity data were extracted.

Plasma metabolite data were expressed as percent unchanged [^{18}F]altanserin for each of the five time points that metabolites were measured, and the resulting percent nonmetabolized over time data were fitted to an exponential function that was, in turn, used to correct the plasma activity data. The corrected regional time-activity curve was processed using a multicompartmental model kinetic analysis (Sheline et al 2002), which accounted for transport of the [^{18}F]altanserin tracer into the brain, transport of [^{18}F]altanserin metabolites into the brain, nonspecific binding of both [^{18}F]altanserin and its metabolites (Smith et al 1998; Lopresti et al 1998) to the brain tissue, and specific binding of [^{18}F]altanserin to the 5-HT_{2A} receptor. The description of this model and comparison to other techniques, such as the Logan graphical analysis (Logan et al 1990), have been well summarized by Price et al (2001). In this approach, the cerebellum is used to generate the nonspecific binding estimates (Biver et al 1997a; Lemaire et al 1991), expressed as the ratios of rate constants k_1/k_2 and k_5/k_6 for the brain tissue (Price et al 2001). The regional 5-HT_{2A} receptor distribution was estimated by calculating the regional [^{18}F]altanserin k_3/k_4 ratio, in which k_3 is the rate of binding to the receptor and k_4 is the rate of dissociation from the receptor. The k_3/k_4 ratio is proportional to binding potential (BP), where BP = B_{max}/K_D (Mintun et al 1984).

Statistical Analysis

Student's unpaired *t* test was used to determine whether age, education, or HAMD scores were significantly different between the MDD group and the control group. A χ^2 statistic was calculated to determine whether the gender distribution across the two groups was significantly different. A Student's unpaired *t* test was used to determine whether the regional values for [^{18}F]altanserin binding to the 5-HT_{2A} receptor, expressed as k_3/k_4, were significantly different between the MDD group and the control group. Also, as late-onset depression is considered by some to have a different etiology than early-onset depression, the statistical analyses were repeated after excluding those patients subgrouped as having late-onset depression. Pearson correlations were conducted to determine the relationship between receptor binding expressed as k_3/k_4 and the MDD patients' severity of depression as measured by the HAMD scores. As the hippocampus region was the only region with significant changes between control and depressed groups, further testing was done to better understand these changes. To evaluate for possible volume effects on k_3/k_4, a stepwise regression was performed with hippocampal k_3/k_4 binding as the dependent variable and hippocampal volume, age, and group (depressed/control) as the independent variables. This analysis was conducted because it is well established that there is an age-dependent decrease in 5-HT_{2A} receptor binding and to determine whether there was an independent effect of hippocampal volume. Previous analysis of the linearity of hippocampal k_3/k_4 binding with age had demonstrated that the hippocampus, in contrast to some other regions of the brain, did not have a significant nonlinear component (Sheline et al 2002). The stepwise analysis demonstrated that volume did not have an independent effect; thus, an analysis of covariance (ANCOVA) was then done with hippocampal k_3/k_4 as the dependent variable, age as a covariate, and group (depressed, control) as

Table 1. Clinical Characteristics of the Patients and Control Subjects

	Controls (n = 29)	MDD (n = 46)	Significance	
Gender (M/F)	9/20	16/30	$\chi^2 = .11$	$p = .74$
Age (years, mean ± SD)	45.8 ± 15.3	49.6 ± 15.3	$t = 1.0^a$	$p = .30$
HAM-D	.31 ± .71	23.13 ± 4.31	$t = 27.4^a$	$p < .0001$
Education (years ± SD)	15.35 ± 2.27	15.15 ± 2.86	$t = .31^a$	$p = .76$

M, male; F, female; MDD, major depressive disorder; HAM-D, Hamilton Rating Scale for Depression.
[a]unpaired *t*-test; degrees of freedom were 73 for all tests.

the independent variable. This allowed the final calculation of effect of group in the hippocampal data. As eight regions were examined in each test, a Bonferroni corrected α of .0064 was used to determine significance based on an uncorrected α of .05. This should be a highly conservative correction given the age-related correlations between regions (Sheline et al 2002).

Results

Clinical characteristics of the 46 depressed patients and 29 healthy subjects are presented in Table 1. Mean ages between the two groups were similar (49.6 vs. 45.8 years, respectively). Ranges of ages between the groups were also similar (depressed: 20–85 years; controls: 22–79 years). There was no significant difference in gender, age, or years of education between these clinical groups (Table 1). Excluding the six patients with LOD resulted in changing the mean MDD patient age to 46.4 years and was also not significantly different from the mean control age (t = .18, df = 67, $p > .8$). Similarly, there was no significant difference in gender composition (MDD without LOD patients were made up of 26 females and 14 males, $\chi^2 = .119$, $p = .73$).

Seventeen depressed subjects had never taken antidepressants before. The remaining depressed subjects had been off antidepressants between 4 and 1352 weeks (mean = 198 weeks, median = 42 weeks, and SD = 369 weeks). The subject who had only been off antidepressants for 4 weeks had previously been treated with nefazodone.

The [^{18}F]altanserin binding to 5-HT_{2A} receptors, expressed as k_3/k_4 values, is presented in Table 2. We found a significant widespread age-dependent decline of 5-HT_{2A} receptor binding in both depressed and control groups, similar to what has been reported previously (Figure 2) (D'haenen et al 1992; Rosier et al 1996; Meltzer et al 1998; Sheline et al 2002).

First, unpaired t tests with Bonferroni corrections (corrected critical α = .0064) were used to detect group differences in all eight regional k_3/k_4 values (Table 2). The hippocampal k_3/k_4 values demonstrated a 29% decrease in the depressed group that was highly significant (t = 3.01, df = 73, p = .004). Other regions demonstrated less pronounced group differences that were not statistically significant ($p > .05$). The age-related distribution of the hippocampal k_3/k_4 values between the depressed and control groups is shown in Figure 2.

Post hoc tests examined the effects of age and hippocampal volume on the group differences in hippocampal k_3/k_4 values. Total hippocampal volumes were found to be significantly different using an unpaired t test between control (mean = 4697 mm^3, SD = 595 mm^3) and depressed subjects (mean = 4229 mm^3, SD = 759 mm^3) (df= 74; t = 2.8; p = .006), with a 9.9% decrease in the depressed subjects. The interaction of hippocampal volume and k_3/k_4 values was evaluated. A stepwise regression with age, group, and hippocampal volume as independent variables and hippocampal k_3/k_4 values as the dependent variable revealed significant effects of age (F = 46.5) and group (F = 8.8) in the final model but no independent effect of hippocampal volume (F = .09) on hippocampal k_3/k_4 values.

Most importantly, the ANCOVA revealed significant effects of group (depressed, control) on hippocampal k_3/k_4 values (df = 1,74; p = .0003), including age as a factor. This significance is much greater than that seen with the unpaired t test due to the removal of age-related variance. Finally, when the MDD group without the LOD patients was analyzed by ANCOVA with the same variables, there continued to be a significant effect of group on hippocampal k_3/k_4 values (df = 1,68; p = .004).

The measure of nonspecific binding (expressed as k_1/k_2 or Distribution Volume [DV]) for [^{18}F]altanserin in the cerebellum compared to plasma was significantly different between the control (mean ± SD = .48 ± .15) and depressed (mean ± SD = .57 ± .17) groups (t = 2.29, df = 73, p = .03); however, the nonspecific binding measure for labeled altanserin metabolites in the cerebellum compared to plasma was not significantly different between the control (mean ± SD = 1.12 ± .31) and depressed (mean ± SD = 1.14 ± .36) groups (t = .16, df = 73, p = .87).

Table 2. [^{18}F]altanserin Binding to the 5-HT_{2A} Receptor for Different Brain Areas in Patients with MDD and Control Subjects

Brain Region	Controls (n = 29)	MDD (n = 46)	Difference (%)	t^a	p
Hippocampus	.96 ± .44	.68 ± .35	−28.9	3.00	.004
Pregenual Anterior Cingulate (BA 24, 32)	2.20 ± 1.17	1.82 ± .93	−17.4	1.57	.12
Subgenual Anterior Cingulate (BA 11, 25)	2.52 ± 1.66	1.98 ± .96	−21.4	1.79	.08
Gyrus Rectus (BA 11)	2.30 ± 1.19	1.98 ± .93	−13.9	1.29	.20
Dorsal Lateral Prefrontal (BA 8, 9)	2.31 ± 1.32	1.93 ± .80	−16.5	1.56	.12
Lateral Temporal (BA 21, 22)	2.69 ± 1.28	2.36 ± .84	−12.4	1.37	.18
Superior Parietal (BA 7)	1.89 ± 1.06	1.56 ± .70	−17.7	1.64	.10
Occipital (BA 17, 18)	2.34 ± 1.26	2.14 ± .66	−8.5	.90	.37

Binding expressed as K_3/K_4 (or $DV_{ratio} - 1$)
BA, Brodmann's area; MDD, major depressive disorder.
[a]unpaired *t*-test, degrees of freedom were 73 for all tests.

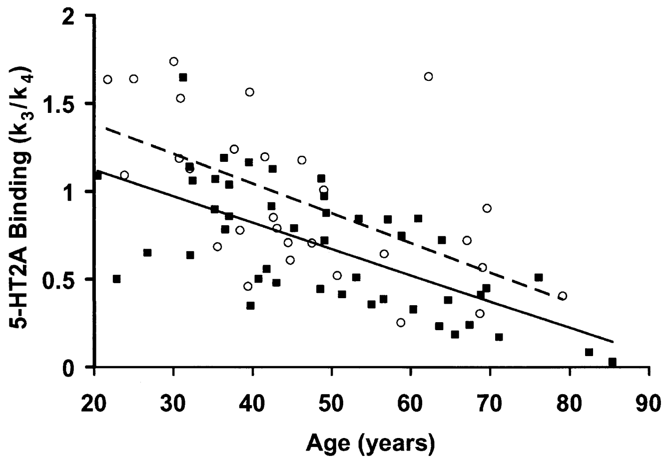

Figure 2. 5-HT_{2A} receptor binding in the hippocampus for control group (open circles, ○) and depressed patient group (solid squares, ■). There is strong age-dependent decline of 5-HT_{2A} receptors in both groups. Depressed patients demonstrated an average decrease of 29% in hippocampal 5-HT_{2A} receptor binding (Table 2). Linear regression analysis for control group data resulted in the following: $y = -.0169\,x + 1.7296$; $R = .590$. Linear regression analysis for the depressed patients group data resulted in the following: $y = -.0151\,x + 1.4247$; $R = .591$.

To explore whether the data suggested a relationship between 5-HT_{2A} receptors and severity of depression, correlations were done between the patient's regional k_3/k_4 values and HAMD rating scores obtained at the time of the PET scan. None of the eight regions of interest demonstrated a significant correlation ($p < .05$) to HAMD rating scores.

Discussion

In the largest group of patients with MDD reported to date with in vivo PET imaging of 5-HT_{2A} receptors, we found a nearly 30% decrease of hippocampal [^{18}F]altanserin binding in depressed patients compared with controls. No other region examined had a similar magnitude of difference, although all regions had a nonsignificant trend, averaging 15.4% less 5-HT_{2A} receptor binding in MDD.

Any finding of decreased hippocampal 5-HT_{2A} receptors in patients with MDD must be interpreted with caution because the number of associated conditions and factors may be involved. For example, in this study, all patients were studied while off any antidepressant medications, as these drugs could theoretically alter 5-HT_{2A} receptors (Charney et al 1981; Leysen 1992; Massou et al 1997; Meyer et al 1999a, 2001; Yatham et al 1999; Yatham et al 2001; Zanardi et al 2001). An additional problem is that prior treatment with antidepressant medications may have caused alteration in 5-HT_{2A} receptors, and such changes could persist for a time while off medication. In our patient population, 17 of 46 gave the history that they had never been treated with antidepressant therapy. Of those previously treated, the median and mean times off antidepressant therapy were 42 weeks and 198 weeks, respectively. Given the lengthy average period off antidepressant therapy, the likelihood that the decreased hippocampal 5-HT_{2A} receptor binding was caused by prior therapy should be very low. To further examine this issue, a post hoc ANCOVA analysis of the 17 antidepressant-naïve patients showed this group had a similar and highly significant decrease in hippocampal 5-HT_{2A} receptor binding compared with the control group (mean difference = −37.2%; $df = 1, 45$; $p = .0006$). Such data indicate previous antidepressant therapy cannot explain the decreased number of receptors.

Another potential confound in interpreting group difference in 5-HT_{2A} receptors is the large effect of age. Several groups have shown age-related decreases in 5-HT_{2A} receptor binding across all brain regions (Sheline et al 2002; Meltzer et al 1998; Rosier et al 1996; Wong et al 1984). In this study, the MDD group had an average age approximately 4 years older than the control group (this difference was nonsignificant); however, in the MDD subgroup after patients with late-onset of depression ($n = 6$) were excluded, the average age of this MDD group and the control group were very well matched (mean age of 46.4 years and 45.8 years, respectively). As shown in the results, the hippocampal 5-HT_{2A} receptors were significantly different not only between the two original groups (MDD and control) but between the MDD (early onset) subgroup and control as well. Furthermore, when the entire data set was assessed with analysis of covariance using age as a covariate, there was a highly significant effect of depression in 5-HT_{2A} hippocampal binding (MDD: $p < .0003$). Thus, in both the age-matched groups and in the analysis that accounts for age-related changes, the finding of decreased hippocampal 5-HT_{2A} receptors in MDD is clearly shown to be independent of the age-related changes.

Volume changes have been reported in patients with MDD (Sheline et al 1999; Bremner et al 2000; Bell-McGinty et al 2002; MacQueen et al 2003). These volume differences were also found in the current investigation. Because of its limited resolution and partial volume effects, PET could theoretically underestimate 5-HT_{2A} hippocampal binding in subjects with substantial gray matter loss. We note that the decrease in 5-HT_{2A} hippocampal binding is of greater magnitude than the hippocampal volume loss (29% vs. 9.9%) and could indicate that while both conditions may coexist, the 5-HT_{2A} binding changes are likely above that expected by simple generalized gray matter loss. Furthermore, to determine whether our measured changes in 5-HT_{2A} hippocampal binding were directly related to hippocampal volume rather than the presence of MDD, we conducted a stepwise multiple regression analysis, using age, volume, and the presence of MDD as independent variables. This analysis showed that both age and the presence of MDD had significant independent effects on 5-HT_{2A} hippocampal binding, whereas there was no significant effect of hippocampal volume. These analyses suggest two coexisting but separate processes may be needed to explain these findings.

The specific methodology for conversion of the PET activity measures into 5-HT_{2A} receptor estimates should also be considered. This work utilized a quantitative four-compartment model with correction for labeled metabolites to calculate 5-HT_{2A} receptor k_3/k_4. The data analysis methods for [^{18}F]altanserin have recently been reviewed and compared by Price et al (2001). Price et al (2001) demonstrated the model used in this work results in very comparable receptor values to other techniques in the literature, including the Logan graphical analysis. The four-compartment model with metabolites showed quantitatively higher k_3/k_4 values than the Logan graphical analysis on average but actually differed very little in rank order of those values (Price et al 2001). Finally, in this data set, the estimation of cerebellar nonspecific binding compared to plasma nonspecific binding (k_1/k_2) was less in the control population than the MDD group. The cerebellar k_1/k_2 is a ratio of the brain nonspecific binding and the plasma nonspecific binding. An argument can be made that the difference in this ratio most likely has little impact on the measures of receptor binding. First, the expression for receptor specific binding, k_3/k_4, was chosen, as this term has no dependence on differences in plasma nonspecific binding (but is sensitive to the actual brain nonspecific binding). Second, it is this plasma nonspecific binding that is typically assumed to be the major source of cerebellar k_1/k_2 variability (Meltzer et al 1998; Price et al 2001), leaving the receptor measures unaffected. Thus, it is unlikely that the lower hippocampal 5-HT_{2A} receptor values seen in MDD could be a result of differences in nonspecific binding or the specific method used for analyzing the tracer curves.

Our results showing a nonsignificant decrease in 5-HT_{2A} receptors throughout multiple brain regions are not inconsistent with the literature. There are reports showing mostly no significant differences in 5-HT_{2A} receptor ligand binding between controls and depressed patients (Attar-Levy et al 1999; Meyer et al 1999b; Meltzer et al 1999), while other reports show decreased 5-HT_{2A} receptor binding in depressed patients in widespread areas of the brain (Larisch et al 2001; Yatham et al 2000; Moresco et al 2000). Biver et al (1997b) found decreased 5-HT_{2A} receptor binding in a single region of the frontal cortex. Unfortunately, methods of analyses and selectivity of radiotracers used varied widely across these reports and may be a source of the variability of the results; however, none have identified significantly decreased hippocampal receptor binding. Only Meltzer et al (1999) specifically commented on the hippocampal region. Using hippocampal/amygdaloid ROIs and [^{18}F]altanserin PET scanning in 11 late-life depressed patients and age-matched controls, Meltzer et al (1999) reported no difference between depressed and control groups. Some postmortem studies of 5-HT_{2A} receptors have included hippocampal regions. Two reports have not found significant changes in hippocampal 5-HT_{2A} receptors (Crow et al 1984; Lowther et al 1994); however, Rosel et al (1998, 2000) reported a 40% decrease in 5-HT_{2A} binding sites within the hippocampus in antidepressant-free suicide victims. These results are in agreement with the 23% decrease in hippocampal 5-HT_{2A} binding sites reported earlier in a study of antidepressant-free suicide victims by Cheetham et al (1988). The decreases in the hippocampal 5-HT_{2A} receptor density in these postmortem suicide studies provides qualified support for our in vivo data in patients with MDD.

This finding of decreased hippocampal 5-HT_{2A} receptors has important biologic implications. The role of the hippocampus in cognitive and mood-related functions is complex and still being defined. It has been well established that the hippocampus is critical to spatial and episodic memory (Zola-Morgan et al 1989; Squire 1993), but there is also evidence for hippocampal involvement in affective modulation. Reports in the literature suggest a role for the hippocampus, within a hippocampal-orbitomedial prefrontal cortex circuit, in attentional monitoring of the internal sensorium (Wall and Messier 2001). Others have described interactions between the amygdala and hippocampus that could partially explain emotional modulation of memory (Abe 2001). Furthermore, the hippocampus is involved in stress reactions due to its well-described role in glucocorticoid regulation. Indeed, some have hypothesized the hippocampus to be specifically involved in the dysregulation of the hypothalamo-pituitary-adrenocortical axis seen in MDD (Sapolsky et al 1991; Young et al 1991).

While serotonin has been demonstrated to be involved in many different aspects of hippocampal function, the exact role of the 5-HT_{2A} receptors in regard to these affective functions is still unclear. 5-HT_{2A} receptors are primarily located on postsynaptic gamma-aminobutyric acid (GABA) interneurons, and alterations in 5-HT_{2A} receptor neurotransmission could potentially affect numerous subsystems; however, localization of 5-HT_{2A} receptors on glia (Xu and Pandey 2000) within the hippocampus has also been demonstrated. Thus, altered 5-HT_{2A} receptors could have a multifactorial origin.

One possible explanation for decreased hippocampal 5-HT_{2A} receptors in MDD is that depressed patients have an underlying serotonergic abnormality. It has often been hypothesized that abnormal serotonin function predates depressive symptoms and predisposes patients to MDD (Maes and Meltzer 1995). Decreased hippocampal 5-HT_{2A} receptors may be one such cause of decreased serotonergic neurotransmission. The efficacy of selective serotonin reuptake inhibitors, which presumably increase serotonergic function, could be interpreted as overcoming the deficit resulting from the decreased postsynaptic 5-HT_{2A} receptors. Our data are consistent with a preexisting decrease in 5-HT_{2A} receptors, given the substantially lower hippocampal 5-HT_{2A} receptor binding in the MDD patients, including the never-medicated MDD patients. We note that our data do not support a direct connection between the severity of the depressed state and a deficiency in 5-HT_{2A} receptors, as there was no significant correlation between HAMD scores and 5-HT_{2A} receptor binding.

An alternative hypothesis is that decreased hippocampal 5-HT_{2A} receptors result from depressive episodes. There are several lines of evidence indicating the hippocampus can be affected by depression. Unrelieved stress in rats, a laboratory model that produces depression-like symptoms, is known to result in damage to the hippocampus, including cell loss (Reagan and McEwen 1997), although it is not known whether this occurs in cells containing 5-HT_{2A} receptors. A variety of receptor changes in the hippocampus has been reported as well from such stress models, but it is not clear if there are changes in the 5-HT_{2A} system in rats (McEwen 1997). Depression in humans has been correlated with subtly smaller hippocampal volumes, and the number of total lifetime days depressed was inversely correlated with hippocampal size (Sheline et al 1996, 1999). While our data replicate the finding of decreased hippocampal volumes (MDD patients had a 9% decrease compared with control subjects), there was no correlation between hippocampal volume and 5-HT_{2A} receptors.

On the other hand, the decreased 5-HT_{2A} receptors may not necessarily represent a detrimental change but rather could be part of a compensatory mechanism. It is useful to consider data on the functional impact of decreased 5-HT_{2A} receptors in the hippocampus. Duman et al (1997, 1999) demonstrated in rats that 5-HT_{2A} receptors in the hippocampus have a unique role in the brain's response to stress. While in other cortical regions the 5-HT_{2A} receptors play a role in increasing brain-derived neurotrophic factor (BDNF) during stress, in the hippocampus the 5-HT_{2A} receptors appear to be critical in producing a *decrease* in BDNF during stress. Decreased BDNF "could contribute to stress-induced atrophy and death of vulnerable" neurons (Duman et al 1999). Thus, decreased hippocampal 5-HT_{2A} in depression may result from a homeostatic reduction or autoregulation in the presence of chronic stress. Perhaps, the efficacy of some antidepressants that directly block 5-HT_{2A} receptors is due, in part, to blocking stress-induced reduction of BDNF in the hippocampus.

In summary, [^{18}F]altanserin PET shows MDD is associated with a substantial decrease in 5-HT_{2A} receptor binding in the hippocampus. Several other cortical regions showed only a nonsignificant trend toward decreased 5-HT_{2A} receptors. The hippocampal receptor decrease is likely indicative of altered serotonergic transmission in major depression. At this time, it is not clear whether such a change predates the depressive episode, is a consequence of the detrimental aspects of depression, or is part of compensatory mechanisms to restore normal function during depression.

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