
PET Imaging of Serotonin 1A Receptor Binding in Depression

Wayne C. Drevets, Ellen Frank, Julie C. Price, David J. Kupfer, Daniel Holt, Phil J. Greer, Yiyun Huang, Clara Gautier, and Chester Mathis

Background: *The serotonin-1A (5HT1A) receptor system has been implicated in the pathophysiology of major depression by postmortem studies of suicide victims and depressed subjects dying of natural causes. This literature is in disagreement, however, regarding the brain regions where 5HT1A receptor binding differs between depressives and controls and the direction of such differences relative to the normal baseline, possibly reflecting the diagnostic heterogeneity inherent within suicide samples. PET imaging using the 5HT1A receptor radioligand, [¹¹C]WAY-100635, may clarify the clinical conditions under which 5HT1A receptor binding potential (BP) is abnormal in depression.*

Methods: *Regional 5HT1A receptor BP values were compared between 12 unmedicated depressives with primary, recurrent, familial mood disorders and 8 healthy controls using PET and [carbonyl-¹¹C]WAY-100635. Regions-of-interest (ROI) assessed were the mesiotemporal cortex (hippocampus-amygdala) and midbrain raphe, where previous postmortem studies suggested 5HT1A receptor binding is abnormal in depression.*

Results: *The mean 5HT1A receptor BP was reduced 41.5% in the raphe ($p < .02$) and 26.8% in the mesiotemporal cortex ($p < .025$) in the depressives relative to the controls. Post hoc comparisons showed the abnormal reduction in 5HT1A receptor BP was not limited to these regions, but extended to control ROI in the occipital cortex and postcentral gyrus as well. The magnitude of these abnormalities was most prominent in bipolar depressives ($n = 4$) and unipolar depressives with bipolar relatives ($n = 4$).*

Conclusions: *Serotonin-1A receptor BP is abnormally decreased in the depressed phase of familial mood disorders in multiple brain regions. Of the regions tested, the magnitude of this reduction was most prominent in the midbrain raphe. Converging evidence from postmortem studies of mood disorders suggests these reductions of 5HT1A receptor BP may be associated with histopathological changes involving*

the raphe. Biol Psychiatry 1999;46:1375–1387 © 1999 Society of Biological Psychiatry

Key Words: Major depressive disorder, bipolar disorder, PET, serotonin, raphe, hippocampus

Introduction

The serotonin-1A (5HT1A) receptor system has been implicated in depression by evidence that depressed subjects have blunted physiological responses to 5HT1A receptor agonists in vivo and decreased 5HT1A receptor binding postmortem (Bowen et al 1989; Lesch 1992; Lopez et al 1998). During 5HT1A receptor agonist challenge, the incremental increases in plasma corticotropin and cortisol concentrations and the decrease in body temperature seen in healthy controls are attenuated in unmedicated subjects with major depressive disorder (MDD) (Lesch et al 1990a, 1990b; Maes and Meltzer 1995). The significance of these data remained unclear, however, because the ceiling cortisol concentration achieved in response to 5HT1A agonist challenge in these studies was similar in depressives and controls, and the smaller change seen in MDD may have been accounted for by the elevated *baseline* cortisol levels in the depressives (Lesch 1992). Postmortem studies of cerebral 5HT1A receptor binding and mRNA expression in MDD and bipolar disorder (BD) subjects provided more direct evidence of 5HT1A receptor dysfunction in mood disorders, but these data remained preliminary, being limited to two studies involving small samples: Lopez et al (1998) showed that 5HT1A receptor mRNA levels were abnormally reduced in the hippocampus in six MDD subjects who died by suicide, and Bowen et al (1989) found reduced 5HT1A receptor binding to [³H]8-OH-DPAT in the temporal polar and frontal opercular cortices in seven medicated depressives with MDD or BD dying of natural causes.

Other postmortem studies investigated 5HT1A receptor binding in samples of unselected suicide victims, that presumably contained subjects suffering from primary mood disorders as well as subjects suffering from depression arising secondary to other psychiatric and medical condi-

From the Department of Psychiatry (WCD, EF, DJK, CG) and Department of Radiology (WCD, JCP, DH, PJG, YH, CM), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Address reprint requests to Wayne C. Drevets, MD, B938 PUH, PET Facility; University of Pittsburgh Medical Center; 200 Lothrop St., Pittsburgh, PA 15213.

Received March 8, 1999; revised July 14, 1999; accepted July 16, 1999.

tions. Results differed widely across these studies, as in the hippocampus the 5HT1A receptor binding in unmedicated suicide victims was reported to be decreased (Cheetham et al 1990), trending toward decreased (Lowther et al 1997), not different (Stockmeier et al 1997), and increased (Joyce et al 1993) relative to controls (the latter result, however, might be accounted for by the 30-year difference in mean age between suicides and controls studied by Joyce et al, because 5HT1A receptor B_{\max} correlated inversely with age in other studies [Dillon et al 1991; Lowther et al 1997; Matsubara et al 1991]). In the raphe, 5HT1A receptor binding was abnormally reduced in one study (Kassir et al 1998) but increased in another (Stockmeier et al 1998) in suicide victims relative to controls. Finally, in the prefrontal cortex (PFC) 5HT1A receptor binding did not differ between suicide and control samples in dorsal or anterior prefrontal cortical areas (Arranz et al 1994; Cheetham et al 1990; Dillon et al 1991; Matsubara et al 1991; Stockmeier et al 1997), but was abnormally increased in the ventrolateral PFC (Arango et al 1995). The dissimilar results across studies suggest that abnormalities of 5HT1A receptor binding may be specific to clinical subsets of subjects prone to suicide, rather than being nonspecifically associated with behaviors such as suicide or depressed mood.

Neuroimaging studies using the 5HT1A receptor radioligand, [*carbonyl*- ^{11}C]WAY-100635, to investigate regional 5HT1A receptor binding potential (BP) in clinically well-characterized depressed subjects hold the potential to determine whether abnormalities of 5HT1A binding are specific to mood disordered subgroups. A preliminary PET study comparing PET measures of [^{11}C]WAY-100635 between depressives ($n = 8$; selected by MDD criteria) and controls ($n = 7$) reported nonsignificant trends toward reduced 5HT1A receptor BP in the MTC and raphe (Sargent et al 1997). Because these subjects were taking selective serotonin reuptake inhibitor (SSRI) at scanning these trends may have been accounted for by drug effects.

Nevertheless, the trend toward reduced mesiotemporal 5HT1A receptor BP in this study was consistent with the finding of Lopez et al (1998) that suicide victims diagnosed as having MDD by psychological autopsy had reduced 5HT1A receptor mRNA in the hippocampus postmortem. Lopez et al (1998) hypothesized that the cortisol hypersecretion associated with MDD reduced 5HT1A gene expression in the hippocampus, because in rats hippocampal 5HT1A receptor mRNA expression is under tonic inhibition by corticosteroid receptor stimulation. In rats 5HT1A receptor density and mRNA levels in the hippocampus decrease in response to corticosterone administration and chronic stress, and increase after adrenalectomy (Bagdy et al. 1989; Chalmers et al 1993; Meijer and DeKloet 1994; Mendelson and McEwen 1991, 1992; Zhono and Ciaranello 1994). The stress-induced down-regulation of 5HT1A receptor expression is prevented by adrenalectomy, showing the importance

of circulating adrenal steroids in mediating this effect (Lopez et al 1998).

In contrast, 5HT1A receptors in the raphe seem insensitive to circulating corticosteroids (Chalmers et al 1993). Abnormal 5HT1A receptor binding in this structure in mood disordered samples may instead reflect neuromorphological abnormalities that affect 5HT1A receptor number. Baumann and Bogerts (1998) observed reduced neuronal counts in the dorsal raphe nucleus (DRN) in MDD and BD subjects studied postmortem, and Kassir et al (1998) found the cumulative area of the DRN abnormally reduced in depressed, nonalcoholic, suicide victims.

These data suggest the sensitivity for detecting 5HT1A receptor abnormalities in depressed samples may be enhanced by selecting subjects with a higher likelihood of having glucocorticoid hypersecretion or serotonergic dysfunction. Selection of major depressives with primary BD or familial MDD may prove useful in this regard. Both bipolar depressives and unipolar depressives with familial pure depressive disease (FPDD: primary MDD subjects who have first degree relatives with MDD, but not BD, alcoholism or antisocial personality disorder [ASPD]) have been more likely to show evidence of LHPA-axis hyperactivity than subjects with depression spectrum disease (DSD: primary MDD subjects who have first degree relatives with alcoholism or ASPD but not BD), sporadic depressive disease (SDD: primary MDD subjects with no first degree relatives with MDD, BD, alcoholism, or ASPD) or depression secondary to other psychiatric conditions (Arana et al 1985; Lewis et al 1984; Winokur 1982). Moreover, Lewis and McChesney (1985) showed that the mean platelet [^3H]imipramine uptake was abnormal in BD and FPDD but not in DSD or SDD, suggesting the former subtypes may more likely manifest serotonergic dysfunction.

In the current study the likelihood of detecting an abnormality of 5HT1A receptor binding was enhanced by selecting subjects with primary mood disorders who had first degree relatives with primary MDD or BD. Because of the small number of subjects imaged, statistical sensitivity was enhanced by reducing comparisons to the two regions where multiple studies demonstrated abnormal 5HT1A receptor BP in depression or suicide, namely the raphe and the hippocampus, and by combining subjects with MDD and BD into a single sample. Differences between depressive subgroups were examined post hoc.

Methods and Materials

Entrance criteria for the depressed sample ($n = 12$) were meeting DSM-IV criteria (American Psychiatric Association 1994) for a current major depressive episode, having a past history of recurrent mood episodes that preceded other medical or psychiatric disorders, and having a first degree relative with primary MDD or BD. Of the

12 subjects entered, 4 had BD, most recent episode depressed (BD-D) and 8 had recurrent MDD (American Psychiatric Association 1994). Of the MDD subjects, 4 had both BD and MDD relatives (MDD/bdr; 3 cases had a first degree relative with BD and the fourth had a first cousin with BD and first degree relatives with MDD). The other 4 MDD subjects had only MDD relatives (MDD/mdr). Diagnosis was established before scanning by a clinical interview with a psychiatrist (WCD). A Structured Clinical Interview for DSM-IV (SCID) was also administered as a screen to aid with establishing inclusion and exclusion criteria.

Exclusion criteria included medical or neurological illnesses likely to affect cerebral physiology or anatomy, gross abnormalities of brain structure evident in magnetic resonance images (MRI), suicidal intent, substance abuse within 1 year, lifetime history of substance dependence (other than nicotine), and exposure to psychotropic or other medications likely to alter cerebral physiology or monoamine neurotransmitter function within 2 weeks (8 weeks for fluoxetine). Two BD-D subjects and one MDD subject had a past history of substance abuse in remission for more than 1 year (American Psychiatric Association 1994).

Healthy control subjects ($n = 8$) who and met these exclusion criteria and did not meet criteria for major psychiatric disorders based upon structured (SCID) and unstructured psychiatric screening interviews were also selected. None of the controls had a history of substance abuse. The controls denied having first degree relatives with primary affective, anxiety, or psychotic disorders.

Subjects were asked to abstain from alcohol for 48 hours before scanning, and reported complying with this request (Dillon et al 1991).

Image Acquisition

After intravascular cannulation, subjects were positioned within the PET scanner gantry so that axial image planes were acquired parallel to the orbital-meatal line. Head position was maintained during scanning using a thermoplastic mask. A dynamic emission scan (29 frames of increasing length over 60 min) was initiated upon IV bolus administration of 7.1 to 15 mCi of high specific activity [*carbonyl*- ^{11}C]WAY-100635 [mean specific activity = 1.50 mCi/nmol (range 0.58–1.69)], synthesized using a modification of the McCarron et al (1996) method (using similar methods for [*carbonyl*- ^{11}C]WAY-100635 imaging, Gunn et al (1998) showed that a 60 min scanning period yields a repeatability coefficient for measuring mesiotemporal BP superior to that obtained using a 90 min scan [Table 3 in Gunn et al 1998]). Images were acquired as subjects rested with eyes-closed using a Siemens/CTI HR+ (63 contiguous slices over 15.2 cm) in 3D mode with septa retracted (FWHM resolution = 5 ± 0.5 mm transverse and 4.5 ± 0.5 mm axially [Brix et al 1997]). A Neuro-insert (CTI PET Systems, Knoxville, TN) was locked into the PET camera gantry to reduce random coincidences (Wienhard et al 1998). Images were reconstructed using a Hanning window with cut-off = Nyquist frequency, resulting in an estimated (including the effect of scatter) true image FWHM resolution of 7.1 mm in the transverse plane and 6.7 mm axially.

Magnetic resonance images (MRI) were obtained using a 1.5 T General Electric (Milwaukee) Signa Scanner and a 3-dimensional spoiled gradient recalled (SPGR) sequence (TE = 5, TR = 24, flip

angle = 40° , slice thickness = 1.5 mm, NEX = 2, field of view = 12 mm, voxel size = $0.94 \times 1.25 \times 1.5$ mm) optimized for delineating gray matter/white matter/CSF boundaries.

Other Assessments

Depression severity was rated using the Hamilton Rating Scale for Depression (HRSD; Hamilton 1960). To examine the relationship between cortisol secretion and 5HT_{1A} receptor BP, the 24-hour urine-free cortisol level (24-hour UFC) was measured before scanning, and “stressed” plasma cortisol concentrations were obtained during scanning (i.e., blood sampled after 2 hours of intravascular cannulation and 80 min of head restraint within the scanner gantry) (Drevets et al 1997b). The 24-hour UFC was considered valid if the urine collection contained a total volume >600 mL (Wallach 1978).

Image Analysis

To facilitate PET-MRI co-registration, PET image frames acquired during the first 15 min after [^{11}C]WAY-100635 injection were aligned and integrated into a single static emission image that overly represented free and nonspecifically bound radiotracer so that cortical outlines were sufficiently evident to guide automated image registration (Woods et al 1993). The PET and MR images were aligned and the MR images were resliced to yield images with identical plane orientation and slice thickness as the PET images using Automated Image Registration (AIR; Wiseman et al 1996; Woods et al 1993). The precision of alignment has a mean absolute error of 2 mm (Wiseman et al 1996; Woods et al 1993).

Because the small sample sizes employed would reduce the sensitivity of statistical comparisons in multiple regions after applying appropriate corrections (Bonferroni), hypothesis testing was limited to the mesiotemporal cortex (MTC) and midbrain raphe. The MTC-ROI was defined by manual tracing around the grey matter of the hippocampus, amygdala, and adjacent parahippocampal and periamygdaloid cortex using ImageTool (CTI PET Systems) in the 6 to 7 planes where both amygdala and hippocampus were evident (Figure 1) (Bronen and Cheung 1991). The globular shape of these structures permitted identification of their anatomical boundaries regardless of interindividual anatomical variability (Bronen and Cheung 1991). Because laterality effects were not hypothesized, ROI from left and right hemispheres were combined. The sensitivity for detecting intergroup differences using MTC measures that emphasized the hippocampus were assessed post hoc in a smaller ROI limited to the head of the hippocampus and adjacent parahippocampal cortex (defined over MTC grey matter posterior to the alveus and the temporal horn of the lateral ventricle; Figure 1) (Bronen and Cheung 1991).

The raphe ROI was centered over the midbrain raphe nuclei, that are collectively evident in [^{11}C]WAY-100635 images because of their very high 5HT_{1A} receptor density relative to that of surrounding tissues (Figure 1) (Hall et al 1997; Pazos et al 1987; Pike et al 1996). The raphe is inadequately visualized in MR images for boundary tracing, so this ROI was defined on PET images constructed by integrating image frames from 15 to 60 min, that predominantly reflect 5HT_{1A} receptor-specific binding. The portion of the raphe evident in the midbrain was selected to emphasize

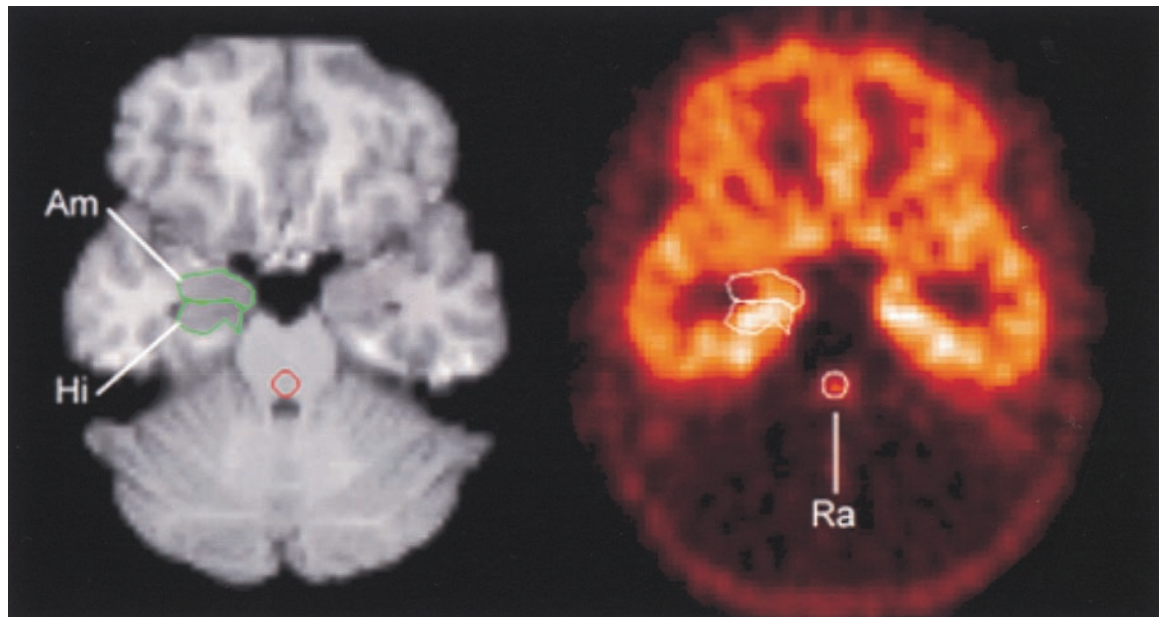


Figure 1. Co-registered PET (right) and MRI (left) sections through the mesiotemporal cortex and midbrain to show placement of the MTC and raphe regions-of-interest. The MTC region included areas of high 5HT_{1A} receptor density (hippocampus) along with areas of moderate 5HT_{1A} receptor density (amygdala and periamygdaloid cortex; Hall et al 1997). By including amygdala and hippocampus together, errors in distinguishing these two structures are avoided and boundary decisions are reduced to delimiting grey matter-white matter and grey matter-CSF interfaces. Measures in the hippocampus alone were assessed post hoc by demarcating amygdala from hippocampus by the alveus and the temporal horn of the lateral ventricle (Bronen and Cheung 1991). For illustration purposes the MTC ROI is shown only for the right hemisphere (readers' left) so that the anatomy is unobscured in the contralateral hemisphere. The raphe also has high 5HT_{1A} receptor density and can be visualized in the PET image because the 5HT_{1A} receptor density in the surrounding mesencephalic and cerebellar tissues is markedly lower (Pike et al 1996). The raphe ROI was defined on the PET image, but as is evident in the co-registered MRI plane, this circular ROI overlies the midbrain area where the dorsal raphe nucleus is known to be situated. Note the virtual absence of [¹¹C]WAY-100635 uptake in the cerebellum (posterior to the raphe) and white matter, consistent with postmortem measures of 5HT_{1A} receptor density in humans (Hall et al 1997). Am, amygdala; Hi, hippocampus; Ra, raphe.

measures from the DRN, that predominantly innervates the forebrain. Midbrain sections were identified in the co-registered MRI image as the 3 consecutive, ventral-most slices where the interpeduncular cistern was evident (Figure 1), and circular ROI (5.1 mm radius) were centered over the brainstem area of high tissue radioactivity in each slice. The diameter of this ROI was larger than that of the actual raphe to accommodate the blurring of the raphe 5HT_{1A} receptor-specific signal in PET images and reduce the effects of position differences in the raphe signal due to movement or misregistration error between image frames.

In addition, ROI were defined in two areas where no differences were expected a priori. A post central gyrus ROI (presumably comprised predominantly of primary somatosensory cortex) was defined by manually tracing around this gyrus in the two MRI slices immediately dorsal to the dorsal convexity of the corpus callosum. An occipital pole ROI (that included part of primary visual cortex) was defined by placing a 20 mm diameter circle over the occipital pole in the transverse MRI slice where the dorsal cerebellum was first evident (while passing ventrally).

The reference region for measuring the concentration of free and nonspecifically bound radioligand was the cerebellar cortex. This ROI was defined in three MR image slices where the cerebellar cortex pixels sampled were located at least twice the FWHM ventral and medial to the ventral occipital cortex and posterior and medial

to the basal temporal cortex to avoid "spilling in" of radioactive counts from these cortices (Links et al 1996), that have markedly higher 5HT_{1A} receptor density than the cerebellum (Hall et al 1997). The posterior border of the cerebellar ROI was situated at least one cm from the brain edge to avoid spilling in of radioactive counts from the venous sinuses coursing along the posterior surface of the cerebellum. The venous sinuses contain a higher concentration of radioactive polar metabolites than the cerebellar cortex itself, that has only a 5% blood volume (Gunn et al 1998; Osman et al 1995, 1998). ROI placement was limited to axial planes at or dorsal to plane 56 (of 63) to obtain measures from planes where the signal-to-noise ratio was relatively uniform in 3D images (Townsend et al 1998).

Tracer Kinetic Modeling of 5HT_{1A} Receptor Binding Potentials (BP)

Decay-corrected, time-radioactivity curves were obtained from the dynamic PET image for each 3-dimensional ROI using a calibrated phantom standard to convert tomographic counts to $\mu\text{Ci/mL}$ for each time point (Figure 2). Regional 5HT_{1A} receptor BP values were fit with a 3-parameter (simplified) reference tissue model (Gunn et al 1998; Lammertsma et al 1996) that fits the BP from measured tissue concentrations in a

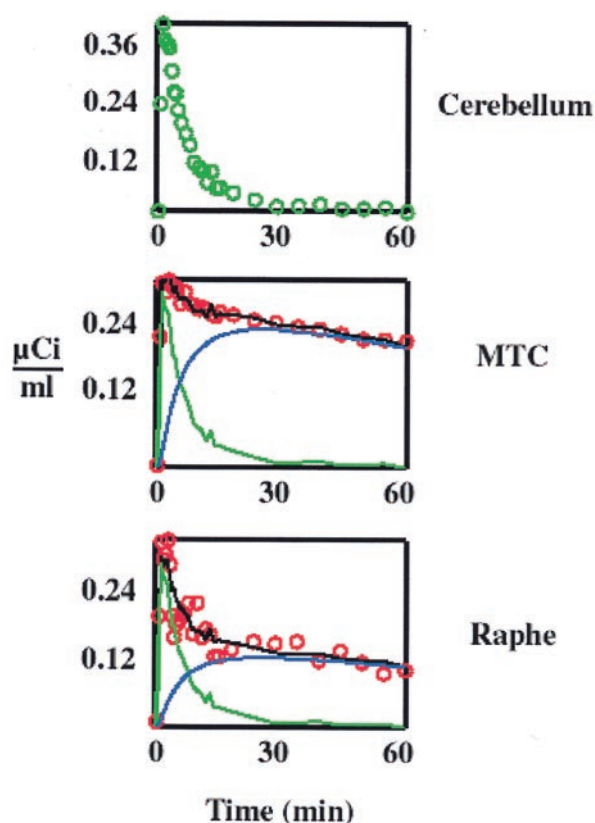


Figure 2. Typical time-radioactivity curves for cerebellum, mesiotemporal cortex (MTC), and midbrain raphe after bolus injection of 10 mCi of [^{11}C]WAY-100635. The tissue concentration in $\mu\text{Ci}/\text{mL}$ (corrected for decay of radioactivity) is shown on the y-axis and the time in min on the x-axis. The black line through the data points for the MTC and raphe (red circles) show curve fits for the simplified reference tissue model. The scaled contribution of the cerebellar reference tissue (green line) and the specific binding component (blue line) are also shown.

region where significant specific binding is present across time relative to a reference tissue where virtually all radioactivity reflects free and nonspecifically bound radiotracer (Figure 2). For [^{11}C]WAY-100635, the cerebellum seems most suitable as the reference tissue because it is nearly devoid of 5HT $_{1A}$ sites and is large enough that ROI can be situated far from tissues with abundant 5HT $_{1A}$ receptors (Hall et al 1997; Pazos et al 1987). The BP values obtained using this model are much less variable than those obtained using compartmental modeling approaches that employ the plasma [^{11}C]WAY-100635 concentration as the input function because the rapid clearance of [^{11}C]WAY-100635 from plasma limits the accuracy of quantitating metabolites at late imaging times (Gunn et al 1998).

Statistical Comparisons

The mean 5HT $_{1A}$ receptor BP in the MTC and midbrain were compared between groups using unpaired t tests. The relationship between 5HT $_{1A}$ receptor BP in the MTC and cortisol secretion were assessed by computing Pearson product moment correlation

Table 1. Demographic, Clinical and Laboratory Characteristics of the Subject Samples

	Control	Depressed
<i>n</i>	8	12
Mean age (\pm SD)	35.3 \pm 13.5	35.8 \pm 9.7
Mean HRSD-17 item (\pm SD)	0.63 \pm 1.4	22 \pm 6.4
Mean HRSD-25 item (\pm SD)	0.88 \pm 2.1	27 \pm 7.0
Number of females (%)	4 (50)	7 (58)
Number of left-handed (%)	1 (14)	2 (17)
Median weeks off psychotropic drugs (range)	N/A	30 (2-130)
Mean 24-hour UFC ($\mu\text{g}/\text{TVol}$)	57 \pm 30	59 \pm 17
Stressed plasma cortisol ($\mu\text{g}/\text{dL}$) ^a	9.7 \pm 2.1	12.5 \pm 2.8 ^b

Abbreviations: HRSD: Hamilton Rating Scale for Depression; UFC: urinary free cortisol; TVol: total 24-hour urine volume.

^aThe plasma cortisol samples for one control and one depressive were lost due to laboratory error.

^bDifference between depressives and controls significant ($p < .05$).

coefficients between the regional BP values and the 24-hour UFC levels and the plasma cortisol concentrations. Post hoc exploratory correlations assessed the relationships between the 5HT $_{1A}$ receptor BP values in the MTC and those in the midbrain and between the 5HT $_{1A}$ receptor BP values and age and depression severity (HRSD scores).

Results

Demographic characteristics, clinical ratings and laboratory data for the depressed and control samples appear in Table 1. The groups were similar with respect to age and gender composition, but significantly differed for HRSD scores and "stressed" plasma cortisol concentrations ($t = 2.4$; $p < .05$; Table 1). The mean volumes of the ROI defined in the MTC did not significantly differ between groups, being 9.73 ± 1.22 mL and 9.01 ± 0.785 mL for the depressives and controls, respectively ($t = 1.61$, ns). The total raphe ROI volume for all 3 planes was 0.594 mL.

Although not used in the kinetic analysis, arterial blood was sampled in all of the controls and 8 of the depressives. [^{11}C]WAY-100635 was rapidly metabolized in both groups. At 10 min the proportion of total plasma radioactivity accounted for by unmetabolized [^{11}C]WAY-100635 was $7.8 \pm 2.8\%$ in the controls and $7.8 \pm 4.0\%$ in the depressives (consistent with Gunn et al [1998] and Osman et al [1998]).

The mean 5HT $_{1A}$ receptor BP was reduced in the depressives relative to the controls by 26.8% in the MTC ($t = 2.50$; $df = 18$; p [2 tail] $< .025$; Table 2) and 41.5% in the midbrain raphe ($t = 2.75$; p [2-tail] $< .02$; Table 2). Post hoc assessment of the ROI in the hippocampus/parahippocampal cortex (excluding amygdala/periamygdaloid cortex) showed a similar, 24.6% decrease in the depressives versus the controls (mean BP = 6.09 ± 1.82 and 8.08 ± 1.72 , respectively; $t = 2.48$; $df = 18$; p [2 tail]

Table 2. Mean 5HT1A Receptor BP Values by Depressive Subgroup

Sample or Subsample	<i>n</i>	Midbrain Raphe (mean BP \pm SD)	Mesiotemporal Cortex (mean BP \pm SD)
Control	8	3.35 \pm 1.17	7.28 \pm 1.78
Depressed	12	1.96 \pm 1.01^a	5.33 \pm 1.58^a
BD-D (BD FHx)	4	1.43 \pm 0.569 ^b	4.09 \pm 1.10 ^b
MDD with BD FHx	4	1.63 \pm 0.816 ^a	5.60 \pm 1.88
MDD without BD FHx	4	2.81 \pm 1.14	6.31 \pm 0.975
Bipolar Spectrum ^c	8	1.53 \pm 0.661 ^b	4.85 \pm 1.64 ^a
All MDD	8	2.22 \pm 1.11	5.96 \pm 1.44

Bold print designates tests of the a priori hypotheses. Abbreviations: BD-D, bipolar disorder-depressed phase; FHx, family history (see Methods); MDD, major depressive disorder.

^a*p* < .05 relative to control.

^b*p* < .01 relative to control.

^cBD-D subjects plus MDD subjects who have BD relatives; see Discussion.

< .025). Similar decreases in depressives versus controls (25.2% and 32.5%, respectively) were also found in the postcentral gyrus (mean BP = 3.18 \pm .902 and 4.25 \pm .650, respectively; *t* = 3.08; *df* = 18; corrected *p* [2 tail] < .05] and the occipital pole (mean BP = 1.89 \pm 0.513 and 2.80 \pm 0.402, respectively; *t* = 4.43; *df* = 18; corrected *p* [2 tail] < 0.01). The rank order of these BP values (hippocampus > MTC > post central gyrus [in parietal cortex] > occipital cortex) was compatible with in vitro measures in human brain (Hall et al 1997; Pazos et al 1987).

The MTC 5HT1A receptor BP values did not significantly correlate with the plasma cortisol concentrations or the 24-hour UFC values in either the entire subject sample (depressives plus controls; (*r* = -0.36 and *r* = -0.14, respectively) or the depressed sample alone (*r* = 0.0070 and *r* = 0.23, respectively). The 5HT1A receptor BP values in the MTC and the raphe were correlated in the entire sample (*r* = 0.63; *df* = 18; *p* < .01), but this relationship did not reach significance in the control (*r* = 0.64; *df* = 6; .05 < *p* < .1) or depressed samples alone (*r* = .35, ns). The HRSD scores (25 item) did not correlate with the 5HT1A receptor BP in either the raphe or the MTC in the depressed group (*r* = 0.35 and *r* = .05, respectively; ns). The 5HT1A receptor BP trended toward showing an inverse correlation with age in the controls in the raphe (*r* = -0.58; .05 < *p* < .1) and the MTC (*r* = -0.55; .05 < *p* < .1), consistent with some postmortem studies (Dillon et al 1991; Lowther et al 1997; Matsubara et al 1991). In contrast, the correlation between age and BP was positive in the MTC (*r* = .67; *p* < .05) and nonsignificant in the raphe (*r* = .46; ns) in the depressives. Time since last antidepressant drug exposure did not correlate with 5HT1A receptor BP in either the raphe (*r* = -0.29, ns) or the MTC (*r* = .026; ns).

Post hoc assessments of the specificity of these findings to particular depressive subgroups provided the results

shown in Table 2. The difference in 5HT1A receptor BP in the raphe between depressives and controls was largely attributable to the BD-D and MDD/bdr subsamples, in whom the mean BP was decreased 57% (*t* = 3.83; *p* [uncorrected] < .01) and 51% (*t* = 2.96; *p* [uncorrected] < .05), respectively, relative to control. The magnitude of the difference in 5HT1A receptor BP in the MTC was also greatest in the bipolar depressives relative to the controls, being decreased 44% in BD-D (*t* = 3.82; *p* [uncorrected] < .01), 23 % in MDD/bdr (*t* = 1.49, ns) and 13% in MDD/mdr (*t* = 1.63, ns).

Discussion

The mean 5HT1A receptor BP was decreased 42% in the midbrain raphe and 25% to 33% in limbic (MTC) and neocortical areas in depressives relative to controls. The magnitude of these differences seemed most prominent in bipolar depressives and unipolar depressives who had bipolar relatives (Table 2, Figure 3). The abnormality in the MTC was consistent with the postmortem data of Lopez et al (1998) showing that the mean 5HT1A receptor mRNA was decreased 31% to 49% across hippocampal subfields in suicide victims with MDD. The reduction in 5HT1A receptor BP in the midbrain in the depressives was similar in magnitude to the 50% reduction in 5HT1A receptor binding capacity found postmortem in the DRN of depressed, nonalcoholic suicide victims by Kassir et al (1998). The reduced 5HT1A receptor BP in the raphe was also compatible with evidence that the hypothermic response to 5HT1A receptor agonist challenge (thought to reflect presynaptic 5HT1A receptor function) is blunted in MDD (Lesch 1992).

Limitations of the Methods

Because sample sizes were small, sensitivity for identifying intergroup differences depended upon selecting a subject sample "enriched" for the likelihood of showing biological abnormalities (Drevets and Todd 1997). The latter approach proved successful in PET studies of blood flow and glucose metabolism, in which subjects meeting BD-D or familial MDD criteria had reproducible abnormalities even when the sample sizes were relatively small (Drevets et al 1992, 1995, 1997a, 1997b, 1999). Nevertheless, future studies involving larger samples are needed to compare 5HT1A receptor BP across subgroups because the differences found were largely accounted for by the BD-D and MDD/bdr subgroups (Table 2; Figure 3), and it remains unclear whether 5HT1A receptor BP is abnormal in MDD subjects without BD relatives.

The sensitivity of the PET measures was limited by the spatial resolution of PET and the inclusion of grey and

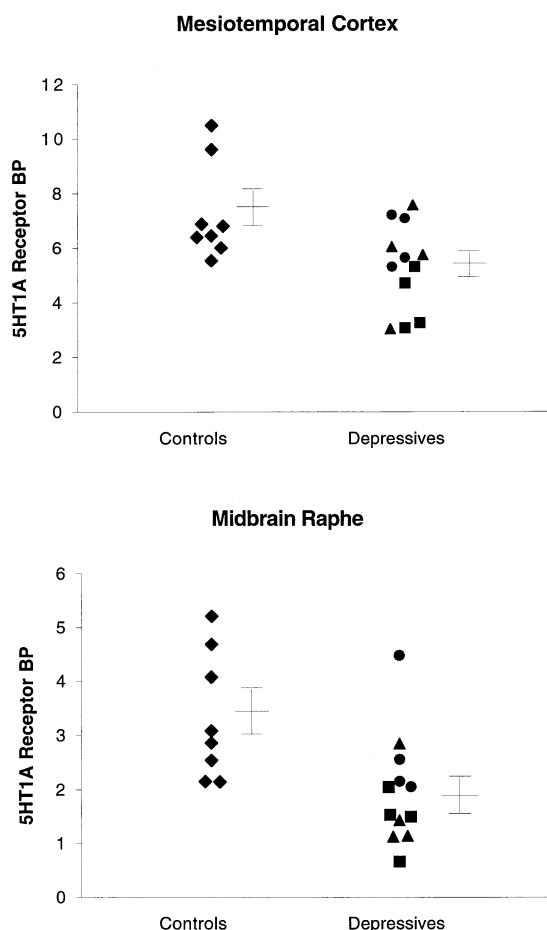


Figure 3. Scatter histograms of the 5HT1A receptor BP values for the depressed and control groups. Within the depressed sample, circles indicate unipolar depressives with only MDD relatives (MDD/mdr), triangles indicate unipolar depressives with BD relatives (MDD/bdr), and squares designate bipolar depressives (BD-D) with BD relatives. Mean and standard error bars appear to the right of each data set.

white matter within MRI-based ROI, such that 5HT1A receptor specific binding measures in PET images were lower than those expected from postmortem autoradiographic studies (Hall et al 1997). These effects particularly reduced the raphe BP values, because the small size of the raphe relative to the spatial resolution of PET resulted in extensive partial volume effects (Links et al 1996; Mazziotta et al 1981). The MTC-BP was also diluted by including areas of low-to-moderate 5HT1A receptor density (amygdala, periamygdaloid cortex) together with areas of high 5HT1A density (hippocampus, parahippocampal cortex) (Figure 1) (Hall et al 1997; Pazos et al 1987). Although higher BP values were obtained by defining ROI over the hippocampus alone (Figure 1), inclusion of both amygdala and hippocampus in the MTC-ROI simplified boundary definition to delineating grey matter-white mat-

ter or grey matter-CSF interfaces, increasing measurement reproducibility.

The use of MRI-based landmarks for defining ROI also reduced the variability of the PET measures. For example, our methods were similar to those of Gunn et al (1998) with respect to the image acquisition, the [*carbonyl*-¹¹C]WAY-100635 dose, and the reference-tissue model derivation of BP, but differed in that Gunn et al (1998) defined ROI directly on PET images without using MRI scans to constrain ROI placement. In healthy humans the magnitude of the mean BP values were similar across studies, but the standard deviations (SD) obtained by Gunn et al (1998) were higher. In the MTC Gunn et al (1998) reported a mean BP \pm SD of 7.65 ± 2.49 , compared to our control group mean of 7.28 ± 1.78 . In the raphe, our mean BP value (3.35) would have been the similar to that of Gunn et al (1998) except that one of their subjects had an outlying BP of 9.68 (Table 4 in Gunn et al 1998). Without this outlier, their mean BP value was 3.48 ± 1.13 , whereas with the outlier the mean BP was 4.52 ± 2.72 . The use of MRI images to select PET planes through the midbrain may thus play a critical role in reducing the variability of raphe measures. Defining ROI via MRI landmarks determined without knowledge of the corresponding PET measures also reduced the possibility that measurement bias contributed to intergroup differences.

The likelihood that abnormalities in regional BP in depression related to differences in the concentrations of free and nonspecifically bound radiotracer was reduced by the high selectivity of [*carbonyl*-¹¹C]WAY-100635 for 5HT1A receptors, as shown by the high specific-to-nonspecific binding, rank order of uptake across regions, and very low radioactivity concentration in cerebellum (Figures 1, 2) (Gunn et al 1998; Pike et al 1996). In vitro, WAY-100635 is >100 -fold selective for 5HT1A receptors relative to a variety of other receptors, reuptake sites, and ion channels (Fletcher et al 1993; Laporte et al 1994) and at 100 nmol/L does not bind significantly to 5HT7 sites (Forster et al 1995). The IC_{50} of WAY-100635 was 1.35 ± 0.44 nmol/L (displacement of specific [³H]8-OH-DPAT binding to hippocampal 5HT1A receptors) and the lowest IC_{50} at any other site tested was 230 ± 30 nmol/L (at the α_1 -adrenoreceptor site) (Fletcher et al 1993). In humans, the only radioactive metabolite of [*carbonyl*-¹¹C]WAY-100635 with high affinity for 5HT1A receptors is [*carbonyl*-¹¹C]desmethyl-WAY-100635, that Osman et al (1998) found detectable at only a "possible trace level" in plasma 60 min after injection. [*Carbonyl*-¹¹C]WAY-100635 is almost exclusively metabolized to [¹¹C]cyclohexane-carboxylic acid and more polar radioactive metabolites not expected to enter the brain or bind strongly to 5HT1A receptors in humans (Osman et al 1995, 1998; Pike et al 1996). [¹¹C]cyclohexane-carboxylic acid con-

tributes up to 8% of the radioactivity in cerebellum at 60 min in monkeys, leading to potential underestimation of BP by up to 8% (Gunn et al 1998). Because the depressives and controls metabolized [*carbonyl*- ^{11}C]WAY-100635 at a similar rate, however, it is unlikely that differences in the intravascular concentration of radiolabeled parent compound or polar metabolites contributed to the differences in mean BP between groups.

It is also unlikely that the BP abnormalities in depression were attributable to differences in cerebral blood volume (CBV) between depressives and controls. Gunn et al (1998) reported that adding a CBV term (fixed at 5%) increased the apparent BP obtained using the simplified reference tissue model by an average of 31%. The BP values obtained using a CBV term were tightly and linearly correlated (essentially at unity) with those computed without a CBV term (Gunn et al 1998), making the addition of a *fixed* CBV term irrelevant for assessing the significance and proportional magnitude of intergroup differences. Although, substantial alterations in CBV in depression may influence BP values, CBV would have to approach zero in depression before BP would decrease to the extent found. Measured CBV has not been evaluated in depression, but the relatively subtle abnormalities of cerebral blood flow found in depression are not expected to be associated with substantial alterations in CBV (Drevets et al 1999; Grubb et al 1978).

The specificity of the regional 5HT_{1A} receptor BP measures was limited most by the dependency of the modeling approach on the cerebellar [^{11}C]WAY-100635 concentration (Gunn et al 1998). The abnormal 5HT_{1A} receptor BP values in depression may thus be accounted for either by a reduction in the 5HT_{1A} receptor specific binding in the target ROI or by an elevation of the cerebellar [^{11}C]WAY-100635 concentration. Selecting between these interpretations may ultimately require postmortem measures of 5HT_{1A} receptor density that are independent of a reference tissue.

Comparisons with Postmortem Studies of 5HT_{1A} Receptor Binding

The limited postmortem data examining 5HT_{1A} receptors in subjects with primary mood disorders support the hypothesis that our findings reflect reductions in 5HT_{1A} receptor specific binding in the MTC and raphe. In six subjects with MDD who died by suicide (and were not chronically medicated before death), Lopez et al (1998) found abnormally decreased 5HT_{1A} receptor mRNA levels (that correlated with receptor density) by 31% to 49% across hippocampal subfields. Our finding that 5HT_{1A} receptor BP is also decreased in neocortical areas agrees with Bowen et al (1989), who observed that 5HT_{1A}

receptor B_{max} was decreased 24.5% in the temporal polar cortex, 24.2% in frontal opercular cortex, and 28.8% in superior parietal cortex in 7 antidepressant-medicated depressives with primary MDD ($n = 6$) or BD ($n = 1$) relative to controls (these differences reached significance only in comparisons of the receptor number for the entire ROI [nmol per region]). The similarity in the magnitudes of the cortical 5HT_{1A} receptor BP abnormalities we found using [^{11}C]WAY-100635 and those Bowen et al (1989) measured using [^3H]8-OH-DPAT binding are noteworthy because 5HT_{1A} receptor densities measured in vitro using [^3H]8-OH-DPAT are proportional to those using [^3H]WAY-100635 (Burnet et al 1997).

In the raphe, 5HT_{1A} receptor binding has not been evaluated in samples limited to primary MDD or BD subjects, but it is noteworthy that Baumann and Bogerts (1998) found reduced Nissl staining neurons in the DRN of both BD and MDD subjects relative to controls postmortem. Such an abnormality may be reflected in PET images by decreased 5HT_{1A} receptor BP because raphe neurons express 5HT_{1A} receptors. Potentially consistent with these data, a single photon emission tomographic study showed decreased brainstem-to-occipital cortex [^{123}I]β-CIT uptake in MDD subjects relative to controls, that was hypothesized to reflect a reduction in 5HT transporter sites (Malison et al 1998).

Our data in the raphe also seem compatible with the postmortem data of Kassir et al (1998) showing that both the total DRN area and the 5HT_{1A} receptor density were decreased in depressed, nonalcoholic suicide victims relative to controls. Both a reduction in DRN area and a reduction in 5HT_{1A} receptor B_{max} would appear in PET images as an abnormally decreased 5HT_{1A} receptor BP (Mazziotta et al 1981). The combined effects of these abnormalities were described by Kassir et al (1998) as a reduction in 5HT_{1A} receptor “binding capacity,” and the magnitude of the reduction in this parameter (50%) was similar to that of the 41.5% reduction in 5HT_{1A} receptor BP we found.

Another postmortem study reported, however, that the 5HT_{1A} receptor density was abnormally *increased* in suicide victims relative to controls (Stockmeier et al 1998). The discrepant results across suicide studies that included cases in whom the depressive syndrome arose secondary to substance dependence, medical illness, or psychiatric disorders other than MDD or BD indicate that the nonspecific presence of the major depressive *syndrome* or suicidal behavior provides neither sensitivity nor specificity for identifying subjects with abnormal 5HT_{1A} binding. Because many of the studies whose data are in conflict used similar methods for measuring 5HT_{1A} receptor density, discrepancies in their results are more likely accounted for by biological heterogeneity among

subjects prone to suicide. For example, whereas nonalcoholic suicide victims had abnormally reduced 5HT1A receptor density in and total area of the DRN (Kassir et al 1998), chronic alcoholics had an abnormally *increased* density (by 2.2-fold) of serotonergic neuronal processes in the DRN (Underwood et al 1998). These observations may explain the discrepant results between Kassir et al (1998) and Stockmeier et al (1998), as the former group studied only nonalcoholic, depressed suicides whereas the latter group included some depressed suicides with a history of alcohol dependence.

Our data more specifically suggest that the sensitivity for detecting abnormal reductions in 5HT1A receptor BP in depression may depend upon selecting depressives who have a family history of BD (Table 2, Figure 3). Family studies attempting to subdivide bipolar and unipolar illness demonstrate that the first degree relatives of adult BD probands are at increased risk for both bipolar and unipolar disorders when compared with relatives of unipolar probands or with the general population (Rice et al 1987). The bipolar genotype may thus be manifested as either BD or MDD phenotypes (Drevets and Todd 1997; Rice et al 1987). Nevertheless, bipolar and unipolar disorders are transmitted to some extent independently, and heritable mood disorders in families containing only unipolar depression may be genetically and pathophysiologically distinct from those in families containing BD (Moldin et al 1991). Future studies may establish whether 5HT1A receptor binding abnormalities distinguish between such disorders or pedigrees.

Possible Neurobiological Correlates of Reduced 5HT1A Receptor BP in Depression

Abnormally decreased 5HT1A receptor BP in depression may reflect either a down-regulation of 5HT1A receptor gene expression, or a reduction in the number of cellular processes expressing 5HT1A receptors. Postsynaptic 5HT1A receptor down-regulation is not expected to reflect a compensatory response to abnormal 5HT release, because reducing 5HT transmission by lesioning the raphe or administering 5HT synthesis inhibitors does not alter 5HT1A receptor binding in the cerebral cortex or the hippocampus (Frazer and Hensler 1990; Hensler et al 1991; Pranzatelli 1994; Verge et al 1986) and increasing 5HT transmission via chronic administration of SSRI or monoamine oxidase inhibitor (MAOI) antidepressant drugs (AD) does not consistently alter 5HT1A receptor density or mRNA concentrations in the cortex, hippocampus, or amygdala (Carli et al 1996; Hensler et al 1991, Spurlock et al 1994, Welner et al 1989). In the raphe, chronic SSRI and MAOI administration desensitizes presynaptic somatodendritic 5HT1A receptors, evidenced in

rats by an increase in the amount of 5-HT released per stimulation-triggered action potential (Chaput et al 1991) and in humans by an attenuation of the hypothermic response to ipsapirone (Lesch et al 1990a, 1991). It is unclear, however, that this AD-induced desensitization of the 5HT1A autoreceptor will be evident in [¹¹C]WAY-100635 images, because [³H]8-OH-DPAT binding in the raphe is not consistently altered by chronic SSRI administration in rats (Frazer and Hensler 1990; Welner et al 1989). Although the 5HT1A receptor BP values did not correlate with the time since last antidepressant exposure, the possibility that the raphe 5HT1A receptor BP was reduced in the depressives because of previous AD exposure must be addressed in longitudinal studies.

Down-regulation of hippocampal 5HT1A receptor gene expression by cortisol hypersecretion in some depressives may contribute to the abnormally reduced 5HT1A receptor BP in the MTC (Lopez et al 1998; Meijer et al 1997). In rats the magnitude of the reduction in 5HT1A receptor mRNA levels induced by chronic unpredictable stress averaged 22% across hippocampal subfields (Lopez et al 1998), similar to the 25% reduction in hippocampal 5HT1A receptor BP we found. A reduction of 5HT1A receptor BP associated with hypercortisolemia may prove specific to subjects with LHPA-axis dysfunction—who are more common among BD and familial MDD subgroups (Arana et al 1985; Lewis et al 1984; Winokur 1982). Nevertheless, the relationship between hippocampal 5HT1A receptor BP and cortisol secretion should be complex, because down-regulation of 5HT1A receptor gene expression is thought to comprise a compensatory mechanism for inhibiting cortisol release (5HT1A receptor stimulation increases plasma corticotropin and cortisol secretion; Lesch 1992; McEwen 1995). Reduced hippocampal 5HT1A receptor BP may thus correlate with the recent propensity to hypersecrete cortisol in depression (Nemeroff et al 1984; Young et al 1993), but to the extent that this response maintains cortisol levels near the normal range, current endocrine measures may no longer reflect cortisol hypersecretion. This interaction may explain the lack of correlation between hippocampal 5HT1A receptor BP and 24-hour UFC or plasma cortisol levels; however, a more widespread reduction of 5HT1A receptor BP would not seem to be explained by a cortisol effect, because chronic stress and corticosteroid administration do not alter 5HT1A receptor binding in the raphe, amygdala or cerebral cortex in rats (Chalmers et al 1993; McKittrick et al 1995).

Serotonin-1A receptors are expressed presynaptically on 5HT neurons in the raphe and postsynaptically on pyramidal neurons, some GABA-ergic interneurons, astrocytes, and some other glia in the limbic and neocortex (Azmitia et al 1996). Neuropathological studies of the

MTC have reported abnormally reduced grey matter wet weight in the parahippocampal gyrus in depressives with MDD or BD (Bowen et al 1989), decreased nonpyramidal neuron counts in sector CA2 of the hippocampus in BD (Benes et al 1998), and reduced cross-sectional area of the right hippocampus in suicide victims (Altshuler et al 1990). Most morphometric MRI studies, however, have not found differences in hippocampal volume between depressives and controls (Axelson et al 1993; Hauser et al 1989; Pearlson et al 1997) and those that have reported abnormalities that are subtle (Sheline et al 1996; Swayze et al 1992). Moreover, in BD the amygdala volume was found to be abnormally decreased by Pearlson et al (1997), but abnormally increased by Altshuler et al (1998). Finally, the whole brain volume was virtually identical in the depressives and controls studied herein, and in most previous studies of depressives in the age range studied (e.g., Drevets et al 1997). The apparently widespread reduction in postsynaptic 5HT_{1A} receptor binding is thus not expected to reflect generalized decrease in neurons or neuropil.

The recent findings that glial cell numbers are abnormally reduced in parts of the ventral PFC (Öngür et al 1998; Rajkowska et al 1998) and amygdala (Öngür and Price, personal communication) in MDD and BD suggests another histopathological process that may be associated with decreased 5HT_{1A} receptor BP. In the limbic forebrain and cerebral cortex glia abundantly express 5HT_{1A} receptors (Azmitia et al 1996), so a reduction in glia may appear in [¹¹C]WAY-100635 images as decreased 5HT_{1A} receptor BP; however, glial and neuronal counts and grey matter volume were all normal in MDD and BD in the postcentral gyrus (specifically BA 3b; Öngür et al 1998), so the reduction in 5HT_{1A} receptor BP in this area would not seem directly accounted for by a reduction in glial-based 5HT_{1A} receptors.

The reduction in glia may, however, more generally affect 5HT_{1A} receptor BP via the role of glia in serotonergic neuronal development and plasticity. Stimulation of astrocyte and radial glial cell-based 5HT_{1A} receptors during fetal development and subsequently during 5HT neuronal injury results in release of the trophic factor S100 β , that promotes 5HT neuronal arborization (Azmitia et al 1991, 1996). If glial function is reduced during 5HT system development in BD and MDD, it is conceivable that arborization of the 5HT axonal tree may be attenuated, potentially resulting in a reduction of synaptogenesis in the limbic and neocortex, as reflected by widespread reductions of postsynaptic 5HT_{1A} and 5HT_{2A} receptor expression (Bowen et al 1989). Such a neuropathological process may also underlie an hypoplasia of the 5HT neuronal structure in the DRN, yielding the observed reductions of DRN Nissl staining neurons, area, and

5HT_{1A} receptor binding (Baumann and Bogerts 1998; Kassir et al 1998).

Implications for Antidepressant Treatment Mechanisms

The PET and postmortem data reviewed herein are compatible with neuroendocrine, CSF 5HT metabolite, and pharmacological evidence showing that 5HT function in general and 5HT_{1A} receptor function in particular are abnormally decreased in mood disorders (Lesch et al 1992; Maes and Meltzer 1995). The effect of chronic SSRI and MAOI administration of desensitizing presynaptic somatodendritic 5HT_{1A} autoreceptors may compensate for blunted 5HT_{1A} receptor function by increasing the amount of 5HT released per action potential (Cervo et al 1988; Chaput et al 1991; Charney et al 1991). Moreover, chronic tricyclic antidepressant and electroconvulsive shock (ECS) administration result in tonic activation of postsynaptic 5HT_{1A} receptors in the hippocampus (Haddjeri et al 1998), potentially compensating for reduced 5HT_{1A} receptor BP (Chaput et al 1991; Detke et al 1995). In the hippocampus and amygdala postsynaptic 5HT_{1A} receptors are particularly abundant on the axon hillock of pyramidal neurons where, when stimulated, they inhibit action potential formation (Andrade and Nicoll 1987; Azmitia et al 1996; Colino and Halliwell 1987; Haddjeri et al 1998; Sprouse and Aghajanian 1988). The tonic activation of postsynaptic 5HT_{1A} receptors produced by chronic AD and ECS results in tonic inhibition of pyramidal neuron firing rates (Haddjeri et al 1998), an effect that may be particularly relevant to the therapeutic mechanisms of AD in the amygdala (Drevets et al 1992, 1995, 1996, 1999; Wang and Aghajanian 1980).

Summary

Using PET and [¹¹C]WAY-100635 we demonstrated abnormal 5HT_{1A} receptor binding in the MTC and midbrain raphe in unmedicated, major depressives. The abnormality in the midbrain may reflect the histological abnormalities in the DRN reported in recent postmortem studies of MDD and BD (Baumann and Bogerts 1998) and suicide (Kassir et al 1998). The reduction in 5HT_{1A} receptor BP in the MTC was similar in magnitude to associated reductions in the neocortex, and may thus reflect a widespread alteration of postsynaptic 5HT_{1A} receptor expression.

Supported by NIH grants MH59769, MH30915, MH51137, MH54715, and MH52247.

The authors thank Dr. Bruce McEwen for discussions related to the design and interpretation of the image data, Dr. Roger Gunn for providing software and advice on implementing the reference tissue

model used in the image analysis, Dr. David Townsend and Dr. Carolyn Meltzer for guidance related to image acquisition and analysis, Shawnette Proper and Rob Sembrat for assistance with image analysis, Sue Morris, RN and Michele Drevets, RN for assistance with subject recruitment and assessment, and the UPMC PET Laboratory staff for technical support.

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