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Antagonist but not agonist labeling of serotonin-1A receptors is decreased in major depressive disorder

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Abstract

Serotonin-1A receptors may play a role in the pathophysiology of depression and suicide. In postmortem brain tissue, agonist binding to serotonin-1A receptors is reportedly increased or unchanged in depression or suicide, while neuroimaging studies report a decrease in antagonist binding to these receptors in subjects with depression. In this study, both agonist and antagonist radioligand binding to serotonin-1A receptors were examined in postmortem orbitofrontal cortex from subjects with major depressive disorder (MDD). Brain tissue was collected at autopsy from 11 subjects with MDD and 11 age- and gender-matched normal control subjects. Two depressed subjects had a recent psychoactive substance use disorder. Six subjects with MDD had a prescription for an antidepressant drug in the last month of life, and, of these six, postmortem bloods from only two subjects tested positive for an antidepressant drug. There was no significant difference between cohorts for age, postmortem interval or tissue pH. The receptor agonist [^{3}H]8-OH-DPAT or the antagonist [^{3}H]MPPF were used to autoradiographically label serotonin-1A receptors in frozen sections from cytoarchitectonically-defined left rostral orbitofrontal cortex (area 47). There was no significant difference between depressed and control subjects in agonist binding to serotonin-1A receptors. However, antagonist binding was significantly decreased in outer layers of orbitofrontal cortex in MDD. This observation in postmortem tissue confirms reports using an antagonist radioligand in living subjects with depression. Decreased antagonist binding to serotonin-1A receptors in outer layers of orbitofrontal cortex suggests diminished receptor signaling and may be linked to corresponding neuronal changes detected previously in these depressed subjects.

Keywords

Serotonin-1A receptor; Depression; Postmortem; Orbitofrontal cortex

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Conflict of Interest

Grazyna Rajkowska, Ph.D., is a consultant for Eli Lilly and Company. Eimear Howley, M.S., is employed by GlaxoSmithKline, Harlow, Essex, UK, a manufacturer of antidepressant medications. All other authors declare that there are no financial interests or conflicts of interest to disclose.

1. Introduction

Various approaches in clinical research implicate serotonin in the pathophysiology of depression. Evidence for the involvement of serotonin in depression and its treatment comes from reports of the efficacy of chronic antidepressant treatments that enhance serotonin neurotransmission, of tryptophan depletion studies in remitted subjects with depression, of diminished neuroendocrine responses to serotonergic stimuli in depression, of changes in the serotonin transporter and serotonin receptors in brain imaging or studies of postmortem brain in depression, and of a polymorphism of the promoter gene for the serotonin-1A receptor associating with depression (Blier and de Montigny, 1999; Mann, 1999; Meyer et al., 2003; Stockmeier, 2003; Lemonde et al., 2003; Drevets et al., 2007).

Probing serotonin receptor binding characteristics in vivo using positron emission tomography (PET) or in postmortem brain tissue is a useful although somewhat indirect means of assessing the serotonergic system in depression. Using the serotonin-1A receptor antagonist [^{11}C] WAY-100635, binding potential is significantly decreased in the orbitofrontal, anterior cingulate, occipital, or parietal cortex in medicated or unmedicated subjects experiencing a major depressive episode and is decreased in subjects whose MDD is in remission and are free of antidepressant medication for six or more months (Drevets et al., 2000, 2007; Sargent et al., 2000; Bhagwagar et al., 2004). However, other neuroimaging studies report either no change in binding potential to serotonin-1A receptors in these regions in depressed subjects exposed to antide-pressants or an increase in binding in antidepressant-naïve subjects with MDD (Meltzer et al., 2004; Parsey et al., 2006).

Serotonin-1A receptors have been examined in postmortem cerebral cortex in suicide victims and subjects with a history of a mood disorder using the selective serotonin-1A receptor agonist [^{3}H]8-hydroxy-2-(di-n-propyl)aminotetralin (8-OH-DPAT). These studies using a radiolabeled agonist have yielded varied results. Matsubara et al. (1991) detected an increase in the number of serotonin-1A receptors in dorsolateral prefrontal cortex (dlPFC, Brodmann areas 8+9) of suicide victims dying of so-called non-violent means. Arango et al. (1995) reported increases in radioligand binding to serotonin-1A receptors in ventrolateral (area 45 and area 46) but not in other areas of prefrontal cortex of suicide victims. In contrast, other studies reported no significant changes in agonist-labeled serotonin-1A receptors in prefrontal cortex, even when suicide victims were classified as either depressed or not depressed (Dillon et al., 1991, dlPFC areas 8+9; Arranz et al., 1994, anterior dorsolateral and orbitofrontal cortex, areas 9+10+11; Lowther et al., 1997, anterior prefrontal area 10, and occipital areas 17+18; Stockmeier et al., 1997, area 10).

One reason for the differing results between studies of living subjects with depression and postmortem tissue from subjects with depression could be in the choice of radioligand. The serotonin-1A receptor can be either coupled to or uncoupled from a GTP binding protein. The serotonin-1A receptor agonist, 8-OH-DPAT, used in all postmortem studies, binds only to the coupled receptor, whereas the receptor antagonist WAY-100635, used in neuroimaging studies, binds to both the coupled and uncoupled receptor (Gozlan et al., 1995; Palego et al., 1997; Lanfumey and Hamon, 2004). Thus, neuroimaging studies in cerebral cortex using a receptor antagonist may be detecting a decrease in the total population of serotonin-1A receptors (coupled plus uncoupled) in depression; whereas agonist binding only to G-protein coupled receptors may not be affected in depression. Independent of the issue of radioligands used for receptor labeling, the expression of mRNA for the serotonin-1A receptor was significantly decreased in superficial layers of dorsolateral prefrontal cortex of subjects with MDD (Lopez-Figueroa et al., 2004).

A newer radioligand antagonist for the serotonin-1A receptor, [^{3}H]4-(2'-methoxy-)-phenyl-1-[2'-(N-2"-pyridal)-p-fluorobenzamido]ethyl-piperazine (MPPF), has been developed (Kung et al. 1996). MPPF has lower affinity than WAY-100635 for the serotonin-1A receptor and, as such, the binding of MPPF may be more responsive to changes in endogenous levels of serotonin (Passchier et al., 2000). However, the binding potential of [^{18}F]MPPF to serotonin-1A receptors is not significantly affected by short-term tryptophan depletion or by physiological increases in synaptic serotonin levels or by chronic treatment with fluoxetine, an antidepressant medication that selectively blocks the reuptake of serotonin (Praschak-Rieder et al., 2004; Udo de Haes et al., 2006; Aznavour et al., 2006).

The objective of this study was to examine agonist vs. antagonist radioligand binding to serotonin-1A receptors in adjacent sections of postmortem orbitofrontal cortex from subjects with MDD as compared to normal control subjects. It was hypothesized that antagonist but not agonist binding to serotonin-1A receptors would be significantly decreased in depression. It was also of interest to determine whether any putative lamina-specific changes in serotonin-1A receptors corresponded to neuroanatomical changes detected in immediately adjacent orbitofrontal cortex of the same depressed subjects; changes such as reduced thickness of gray matter and altered neuronal and glial density and neuronal soma size (Rajkowska et al., 1999).

2. Method

2.1. Human subjects and tissue selection

Brain tissue was obtained at autopsy at the coroner's office of Cuyahoga County, Cleveland, OH. The study was performed according to the declaration of Helsinki and with a protocol approved by the University Institutional Review Board. Informed written consent was obtained from the next-of-kin for all subjects. Characteristics of the control and depressed subjects are recorded in Table 1 and Table 2. A coronal block of left orbitofrontal cortex (rostral part of Brodmann area 47) was collected, frozen in isopentane cooled by dry ice and stored at −80 °C. The frozen piece of orbitofrontal cortex was located immediately caudal to fixed coronal sections defined by cytoarchitectonic criteria and taken from all of the same depressed and many of the same control subjects for cell counting examination by Rajkowska et al. (1999).

Psychiatric assessments of all subjects were performed via informant-based retrospective interviews as previously described (Stockmeier et al., 1998). A trained interviewer administered the schedule for affective disorders and schizophrenia: lifetime version (SADS-L; Spitzer and Endicott, 1978) to next-of-kin about three months after the death to determine current and lifetime axis I psychopathology. Diagnoses for axis I major mental disorders were independently assessed by a clinical psychologist and a psychiatrist, and consensus diagnosis was reached in conference using information from the knowledgeable informants, the coroner's office, and available inpatient and outpatient records. Eleven subjects met the DSM-IV criteria for MDD (American Psychiatric Association, 1994). Ten of these subjects were experiencing a depressive episode within the last two weeks of their lives, and one subject with MDD was in remission. Six subjects with MDD had an antidepressant drug prescription in the last month of life, while postmortem blood from only two subjects tested positive for an antidepressant drug. Medication histories for the depressed subjects are included in Table 2. One subject with MDD also met criteria for polysubstance dependence and another subject for polysubstance abuse.

There was no significant difference between subject groups in age, postmortem interval or tissue pH. The average age (years, mean ± SEM) of the two groups was – Control: 44.1 ± 4.3, MDD: 47.5 ± 4.8. The average postmortem interval (hours, mean ± SEM) of the two groups

was – Control: 19.9 ± 2.0, MDD: 20.9 ± 1.3. The average tissue pH (mean ± SEM) was – Control: 6.75 ± 0.06, MDD: 6.51 ± 0.09.

2.2. Receptor autoradiography

Autoradiographic measurement of serotonin-1A receptor binding was performed using the receptor agonist [^{3}H]8-hydroxy-2-(di-n-propyl)aminotetralin (8-OH-DPAT) (Pazos et al., 1987; Stockmeier et al., 1998) or the antagonist [^{3}H]4-(2'-methoxy-)-phenyl-1-[2'-(N-2"-pyridal)-p-fluorobenzamido]ethyl-piperazine (MPPF) (Kung et al., 1996). Triplicate (total binding) and duplicate (nonspecific binding) frozen sections (20 μ) of rostral orbitofrontal cortex were thawed and air-dried. After a 30 min pre-incubation at room temperature in buffer containing 170 mM Tris-HCl, 4 mM $CaCl_2$, and 0.01% ascorbic acid (pH 7.7, 24 °C), three sections were incubated in fresh buffer for one hour at 24 °C with [^{3}H]8-OH-DPAT (2 nM, 162.9 Ci/mmol, New England Nuclear, Boston, MA). Nonspecific binding was measured in duplicate serial sections co-incubated with 10 μM serotonin (serotonin–creatinine sulfate complex, Sigma Chemical, St. Louis, MO). Citalopram–hydrobromide (1 μM, Lundbeck, Copenhagen, Denmark) was included in the incubation with [^{3}H]8-OH-DPAT to prevent radioligand binding to the serotonin transporter. Adjacent sections were similarly processed for antagonist radioligand binding. For antagonist binding, after a 30 min pre-incubation at room temperature in buffer containing 50 mM Tris-HCl and 2 mM $MgCl_2$ (pH 7.4, 24 °C), three sections were incubated in fresh buffer for 90 min at 24 °C with [^{3}H]MPPF (1.9 nM, 70.5 Ci/mmol, New England Nuclear). Nonspecific binding was measured in duplicate serial sections co-incubated with 10 μM serotonin (serotonin–HCl, Sigma, St. Louis, MO). Prazosin-HCl (100 nM, RBI, Natick, NJ) and S(–)-sulpiride (1 μM, RBI) were included in the incubation with [^{3}H]MPPF to prevent radioligand binding to alpha-1 adrenergic and dopamine-2 receptors, respectively.

After the incubation, the [^{3}H]8-OH-DPAT-labeled sections were washed twice at 4 °C for five minutes in non-radioactive assay buffer (pH 7.7 at 4 °C), and the [^{3}H]MPPF-labeled sections were washed twice at 4 °C for ten minutes in non-radioactive assay buffer (pH 7.4 at 4 °C). Sections were then dipped in ice-cold water, air-dried, and stored for 24–48 h at 4 °C in sealed slide boxes with capsules containing desiccant. The sections and tritiated plastic standards (American Radiolabeled Chemicals, St. Louis, MO) were apposed to Hyperfilm-^{3}H (Amersham, Arlington Heights, IL) in X-ray cassettes for 8 weeks ([^{3}H]8-OH-DPAT) or 8.5 weeks ([^{3}H]MPPF). Films were processed with Kodak D-19 developer and fixed with Kodak Rapid Fix (Eastman Kodak, Rochester, NY).

Autoradiographic images were digitized and quantified using the microcomputer controlled imaging device (MCID-M5, Imaging Research, Inc., St. Catherines, Ontario). Sections used for radioligand binding were later stained for Nissl substance with cresyl echt violet to facilitate the cytoarchitectonic identification of individual cortical laminae. Only cortical laminae with autoradiographic images registering a relative optical density between 0.2 and 0.8 were quantified. At the optimized exposure times listed above, only antagonist binding in layers I–III and agonist binding in layers I–II met this criterion. Calibrated images of total and nonspecific radioligand binding were digitized and sampled, and nonspecific binding was subtracted from total binding to yield specific binding. Specific binding was between 90 and 96 percent of total binding. The results are expressed in terms of calibrated standards. Both the tissues and the resultant autoradiograms from the matched pairs of subjects were processed and analyzed in parallel, and laboratory personnel were blind to the clinical diagnoses of the subjects.

2.3. Statistical analysis

The main statistical analysis of all 22 subjects was performed with a mixed model ANOVA (SAS, Cary, NC) with one of five dependent measures (antagonist labeling in layers I, II, and III, and agonist labeling in layers I and II). Diagnosis was modeled as a fixed effect and between cohorts pairing of subjects (by age) as a random effect. In addition to these three ANOVA models, 15 ANCOVA models were also evaluated, using the three covariates (age, postmortem interval, and tissue pH) one at a time, along with the five dependent measures listed above. When examining the potential effect of gender, suicide or clinical variability, Student's *t*-test was used because of a relatively small number of subjects. Six male depressed subjects were compared with six male controls, and five female depressed subjects were compared with five female controls. Because of the clinical variability of four depressed subjects, the 11 controls were also compared with seven depressed subjects with the following depressed subjects omitted in this statistical test: one subject with MDD that was in full remission, one subject with polysubstance dependence, one subject with sertraline present in plasma, and one subject with polysubstance abuse and amitriptyline present in plasma. The main effect of depression persisted when controlling for the potential confounds of gender or clinical variability. Spearman's rank correlation coefficients were calculated to evaluate potential interactions between [^{3}H]MPPF binding and age of onset or duration of depression.

3. Results

Subjects with MDD showed a statistically significant decrease in radioligand binding of [^{3}H]MPPF, the serotonin-1A receptor antagonist, in the rostral orbitofrontal cortex (Fig. 1 and Fig. 2). There was a significant MDD-related decrease in antagonist binding in layer I (16 percent decrease, $F = 16.88$, df = 20, $p = 0.0005$), layer II (18 percent decrease, $F = 13.11$, df = 20, $p = 0.0017$), and layer III (21 percent decrease, $F = 5.43$, df = 20, $p = 0.0304$). In contrast, there was no statistically significant difference between controls and depressives in the binding of [^{3}H]DPAT, the serotonin-1A receptor agonist, in layer I ($F = 3.81$, df = 19, $p = 0.0658$) or in layer II ($F = 3.42$, df = 19, $p = 0.0799$) (Fig. 1 and Fig. 2). In the mixed model covariate analysis, none of the three covariates (age, postmortem interval, or tissue pH) made a statistically significant contribution to the model, and the statistical differences noted above with the ANOVA were essentially replicated (data not shown). Specifically, subjects with MDD showed a significant decrease in antagonist but not agonist binding to serotonin-1A receptors when co-varying for age, postmortem interval, or tissue pH.

The potential influence of gender, clinical variability, suicide or allele or genotype frequency was also considered. The observation of a statistically significant decrease in [^{3}H]MPPF but not [^{3}H]DPAT binding was noted in both male and female depressed subjects (mean ± SEM, fmol/mg; layer I, control males: 163.6 ±04.6, MDD males: 147.2 ± 3.4, $p = 0.0157$; layer II, control males: 189.1 ± 4.6, MDD males: 161.4 ±10.0, $p = 0.0304$; layer I, control females: 166.6 ± 9.9, MDD females: 127.3 ±5.8, $p = 0.009$; layer II, control females: 183.1 ± 12.4, MDD females: 144.4 ± 9.2, $p = 0.0329$. A statistically significant decrease in [^{3}H]MPPF but not [^{3}H]DPAT binding was also noted when the following four depressed subjects were omitted: one subject with MDD that was in full remission, one subject with comorbid polysubstance dependence, one subject with sertraline present in plasma, and one subject with comorbid polysubstance abuse and amitriptyline present in plasma (mean±SEM, fmol/mg; layer I, control: 165.0 ± 4.9, MDD: 139.9 ± 4.8, $p = 0.0032$; layer II, control: 186.8 ± 5.8, MDD: 156.6 ± 9.2, $p = 0.0101$). The binding value for the depressed subject in remission was less than the mean of the depression cohort. There were no significant correlations between the age of onset (layer I, $r = 0.0137$, $p = 0.967$; layer II, $r = -0.137$, $p = 0.967$) or the duration (layer I, $r = -0.3503$, $p = 0.286$; layer II, $r = 0.0599$, $p = 0.860$) of depression and binding values for [^{3}H]MPPF. Regardless of whether the depressed subject committed suicide, the binding to

antagonist-labeled serotonin-1A receptors was also decreased in depression, compared to control subjects (mean ± -SEM, fmol/mg; layer I, control: 165.0 ± 4.9, suicide: 139.4 ± 5.3, $p = 0.0047$; control: 165.0 ± 4.9, non-suicide: 136.6 ± 7.8, $p = 0.0067$; layer II, control: 186.8 ± 5.8, suicide: 159.9 ± 9.9, $p = 0.0243$; control: 186.8 ± 5.8, non-suicide: 146.0 ± 10.0, $p = 0.0023$). Based on χ^2 analysis of genotype frequencies and comparing allele frequencies with Fisher's exact test, no statistically significant difference in the allele or genotype frequency between control and depressed subjects for the novel C(-1019)G polymorphism was observed (A. Burns and P. Albert, unpublished observation).

4. Discussion

Serotonin-1A receptors were examined autoradiographically in the rostral orbitofrontal cortex of subjects with MDD and age- and gender-matched control subjects. There was a statistically significant effect of diagnosis, in which subjects with MDD had decreased antagonist but not agonist binding to serotonin-1A receptors in superficial layers of orbitofrontal cortex. There was a trend for a reduction in agonist binding to serotonin-1A receptors but it did not reach statistical significance. Covariate statistical analysis revealed that age, postmortem interval or tissue pH did not significantly contribute to the variance among subjects of either cohort. Although the sample size was small for considering a potential influence of gender, antagonist binding to serotonin-1A receptors was significantly decreased in both male and female subjects with depression. A reduction in autoradiographic image intensity in MDD could correspond to either a decrease in serotonin-1A receptor number or affinity, and the current data set does not distinguish between these two possibilities. A reduction in receptor number in depression might be supported by the observation of Lopez-Figueroa et al. (2004) that the expression of mRNA for serotonin-1A receptors was significantly decreased in superficial layers of dorsolateral prefrontal cortex (areas 9 and 46) in MDD. A reduced number of receptors in depression may limit the ability of the brain to compensate under stressful situations where glucocorticoids are known to downregulate serotonin-1A receptor expression (Chalmers et al., 1993; Ou et al., 2001).

A decrease in function in serotonin-1A receptors in orbitofrontal cortex in depression may have a significant effect on prefrontal and subcortical circuitry involved in depression. PET studies record abnormal blood flow or glucose metabolism in the orbital and medial prefrontal cortex, dorsolateral prefrontal cortex, subgenual cingulate cortex and amygdala in depression (Mayberg et al., 1999; Drevets, 2000). Studies in non-human primates reveal that layer III of the orbitofrontal cortex is the main connection between this region and associational and limbic cortical regions (Carmichael and Price, 1995; Rempel-Clower and Barbas, 2000). Layers III and V of the orbitofrontal cortex send axons to the amygdala and layers II and VI receive projections from the amygdala (Aggleton et al., 1980; Porrino et al., 1981). Hence, altered serotonin-1A receptor binding in outer layers of orbitofrontal cortex in depression may significantly contribute to disrupted forebrain circuitry involved in depression.

All of the subjects with MDD and several of the control subjects in the current study were examined morphometrically in an adjacent, formalin-fixed portion of orbitofrontal cortex (Rajkowska et al., 1999). In that study, subjects with depression exhibited a reduction in the thickness of cortical gray matter, smaller sizes of neuronal cell bodies and a lower density of large neurons accompanied by an increase in the density of smaller neurons. This neuronal pathology was most prominent in outer cortical layers II and III where antagonist binding to serotonin-1A receptors is down-regulated. In contrast, changes in glial density were noted in mid to deeper cortical layers in the same depressed subjects (Rajkowska et al., 1999).

The neuropathological and serotonin-1A receptor changes observed postmortem in superficial prefrontal cortical layers may be linked with changes in GABA and glutamate detected in the

cerebral cortex of depressed subjects (Sanacora et al., 2004; Hasler et al., 2007; Maciag et al., 2007; Rajkowska et al., 2007). The level of GABA is reduced in depressed subjects not taking antidepressant drugs but these levels normalized with treatment by selective serotonin reuptake inhibitors (SSRI)(Sanacora et al., 2002). The therapeutic effect of SSRI's may involve interactions between serotonin-1A receptors, GABA and glutamate in outer layers of prefrontal cortex.

The serotonin-1A receptor gene contains a novel C(-1019)G polymorphism and the homozygous G(-1019) allele is enriched two-fold in depressed subjects and enriched four-fold in suicide victims relative to control subjects (Lemonde et al., 2003). However, in our small number of samples, no statistically significant difference in the allele or genotype frequency was observed between control or depressed subjects (A. Burns and P. Albert, unpublished observation). Although the genotype frequency analysis is significantly underpowered, these data suggest that the decrease in antagonist binding to serotonin-1A receptors is likely not due to a mismatch in allele or genotype frequency between the control and depressed subjects.

Serotonin-1A receptor protein was recently measured in prefrontal cortex (area 10, right hemisphere) by Western blotting in depressed and control subjects not included in this study. Receptor protein levels were significantly decreased in females but not males with depression (Szewczyk et al., 2008), whereas the present study revealed a decrease in serotonin-1A binding sites in both females and males with MDD. A possible reason for the difference in results between Szewczyk et al. (2008) and the current study is that two different cortical regions (orbitofrontal vs. frontal pole) were examined in different hemispheres.

There are limitations in this report of decreased antagonist binding to serotonin-1A receptors in MDD. The number of subjects in the study is relatively small, and the results are presented for only one cerebral cortical region. However, our finding of diminished antagonist binding to serotonin-1A receptors is strengthened by the 3-dimensional cell counting study in the same brain region in these depressed subjects (Rajkowska et al., 1999), and are consistent with the PET studies of Drevets et al. (2000; 2007) and Sargent et al. (2000).

Another limitation of this study is the clinical variability in the depressed subjects. While suicide was ruled the cause of death for six of the eleven depressed subjects, it did not alter the main effect of depression. In addition, MDD was in full remission in one subject, and two of the subjects with MDD had comorbid diagnoses of polysubstance dependence or polysubstance abuse. The statistically significant finding of a decrease in antagonist radioligand binding to serotonin-1A receptors in all eleven depressed subjects persisted when data were reanalyzed after excluding four subjects (with a comorbid psychoactive substance use disorder, with an antidepressant medication present in blood, or with MDD in full remission). It does not appear likely that such a prescription alone would precipitate a decrease in antagonist binding to serotonin-1A receptors in MDD as there was no difference in binding between depressed subjects with a history of antidepressant treatment and those without. Furthermore, serotonin-1A binding potential is significantly decreased in orbitofrontal and other prefrontal cortical regions in 1) depressed subjects taking fluoxetine or sertraline, 2) depressed subjects that were medication-free for over one year, and 3) depressed subjects never medicated (Sargent et al., 2000). However, Parsey et al. (2006) reported no change in serotonin-1A binding potential in subjects with MDD recently treated with antidepressant medications and an increase in this binding potential in never medicated subjects with MDD. These varying neuroimaging results may be related to a methodological distinction in that Sargent et al. (2000) used cerebellar gray matter and Parsey et al. (2006) used cerebellar white matter as a reference tissue for determining free ligand concentration and nonspecific binding.

The question arises as to the significance of a decrease in antagonist but not agonist radioligand binding in cerebral cortex in MDD. The serotonin-1A receptor exists in two states: either coupled or uncoupled to a GTP-binding protein such as Gαi or Gαo (Gozlan et al., 1995; Lanfumey and Hamon, 2004). The agonist 8-OH-DPAT binds only to the coupled receptor, while antagonists such as WAY-100635 or MPPF bind to both the coupled and free receptor. Consequently, the density of antagonist-labeled sites is 60–70% higher than agonist-labeled sites in human brain (Burnet et al., 1997). Thus, the current data reporting a decrease in antagonist but not agonist binding in depression suggest a decrease in the number or affinity of serotonin-1A receptors not coupled to Gαi/o protein. A decrease in the number or affinity of the total receptors, as assessed with the receptor antagonist, may restrict the reserve of receptors available to couple to Gαi/o proteins, and thus diminish receptor signaling when additional reserve is needed. There was a trend for a reduction in agonist binding to serotonin-1A receptors, suggesting that a small reduction in receptors coupled to a GTP-binding protein may have contributed to the decrease in total serotonin-1A receptors as measured with the antagonist.

The cellular and laminar location of serotonin-1A receptors is a critical issue regarding the functional significance of a decrease in serotonin-1A receptor binding in depression. In response to stimulation of the dorsal and median raphe nuclei and the release of serotonin in cerebral cortex, serotonin-1A receptors mediate the inhibitory effect of serotonin on cortical pyramidal neurons (Amargos-Bosch et al., 2004). In monkey and human temporal cortex, DeFelipe et al. (2001) found serotonin-1A receptor immunoreactivity on axons of pyramidal neurons proximal to input from GABA-containing chandelier interneurons. These authors also report that soma and proximal portions of dendrites of other neurons were also immunostained but with lower intensity than labeling of the initial segments of pyramidal axons. The neurons immunostained with lower intensity may correspond to the GABA interneurons in rat cerebral cortex that express mRNA or immunoreactivity for the serotonin-1A receptor (Aznar et al., 2003; Santana et al., 2004). Thus, the decrease observed here in antagonist binding to serotonin-1A receptors may reflect a decrease in serotonin-1A receptors localized to GABA interneurons or to the axon initial segments of pyramidal neurons. Consistent with this suggestion, Rajkowska et al. (2007) detected a significant decrease in presumptively-immunolabeled GABA neurons in dorsolateral prefrontal cortex and a strong trend for a decrease in these neurons in orbitofrontal cortex in depression.

In conclusion, subjects with MDD had decreased antagonist but not agonist binding to serotonin-1A receptors in superficial layers of orbitofrontal cortex. The decrease in antagonist binding to serotonin-1A receptors appears to be related to depression and not to suicide or antidepressant therapy. The observation in postmortem tissue confirms reports using an antagonist radioligand in living subjects with depression and suggests diminished receptor signaling.

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Contributors

C.A. Stockmeier and G. Rajkowska designed the study, wrote the protocol, analyzed the data and wrote the first draft of the manuscript. L. Friedman statistically analyzed the data and assisted in writing the first draft of the manuscript.

X. Shi performed the radioreceptor binding assays. E. Howley, G. Clarke and A. Sobanska analyzed the autoradiograms. All authors contributed to and have approved the final manuscript.

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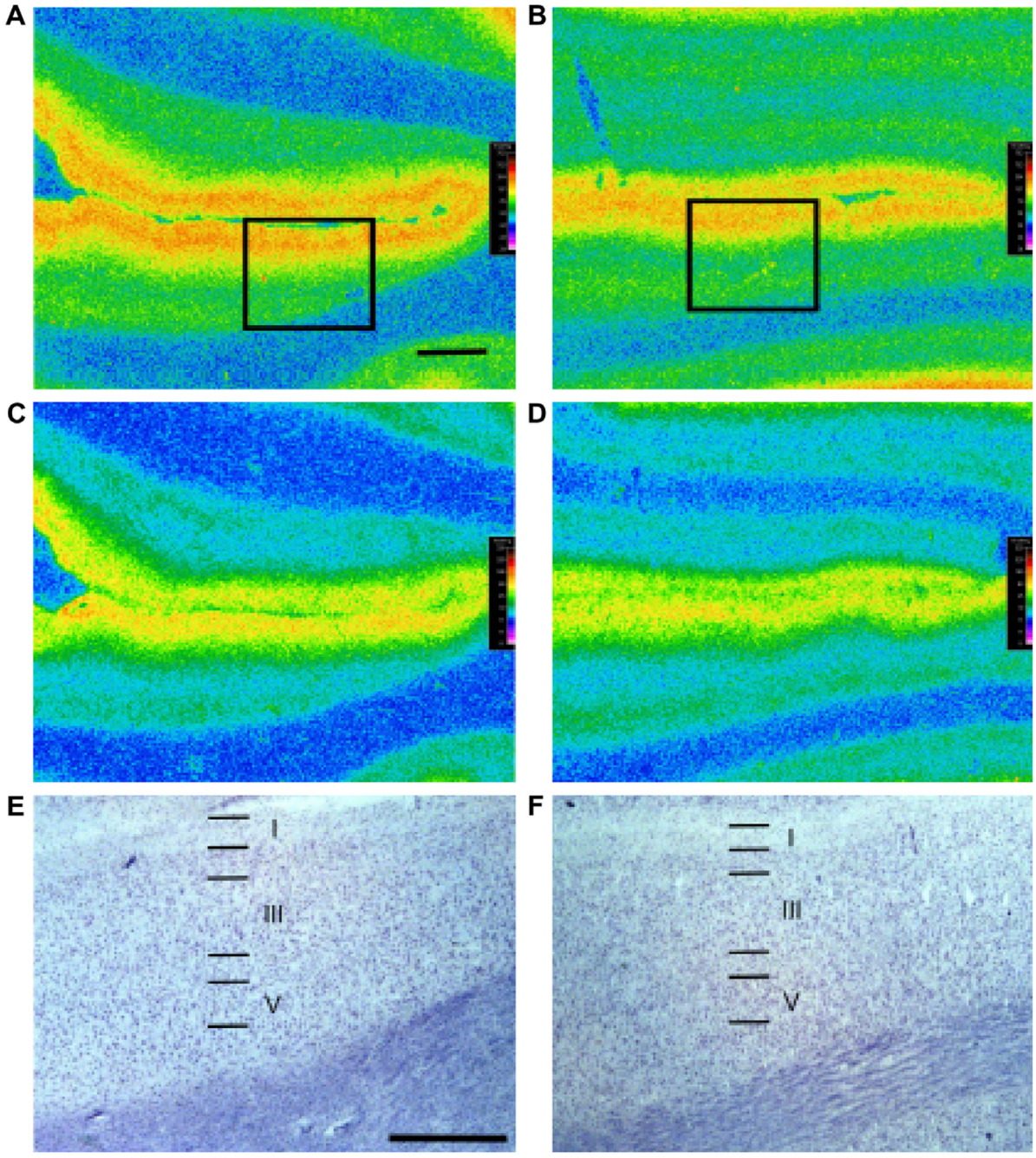

Fig. 1.

Autoradiograms of [^{3}H]MPPF (A, B) and [^{3}H]8-OH-DPAT (C, D) binding to serotonin-1A receptors in adjacent sections of orbitofrontal cortex. Calibrated serotonin-1A autoradiograms from a control subject (A, C) and the age-matched subject with MDD (B, D) are presented. Note the decrease in autoradiographic density in outer cortical layers in the depressed subject compared to the control subject as measured with [^{3}H]MPPF but not with [^{3}H]8-OH-DPAT. The boxes in (A) and (B) are placed over cytoarchitectonically-identified rostral orbitofrontal cortex (Brodmann area 47). Radiolabeled sections used to generate the autoradiograms were subsequently stained for Nissl substance to identify cortical laminae. Laminae (I–VI) for

cresyl-violet-stained cortex corresponding to the boxed regions in (A) and (B) are presented in (E) and (F), respectively. The scale bars in (C) and (E) are 2 and 1 mm in length, respectively.

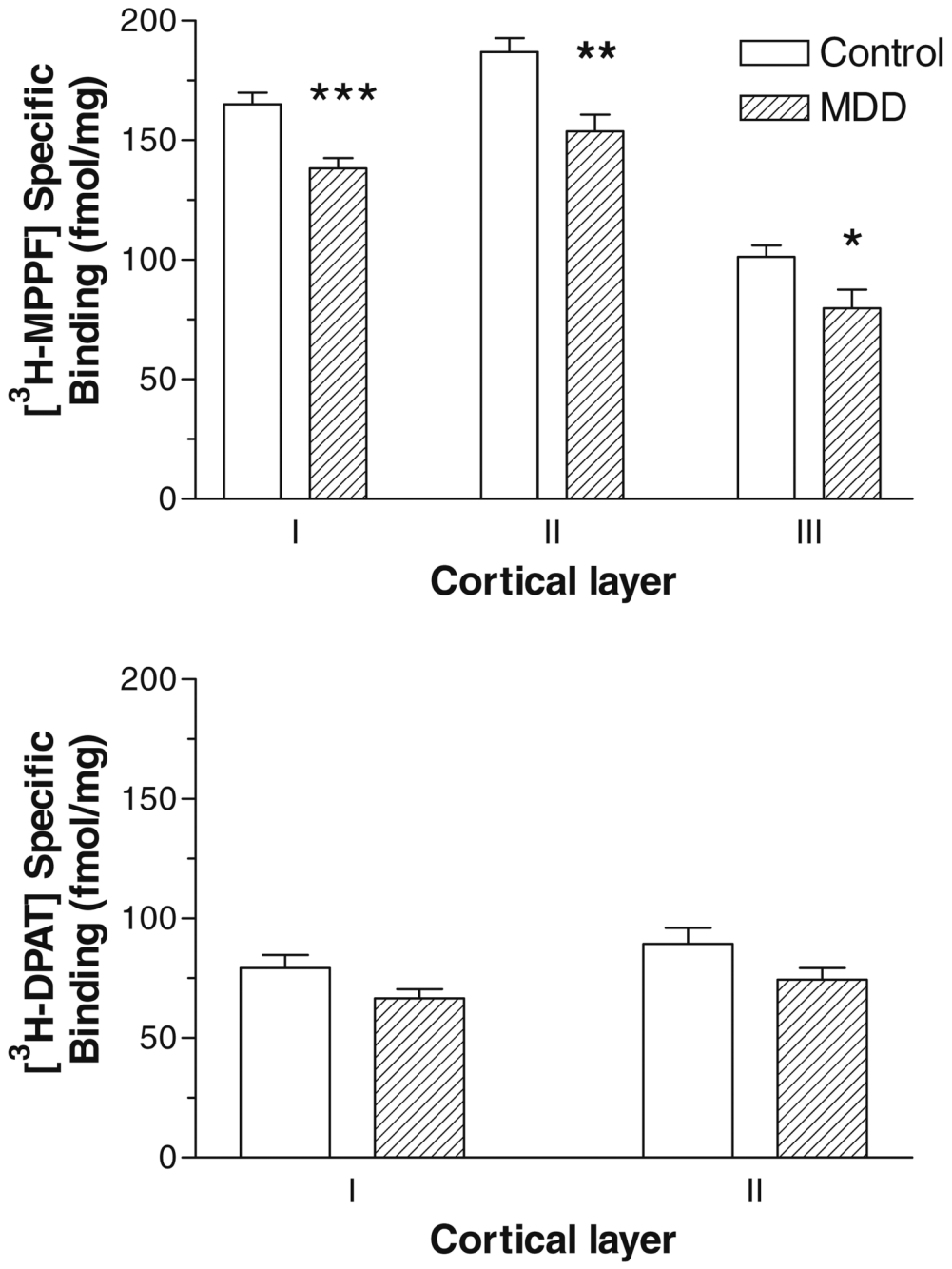

Fig. 2.

Radioligand binding to serotonin-1A receptors in orbitofrontal cortex in subjects with major depressive disorder (MDD) compared with psychiatrically-normal control subjects. [^{3}H]MPPF (top) and [^{3}H]8-OH-DPAT (bottom) binding in the outer cortical laminae is represented on the y-axis as fmol/mg (mean ± SEM). Sections used for receptor autoradiography were subsequently stained for Nissl substance and used to identify the cortical laminae. *** p = 0.0005, ** p = 0.0017, * p = 0.03 vs. control subjects.

Table 1

Characteristics of the normal control subjects.

Age/gender	Cause of death	PMI (hrs) /pH	Toxicology	Medications[a]	Axis I diagnosis
23/f	Motor vehicle accident	11/6.84	Nothing detected	None	none
27/f	CVD	15/7.01	Nothing detected	None	None
30/m	CVD	19/6.98	Nothing detected	None	None
35/m	CVD	25/6.74	Nothing detected	None	None
46/m	CVD	11/6.95	Nothing detected	None	None
46/f	Homicide	24/6.32	Nothing detected	None	None
49/f	CVD	29/6.57	Nothing detected	None	None
50/f	CVD	27/6.74	Nothing detected	None	None
50/ m	CVD	12/6.54	Nothing detected	None	None
58/m	CVD	22/6.78	Nothing detected	None	None
71/m	Cardiac rupture	24/6.82	Nothing detected	None	None

Abbreviations: CVD, cardiovascular disease; f, female; m, male; pH, tissue pH; PMI, postmortem interval.

[a]Psychoactive medications prescribed in the last month of life. Only psychoactive compounds are listed under toxicology.

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Table 2

Characteristics of the subjects with major depressive disorder.

Age/ gender	Cause of death	PMI (hrs) / pH	Toxicology	Medications[a]	Axis I diagnosis	Duration of illness (years)
30/m	Sigsw – chest, suicide	18/6.91	Ethanol 0.07%	None	MDD, chronic, moderate	3
34/f	Carbon monoxide, suicide	24/6.27	Ethanol 0.12%, CO, alprazolam	Alprazolam[a], valproate[a], trazodone, risperidone	MDD, recurrent, severe; panic attacks with agoraphobia	20
36/m	Undetermined	11/6.96	Diphenhydramine	None	MDD, recurrent, moderate	3
40/f	CVD	25/6.32	Morphine, codeine, hydrocodone	Fluoxetine[a], temazepam[a], hydrocodone[a]	MDD, recurrent, in full remission	5
42/f	Suicide overdose – propoxyphene and acetaminophen	24/6.62	Propoxyphene, acetaminophen,	Propoxyphene[a], fluoxetine[a], amitriptyline[a], paroxetine[a], diazepam[a],	MDD, chronic, moderate; polysubstance dependence; bulemia nervosa	26
42/m	Drowning, suicide	20/6.64	Sertraline, ethanol 0.02%	Sertraline[a]	MDD, single episode, severe	0.25
46/m	Gunshot, homicide	17/6.26	Nothing detected	None	MDD, single episode, mild	1
50/f	Hanging, suicide	23/6.83	Nothing detected	Clomipramine, fluoxetine, thiothixene	MDD, recurrent, moderate, with psychosis, mood congruent	5
54/m	Accidental CO poisoning	23/6.24	CO, phenobarbital, phenytoin	Sertraline[a]	MDD, single episode, moderate, chronic	3
63/f	Pulmonary thromboembolism	24/6.32	Amitriptyline, chlorpromazine, amantadine, lidocaine	Amitriptyline[a], chlorpromazine[a], clonazepam[a], amantidine[a]	MDD, recurrent, moderate; Polysubstance abuse	30
86/m	Stab to chest and throat, suicide	21/6.23	Nothing detected	Fluoxetine[a], atenolol[a], leuprolide	MDD, recurrent, severe, with melancholia	20

Abbreviations: CO, carbon monoxide; CVD, cardiovascular disease; f, female; MDD, major depressive disorder; m, male; pH, tissue pH; PMI, postmortem interval; sigsw, self-inflicted gunshot wound.

[a]Psychoactive medications prescribed in the last month of life. Only psychoactive compounds are listed under toxicology.