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ELEVATED 5-HT 2A RECEPTORS IN POSTMORTEM PREFRONTAL CORTEX IN MAJOR DEPRESSION IS ASSOCIATED WITH REDUCED ACTIVITY OF PROTEIN KINASE A

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Abstract

Previous human postmortem brain tissue research has implicated abnormalities of 5-HT receptor availability in depression and suicide. Although altered abundance of 5-HT 1A, 5-HT 2A, and 5-HT 2C receptors (5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C}) has been reported, the causes remain obscure. This study evaluated the availability of these three receptor subtypes in postmortem brain tissue specimens from persons with a history of major depression (MDD) and normal controls and tested the relationships to protein kinases A and C (PKA, PKC). Samples were obtained from postmortem brain tissue (Brodmann area 10) from 20 persons with a history of MDD and 20 matched controls as determined by a retrospective diagnostic evaluation obtained from family members. Levels of 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptor were quantitated via Western blot analyses. Basal and stimulated PKA and PKC activity were also determined. The depressed samples showed significantly increased 5-HT_{2A} receptor abundance relative to controls, but no differences in 5-HT_{1A} or 5-HT_{2C} receptors. Basal and cyclic AMP-stimulated PKA activity was also reduced in the depressed sample; PKC activity was not different between groups. 5-HT_{2A} receptor availability was significantly inversely correlated with PKC activity in controls, but with PKA activity in the depressed sample. Increased 5-HT_{2A} receptor abundance and decreased PKA activity in the depressed sample are consistent with prior reports. The correlation of 5-HT_{2A} receptor levels with PKA activity in the depressed group suggests that abnormalities of 5-HT_{2A} receptor abundance may depend on receptor uncoupling and heterologous regulation by PKA.

Keywords

5-HT receptors; protein kinases; protein kinase A; protein kinase C; depression; postmortem

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Major depression (MDD) is a serious, potentially disabling, and even life threatening disorder. Although the underlying pathophysiology is undoubtedly multifactorial, a huge literature implicates the 5-HT system as a possible causal factor in depression in general and suicidal behavior in particular (Stockmeier, 2003; Pandey et al., 2003b; Mann et al., 2001; Jans et al., 2007; Arango et al., 2003). 5-HT serves a modulatory role with regard to mood, and a variety of factors may lead to dysfunction of the 5-HT system, leading to abnormal mood states. A variety of mechanisms regulate 5-HT response in brain, including enzymes involved in the synthetic pathway (e.g. tryptophan hydroxylase, indoleamine 2,3-dioxygenase), synaptic regulation (e.g. the 5-HT transporter protein) and a variety of 5-HT receptors.

5-HT 2A receptors ($5HT_{2A}$) have been shown to be elevated in frontal cortex of depressed persons and suicide victims (Turecki et al., 1999; Stanley and Mann; Pandey et al., 2002; Hrdina and Du, 2001; Hrdina et al., 1993; Arranz et al., 1994; Arango et al., 1990, 1997), particularly involving pyramidal cells of cortical layer V (Pandey et al., 2002). 5-HT_{2A} receptors have been shown to have a significant role in the modulation of mood state, consistent with their widespread distribution in brain regions known to modulate mood responses, including cortex, hippocampus, and amygdala (Weisstaub et al., 2006). Activation of 5-HT_{2A} has been shown to enhance anxious responding in animal and human studies (Mora et al., 1997; Graeff et al., 1996), whereas selective blockade (Kleven et al., 1997; Griebel et al., 1997), antisense inhibition (Sibille et alt., 1997), or disruption (Weisstaub et al., 2006) has been shown to reduce anxiety and learned helplessness behavior. Furthermore, learned helplessness in rats related to chronic inescapable shock, a putative model for human depression, is associated with significant up-regulation of 5-HT_{2A} mRNA and protein expression in frontal cortex (Dwivedi et al., 2005).

Elevated availability of 5-HT_{2A} receptors in brains of depressed and suicide victims has been attributed to decreased 5-HT leading to receptor upregulation (Jans et al., 2007). However, the regulation of 5-HT_{2A} receptors is complex and, under certain conditions, paradoxical (Van Oekelen et al., 2003). For example, both agonists and antagonists induce down-regulation in 5-HT_{2A} receptor availability (Van Oekelen et al., 2003). 5-HT_{2A} receptors are also susceptible to both homologous and heterologous receptor-mediated down-regulation via protein kinases (Saucier et al., 1998; Saucier and Albert). Although homologous desensitization can occur via protein kinase C (PKC)–dependent phosphorylation by activation of the 5-HT_{2A}–$G_{q/11}$–phospholipase C–diacylglycerol cascade, heterologous desensitization of 5-HT_{2A} receptors by other enzymes including protein kinase A (PKA) or calcium-calmodulin kinase (CaMK) also occurs (Van Oekelen et al., 2003). Phosphorylation-dependent internalization to endosomes results in either dephosphorylation leading to resensitization or degradation (Van Oekelen et al., 2003). Therefore, regulation of 5-HT_{2A} receptors via phosphorylation may significantly affect availability.

Our research group and others have demonstrated decreased activity and protein availability for PKA (Shelton et al., 1996, 1999; Perez et al., 1995, 1999, 2001; Pandey et al., 2007; Manier et al., 1996, 2000; Dwivedi et al., 2002, 2004b; Akin et al., 2004, 2005) and PKC

(Pandey et al., 1997, 1998; Coull et al., 2000; Akin et al., 2005) in a significant subset of patients with MDD using both peripheral tissue models and postmortem brain tissue. This has also been tested functionally by demonstrating decreased phosphorylation of target proteins such as cyclic AMP response element binding protein (CREB) (Pandey et al., 2007; Manier et al., 2001) and myristoylated alanine-rich C kinase substrate (MARCKS) (Pandey et al., 2003a). However, to our knowledge, the relationship between reduced PKA and PKC and 5-HT receptor availability has not been previously tested.

Other 5-HT receptors have been implicated in the regulation of mood. For example, presynaptic 5-HT_{1A} receptors inhibit release of 5-HT and down-regulation is required for the antidepressant response to 5-HT selective reuptake inhibitors (SSRIs) (Lemonde et al., 2003; Blier et al., 2001). Post-synaptic 5-HT_{1A} receptors also appear to mediate some of the antidepressant actions of SSRIs and related drugs. For example, activation of 5-HT_{1A} receptors enhances the activity of both norepinephrine and dopamine neurons (Szabo and Blier, 2001; Ichikawa and Meltzer, 1999; Ichikawa et al., 2001), which is likely to be involved in antidepressant effects (Szabo and Blier, 2001; Stahl and Shayegan; Simon and Nemeroff; Haddjeri et al., 1997). 5-HT_{1A} also has been studied in depressed and suicide samples using both brain imaging and postmortem brain tissue methods (Tochigi et al., 2008; Szewczyk et al., 2008; Stockmeier et al., 1997, 1998; Matsubara et al., 1991; Lemonde et al., 2003; Hsiung et al., 2003; Drevets et al., 1999, 2007; Arranz et al., 1994; Arango et al., 2001), with variable results (for a review, see Stockmeier, 2003). A number of studies have demonstrated reduced binding, availability, or activity of 5-HT_{1A} receptors, but this appears to vary depending on both the brain region analyzed and the clinical subtype tested (Stockmeier, 2003; Drevets et al., 2007). For example, Drevets et al. conducted two different positron emission tomography (PET) studies of 5-HT_{1A} binding using carbonyl-[^{11}C]WAY-100635, a relatively selective 5-HT_{1A} ligand in depressed and control samples. They showed decreased 5-HT_{1A} binding in medial temporal cortex and raphe nuclei, but not in other brain regions, a finding that appeared to be specific for recurrent, familial depression (Drevets et al., 1999, 2007). Stockmeier et al. (1997) did not find any differences [^{3}H]8-hydroxy-2-(di-n-propyl)-aminotetralin ([^{3}H]8-OH-DPAT) binding to 5-HT_{1A} receptors in right anterior prefrontal cortex (PFC) (Brodmann area [BA] 10) from depressed suicide victims in comparison to controls, although in a related study, 5-HT_{1A} protein abundance was found to be reduced in PFC samples from depressed females (Szewczyk et al., 2008).

By contrast, 5-HT_{2C} receptors have received less attention, in spite of their apparent involvement in mood regulation. Activation of 5-HT_{2C} receptors attenuates PFC norepinephrine and dopamine release in rodent models (Pozzi e al., 2002; Li et al., 2005; Gobert et al., 2000), and blockade of 5-HT_{2C} receptors by atypical antipsychotics has been hypothesized to underlie their antidepressant properties (Shelton and Papakostas). There have been limited postmortem studies of 5-HT_{2C} receptors; studies (Schmauss, 2003; Niswender et al., 2001; Gurevich et al., 2002) have shown an increase in an edited form of 5-HT_{2C} receptor (isoleucine–asparagine–isoleucine to valine–glycine–valine editing at positions 156, 158, and 160) that is associated with decreased coupling of the receptor to G-proteins in samples from persons with MDD. One study of 5-HT_{2C} receptors in various human postmortem brain regions contrasted samples from suicide victims and controls

(Pandey et al., 2006). 5-HT_{2C} receptors were found to be widely distributed, with greater abundance in choroid plexus, hypothalamus, and nucleus accumbens, and lesser availability in PFC and cerebellum. However, only PFC (BA8/9) showed decreased abundance of 5-HT_{2C} receptors in the depressed sample relative to controls.

The primary purpose of the current study was to contrast the abundance of 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors and to test the association of these receptors with PKA and PKC levels in postmortem orbitofrontal cortex tissue (BA10) from depressed and control samples. BA10 is involved in a variety of functions of significance to depression, including executive function (Rogers et al., 1999; Okuda et al., 2007; Leung et al., 2005; Konishi et al., 2000) and reward behavior (Rogers et al., 1999). BA10 is also relatively selectively activated with administration of cocaine (Kufahl e al., 2005) and amphetamine (Devous et al., 2001), which is consistent with the rich innervations of this region by norepinephrine and dopamine containing neurons (Volkow et al., 2000). We hypothesized that there would be alterations in the availability of these receptor subtypes in depressed subjects versus controls, consistent with previous observations in postmortem samples. We also hypothesized that there would be reduced PKA and PKC activity and that the activity of these enzymes would be correlated with the abundance of 5-HT receptors, particularly 5-HT_{2A}. A final goal of this study was to test whether altered 5-HT receptors and kinase activity are specific to depressed patients who died by suicide.

EXPERIMENTAL PROCEDURES

Brain specimens were obtained from the Brain Tissue Donation Program at the University of Pittsburgh Medical Center and were acquired during autopsies after consent was given by the next of kin. Samples of PFC (BA10) were collected from 20 persons with a history of MDD and 20 age, sex, and postmortem interval (PMI) matched controls (Table 1). All procedures were conducted in accordance with the Declaration of Helsinki and were approved by the University of Pittsburgh's Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research and the Vanderbilt University Health Sciences Institutional Review Board. An independent diagnostic conference was conducted with a committee of experienced research clinicians who assigned DSM-IV diagnoses by consensus for each subject on the basis of all information obtained from a standardized psychological autopsy which incorporated structured interviews (e.g. the Structured Clinical Interview for DSM-IV; First et al., 1996), conducted by trained and experienced clinicians with family members of the index case (patients and controls), to assess diagnosis, psychopathology, medical, developmental, social and family history, medication history, history of alcohol, tobacco and other substance use, and handedness. The use of multiple informants, including physicians and other health care workers, provided both an extensive range of detailed information and the opportunity to corroborate critical elements of the history. Ancillary data from clinical records, toxicology and neuropathology examinations, and the Medical Examiner's investigation were also reviewed. Written informed consent was obtained from all participants. Based on the consensus findings, the deceased were given primary (e.g. MDD) and subtype (e.g. melancholia) diagnoses. All samples came from persons free from all known psychotropic

agents based on toxicology and recent substance abuse. The right hemisphere of each brain was blocked coronally, immediately frozen and stored at −80 °C.

Western blot analysis

Tissues were homogenized by sonication in 10 volumes of ice-cold buffer containing 20 mM tris(hydroxymethyl)aminomethane (Tris)–HCl, (pH 7.4 at 25 °C), 2 mM EDTA, 25 mM 2-mercaptoethanol, 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, plus 0.5% Triton X-100, 2 μg/ml leupeptin, 3 μg/ml aprotinin, and 0.2 mg/ml soybean trypsin inhibitor. The homogenate was centrifuged at 12,000×*g* for 10 min at 4 °C. Equal volumes of supernatant (20 μl containing 30 μg of protein) and gel loading solution (50 mM Tris–HCl, pH 6.8, 4% β-mercaptoethanol, 1% sodium dodecyl sulfate [SDS], 40% glycerol, and a trace amount of Bromphenol Blue) were mixed, then boiled for 3 min and kept on ice for 10 min. Samples were loaded onto 10% (w/v) SDS–polyacrylamide gel using the Mini Protein II gel apparatus (Bio-Rad, Hercules, CA, USA). The gels were run using 25 mM Tris-base, 192 mM glycine, and 0.1% (w/v) SDS at 200 V. The proteins were transferred electrophoretically to an enhanced chemiluminescence (ECL) nitrocellulose membrane (Amersham) using the Mini TransBlot transfer unit (Bio-Rad) at 0.25 amp constant current. Membranes were washed with phosphate-buffered saline containing 0.05% Tween 20 for 10 min. The blots were blocked by incubation with 3% (w/v) powdered nonfat milk in phosphate-buffered saline. They were incubated overnight at 4 °C with primary antibody (anti-5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, [all 5-HT receptor antibodies were from Santa Cruz Biotechnologies, Santa Cruz, CA, USA] or phosphorylated cyclic AMP response element binding protein (CREB-P) [Upstate Cell Signaling Solutions, Charlottesville, VA, USA]) at a dilution of 1:1000–1:3000 depending on the antibody used. The membranes were washed with phosphate-buffered saline and incubated with horseradish peroxidase–linked secondary antibody (anti-rabbit immunoglobulin G; 1:3000) for 1.5 h at room temperature. The membranes were washed with water followed by phosphate-buffered saline containing 0.05% Tween 20 and exposed to ECL film, then standardized using 10–100 μg of protein. The optical density of the bands varies linearly with a concentration of up to 100 μg of protein. The band optical density was quantified using Un-Scan-It gel digitizing software (Orem, UT, USA); the optical density is corrected by the total protein in the sample, determined by the methods of Lowry et al. (1990). All samples were done in triplicate. For representative blots, see Fig. 1.

PKA and PKC activity

Brain specimens were homogenized using a glass-Teflon homogenizer (1000 rpm, five strokes) in 50 vol buffer containing 30% sucrose). PKA activity was determined in the soluble fraction (±100 μM cAMP), as previously described (Shelton et al., 1996; Manier et al., 1996). PKA activity was defined as the transfer of PO_4 from ATP (100 μM; ^{32}P tracer, 300 counts per minute/pmol) to the heptapeptide Kemptide (leucine–arginine–arginine–alanine–leucine–glycine; 50 μM) and normalized per units protein and time.

The PKC assay was based on the phosphorylation of CREB in tissue homogenates after treatment by phorbol 12-myristate 13-acetate (PMA) (100 μM, 10 min at 37 °C). Stimulated

and endogenous phosphorylation of CREB was determined. CREB-P was quantitated by Western blotting, using methods described above.

Statistical analysis

Demographic and postmortem (e.g. PMI) variables were compared via independent samples *t*-test or chi square analysis as appropriate. Western blot data and kinase activity were compared via independent samples *t*-test. Within-groups analyses (e.g. suicide versus non-suicide) were done with independent samples *t*-tests. Missing data were handled pairwise. The primary outcome contrast determined a priori was a comparison of 5-HT receptors in samples from persons with MDD against matched controls, with 5-HT_{2A} being the first entered. The relationships between PKA and PKC activity versus 5-HT receptor availability was tested via the Pearson product moment correlation coefficient. The a priori hypotheses were as follows: 1. The availability of 5-HT_{1A} receptors would be reduced in depressed versus control samples; 2. 5-HT_{2A} receptors would be increased relative to controls; 3. 5-HT_{2C} receptors would be decreased in depressed versus controls; 4. PKA and PKC activity would both be reduced in depressed relative to control samples; 5. Both PKA and PKC activity would be significantly correlated with 5-HT receptor abundance; 6. The contrast of suicide versus non-suicide depressed samples would not show any significant differences in 5-HT receptors or kinase activity. All hypotheses were tested independently. The effects of age, PMI, and brain pH on protein levels and kinase activity were tested by Pearson product moment correlation analysis. Data were analyzed using SPSS 15.0 (SPSS, Chicago, IL, USA).

RESULTS

Demographic characteristics and tissue sample descriptions are shown in Table 1 and the characteristics of the individual samples are shown in Table 2. There were no significant differences in age (t=−317, df=38, P=0.75), PMI (t=1.47, df=38, P=0.88), pH of the samples (t=−1.184, df=38, P=0.024), or sex (both groups were 15% female). All samples were from Caucasians.

Immunolabeling of 5-HT receptors

There were no statistically significant differences between depressed and control samples in 5-HT_{1A} receptors (t=0.358, df=38, P=NS) or 5-HT_{2C} receptors (t=0.043, df=38, P=NS) (Table 3). Alternatively, 5-HT_{2A} receptors were dramatically and significantly increased in depressed versus controls (t=2.767, df=38, P=0.009); the depressed samples were increased to 217.30% of matched controls.

PKA and PKC activity

Basal and cyclic AMP–stimulated activity of PKA was compared between depressed and control samples. Depressed patients showed a statistically significant reduction of basal PKA activity (depressed = 1023 [S.D. =290.54], controls= 1273 [S.D.=302.97] pmol/min/mg protein [t=−2.664, df=38, P=0.011]), and a trend toward reduction of cyclic AMP–stimulated PKA activity (depressed=2953.90 [S.D.=768.23], controls=3218.65 [S.D.=759.65] pmol/min/mg protein [t=−1.510, df=38, P=0.139]).

Basal- and PMA-stimulated PKC activity was also compared between depressed and control groups (n=16, each group). No significant differences were found between groups on basal (depressed=3379.00 [S.D.=2356.62], controls=4439.75 [S.D.=3977.23]) or PMA activated (depressed=3475.56 [S.D.=2975.02], controls=4874 [S.D.=3787.50]) PKC activity.

Correlations between PKA and PKC and 5-HT receptors

The relationships between abundance of 5-HT receptors and both PKA and PKC were tested by Pearson product moment correlation. There were no statistically significant correlations between 5-HT_{1A} or 5-HT_{2C} receptors and either basal or activated PKA or PKC in the total sample, depressed, or control groups (Table 3). There were strong trends for a significant positive correlations between 5-HT_{2C} receptor versus PKA in the depressed sample; basal PKA (r=0.432, P=0.057), activated PKA (r=0.437, P=0.054). However, when one depressed outlier sample with an extremely low 5-HT_{2C} receptor value was removed, the association disappeared (PKA basal, r=0.118, P=0.473; PKA activated, r=0.235, P=0.150).

Alternatively, the 5-HT_{2A} receptor showed significant, inverse correlations with PKA activity in the total sample (basal PKA, r=−0.365, P=0.021); activated PKA, r=−0.348, P=0.028). However, when examined separately, only the depressed group showed significant associations, including a strong trend for basal PKA (r=−0.421, P=0.065) and a significant correlation for activated PKA (r=−0.560, P=0.010) (Fig. 2A). The control sample did not show significant correlations between PKA and 5-HT_{2A} receptor (basal PKA, r=0.051, P=0.831; activated PKA, r=0.031, P=0.896) (Fig. 2B).

There were no significant correlations between PKC activity and any 5-HT receptor in the total sample or depressed subgroup (Table 4). There were no significant correlations between PKC activity and either 5-HT_{1A} or 5-HT_{2C} receptor abundance. However, there was a significant inverse correlation between 5-HT_{2A} receptor availability and basal PKC activity (r=−0.515, P=0.041), with a trend for significant association with activated PKC (−0.435, P=0.092).

5-HT receptors in depressed suicide versus non-suicide groups

Within the depressed sample, there were no statistically significant differences between those who died by suicide (n=11) and those who died by other causes (n=9) for any of the 5-HT receptors. Similarly, the groups did not show any differences in basal or activated PKA or PKC.

Effects of age, sex, PMI, and pH on 5-HT receptors and kinases

There were no statistically significant correlations between age, PMI, or pH on availability of 5-HT_{1A}, 5-HT_{2A}, or 5-HT_{2C} receptors or either PKA or PKC activity in the total sample, depressed, or control groups, although there was a trend for a significant correlation between age and 5-HT_{1A} receptors in the depressed sample (r=−437, P=0.058). There were no significant differences by sex on any 5-HT receptors or either PKA or PKC in the total, depressed, or control samples, although the number of females was small (n=3 in depressed and controls).

Increased 5-HT_{2A} receptor availability still held when males only (n=17 per group) were analyzed. Males were increased at 233% of matched controls. Females were a mean of only 127% of controls, but, as noted earlier, the sample size was too low to draw any conclusions. When only depressed males were analyzed, the significant inverse correlation between 5-HT_{2A} receptor protein and activated PKA still held (r=−0.583, P=0.014), with a trend for basal PKA (r=−0.474, P=0.058).

DISCUSSION

In this study, we evaluated the abundance of specific 5-HT receptors and activity of two key enzymes, PKA and PKC, in human postmortem brain tissue specimens from PFC (BA10), which produced several significant findings. First, like a number of prior studies (Turecki et al., 1999; Stanley and Mann; Pandey et al., 2002; Hrdina and Du, 2001; Hrdina et al., 1993; Arranz et al., 1994; Arango et al., 1990, 1997), we found a significant increase in 5-HT_{2A} receptors in frontal cortex specimens in depressed persons relative to controls. The mean value for 5-HT_{2A} receptors was increased to 157% in the depressed sample; when the depressed individuals were compared against their matched controls, the increase was 217%. Increased 5-HT_{2A} receptors have significant implications with regard to depression vulnerability. As noted earlier, activation of 5-HT_{2A} receptors has been shown to increase anxiety (Mora et al., 1997; Graeff et al., 1996), which is reversed by disruption (Weisstaub et al., 2006), inhibition (Sibille et al., 1997), or blockade (Kleven et al., 1997; Griebel et al., 1997). Anxiety is an important part of the complex of features of depression (Brown et al., 1998); anxious temperament (i.e. trait neuroticism) and anxiety disorders also are known to increase the risk for depression (Jorm et al., 2000; Hettema et al., 2006). Hence, increased availability of 5-HT_{2A} receptors, which may increase anxious responding, may mediate depressive vulnerability in some individuals.

Although brain 5-HT_{2A} receptors have been shown to be elevated in depression, the causal pathway is obscure. Both genetic variation of the 5-HT_{2A} receptor (Turecki et al., 1999) and decreased 5-HT innervation (Pandey et al., 2002) have been hypothesized as possible causal models. However, to our knowledge, the relationship between kinase activity and 5-HT_{2A} receptor abundance has not been previously investigated. PKA activity was decreased in the samples from depressed persons relative to controls, as demonstrated in other postmortem studies in PFC (Pandey et al., 2005, 2007). Consistent with the overall findings, Dwivedi et al. (2004a) have found that stress-induced learned helplessness in rats results in both decreased PKA and increased 5-HT_{2A} receptor (Dwivedi et al., 2005) availability in frontal cortex.

In the present study, a statistically significant association was found between cyclic AMP–stimulated PKA activity and 5-HT_{2A} receptor abundance. This suggests three possible causal models. First, as discussed earlier, PKA is involved in heterologous regulation of 5-HT_{2A} receptors; phosphorylation of 5-HT_{2A} receptors leads to internalization, followed by dephosphorylation and either recycling to the cell surface or degradation (Van Oekelen et al., 2003; Roth et al., 1998). Therefore, decreased phosphorylation of cell surface receptors could increase availability, as in this study. Second, increased 5-HT_{2A} receptors could lead to reduced PKA, although the mechanism is unclear. Finally, an independent factor

could be involved in the regulation of both PKA and 5-HT_{2A} receptors. For example, increased glucocorticoid activity has been shown to decrease PKA availability (Dwivedi and Pandey, 2000) and increase 5-HT_{2A} receptor responsiveness (Umeda et al., 2007; Katagiri et al., 2001; Dwivedi and Pandey, 2000) in rats. It is therefore possible that increased glucocorticoids induce both findings, given the fact that hypothalamic–pituitary–adrenal axis activity is enhanced in many depressed patients (Plotsky et al., 1998).

It should be noted that only a portion of the variance of 5-HT_{2A} (approximately 31%) in the depressed sample is accounted for by PKA activity. Therefore, other factors, such as reduced 5-HT availability, may also contribute to the reduction. However, the relationship between 5-HT_{2A} abundance and PKA activity in the depressed sample is potentially significant since, essentially, none of the 5-HT_{2A} variance was explained by PKA in the controls. In addition, since this is a static finding in postmortem tissue, a direct causal link between PKA and 5-HT_{2A} variance cannot be definitively established, only inferred.

We did not find a decrease in PKC activity in the depressed group, unlike prior reports (Pandey et al., 1997, 1998; Coull et al., 2000; Akin et al., 2005). The reason for this discrepancy is unclear. Our group has reported reduced PKC in peripheral fibroblasts from living depressed persons (Akin et al., 2005), and other groups have shown low PKC in both peripheral and brain tissue samples (Pandey et al., 1997, 1998). Most brain tissue studies have been done in samples from BA8 or 9, whereas the current study examined BA10. To our knowledge, only one previous study examined PKC in this region, and found no differences in levels of PKC isoforms in samples from depressed persons (Hrdina et al., 1998), which is consistent with the current results.

We did find a significant association between basal PKC activity and 5-HT_{2A} receptor availability, but only in the control group. Agonist binding to 5-HT_{2A} receptors leads to coupling of $G_{q/11}$ proteins and activation of phospholipase C, which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PI) catalyzing the formation of inositol-triphosphate and diacylglycerol. The latter binds to and activates PKC and homologous phosphorylation of 5-HT_{2A} receptors by PKC leads to down-regulation (Saucier et al., 1998). Hence the availability of the 5-HT_{2A} receptor is dependent, in part, on PKC activity, as was found in the control group. The reason for the lack of correlation in the depressed sample is unclear. One possible explanation is a decreased availability of 5-HT in depressed subjects, leading to lessened homologous desensitization via PKC. Alternatively, previous research in our laboratory (Akin et al., 2004) has shown reduced PI hydrolysis after activation of 5-HT_{2A} receptors in cultured fibroblasts from depressed patients relative to controls. Therefore, a second possible explanation is uncoupling of 5-HT_{2A} receptors from post-receptor mechanisms in the depressed subjects, which would obviate the relationship between receptor and intracellular signaling.

We did not find significant differences between brains from depressed subjects versus controls in 5-HT_{1A} or 5-HT_{2C} receptor abundance. Prior studies have shown lower 5-HT_{1A} receptors in specific brain regions (Stockmeier, 2003; Drevets et al., 2007). For example, using positron emission tomography imaging, Drevets et al. (2007) showed reduced 5-HT_{1A} ligand binding in medial temporal cortex and raphe nuclei, but not in other brain regions

(Drevets et al., 1999, 2007). Also consistent with the current study (Stockmeier et al., 1997), did not find any differences in [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in BA10. A recent postmortem study in BA10 found reduced 5-HT$_{1A}$ in females only, which may also be consistent with our results since our sample was predominantly male; it should be pointed out, however, that the absolute mean values in males and females did not differ substantially in the present study. In fact, both males and females were slightly higher than their matched controls (116.3% and 109.3% respectively).

To our knowledge, only one study (Pandey et al., 2006) has examined 5-HT$_{2C}$ receptors in human postmortem brain tissue in suicide victims versus controls; the suicide sample showed higher 5-HT$_{2C}$ availability in BA8/9 (dorsolateral PFC) but not in hippocampus or choroid plexus. The fact that the present study did not show differences in depressed or suicide samples in BA10 suggests that the differences previously shown may be regionally specific, even within the cortex.

Previous research has suggested that elevated 5-HT$_{2A}$ receptor binding is present in suicide victims, and may not depend on the presence of MDD per se (Turecki et al., 1999; Stockmeier et al., 1997; Rosel et al., 2000; Pandey et al., 2002; Hrdina and Du, 2001; Du et al., 2000; Arango et al., 1997). In the present study, there were no differences in the depressed sample between those who died by suicide versus other causes. Elevated 5-HT$_{2A}$ receptors may, in fact, not be specific to MDD, but may be a vulnerability factor in people otherwise predisposed to being depressed. 5-HT$_{2A}$ receptor activation is associated with anxiety-related symptoms and behaviors in both animal and human models (Mora et al., 1997; Graeff et al., 1996). Anxiety has been shown to be associated with risk for depression (Kendler et al., 2003; Kendler, 1996) and suicide (Hawgood and De Leo, 2008). Elevations in 5-HT$_{2A}$ receptor abundance may be associated with increases in stress-related dysphoric responses which may increase risk for depression, but may also enhance risk for suicide in either depressed or non-depressed samples.

This study is limited by the fact that it only involved 20 depressed and control samples, all were from Caucasians, and all but three in each group were from men. Therefore, the results may not be representative of all depressed patients. The samples were only from BA10, and may not reflect the state in other brain regions. In addition, diagnostic classification was made retrospectively and by second-hand report. However, the data are consistent with those from previous studies, which support the findings.

CONCLUSION

In conclusion, the present study found an elevation in 5-HT$_{2A}$ receptors in human postmortem brain tissue specimens from BA10 in depressed relative to control groups. This elevation was inversely correlated with PKA activity, suggesting that abnormalities of PKA may, at least in part, explain the abnormalities in 5-HT$_{2A}$ receptors. Further research is needed to more completely understand the causal pathways for these findings.

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Abbreviations:

BA	Brodmann area
CREB	cyclic AMP response element binding protein
CREB-P	phosphorylated cyclic AMP response element binding protein
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
MDD	major depression
PFC	prefrontal cortex
PI	phosphatidylinositol-4,5-bisphosphate
PKA	protein kinase A
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
PMI	postmortem interval
SDS	sodium dodecyl sulfate
SSRI	5-HT selective reuptake inhibitor
Tris	tris(hydroxymethyl)aminomethane
[^{3}H]8-OH-DPAT	[^{3}H]8-hydroxy-2-(di-n-propyl)-aminotetralin

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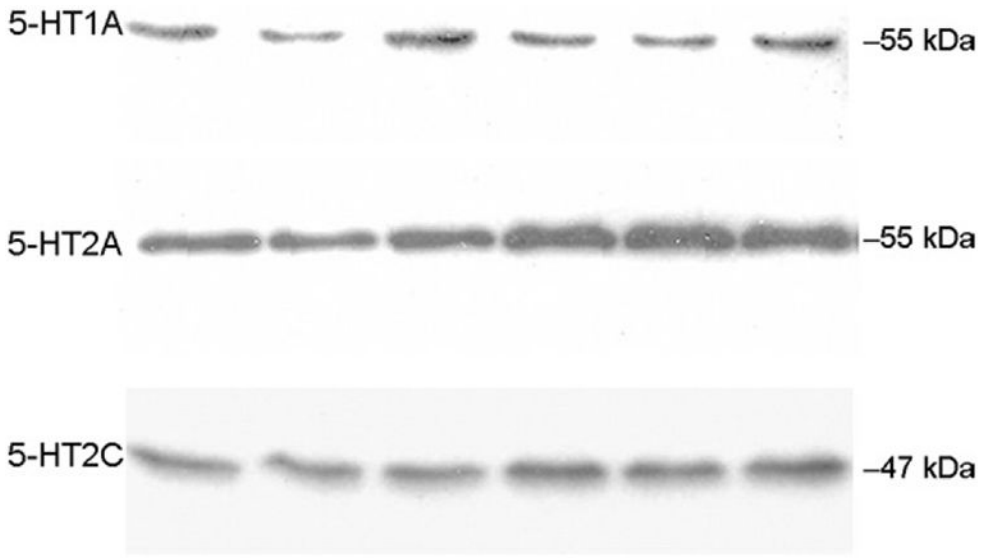

Fig. 1.

Representative Western blots for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors (triplicates, controls left, depressed right).

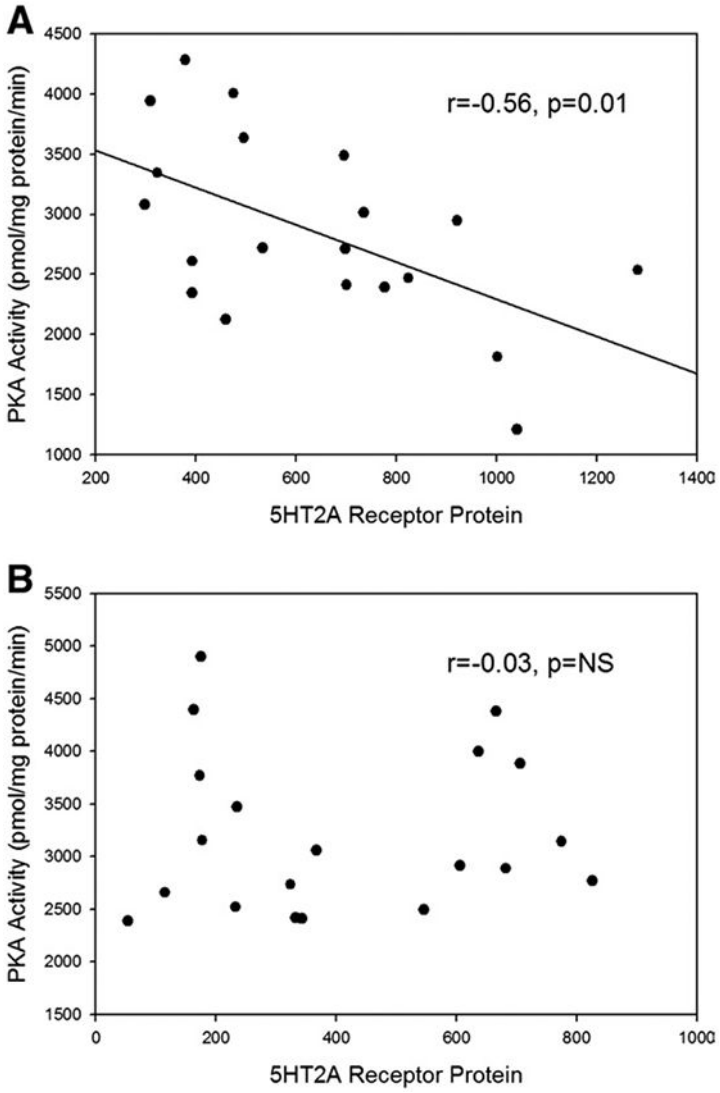

Fig. 2.
Correlations, PKA activity versus 5-HT2A receptor protein. A=Depressed, B=Controls.

Table 1.

Demographic and postmortem data

	Depressed	Controls
N	20	20
Age (S.D.)	45.5 (14.2) years	46.9 (13.4) years
PMI (S.D.)	18.2 (6.4) hours	17.9 (5.6) hours
Brain pH (S.D.)	6.65 (0.23)	6.74 (0.27)
Sex (%)	3 Females (15%)	3 Females (15%)
Race (%)	20 White (100%)	20 White (100%)

Table 2.

Sample characteristics[a]

Subject number	Group	Pair	Sex	Race	Age	PMI (hours)	pH	Cause of death	Manner of Death
511	MDD	1	M	W	43	17.9	6.91	Arteriosclerotic cardiovascular disease	Natural
596	MDD	2	M	W	68	20.5	6.88	Gunshot wound	Suicide
600	MDD	3	M	W	63	9.9	6.72	Hanging	Suicide
613	MDD	4	M	W	59	15.6	6.95	Gunshot wound	Suicide
614	MDD	5	M	W	39	19.5	6.67	CO poisoning	Suicide
628	MDD	6	M	W	26	21.6	6.73	CO poisoning	Suicide
668	MDD	7	M	W	34	24.3	7.00	Hanging	Suicide
699	MDD	8	M	W	65	5.5	6.71	Gunshot wound	Suicide
735	MDD	9	F	W	40	14.0	6.84	Pulmonary embolism	Accidental
927	MDD	10	M	W	58	24.9	6.11	Arteriosclerotic cardiovascular disease	Natural
949	MDD	11	M	W	38	25.0	6.23	Cardiac arrhythmia	Natural
1013	MDD	12	M	W	46	16.1	6.27	Nail-gun wound	Suicide
1017	MDD	13	M	W	27	18.8	5.69	Diabetic ketoacidosis	Natural
1028	MDD	14	M	W	39	14.5	6.18	Gunshot wound	Suicide
1053	MDD	15	M	W	47	24.0	6.57	Arteriosclerotic cardiovascular disease	Natural
1131	MDD	16	M	W	29	26.6	6.92	Gunshot wound	Suicide
1186	MDD	17	M	W	45	6.6	6.25	Traumatic asphyxiation	Accidental
1215	MDD	18	M	W	44	11.0	6.54	Arteriosclerotic cardiovascular disease	Natural
1221	MDD	19	F	W	28	24.8	6.61	Pulmonary embolism	Natural
10028	MDD	20	F	W	72	23.1	6.66	Gunshot wound	Suicide
739	Control	1	M	W	40	15.8	6.52	Arteriosclerotic cardiovascular disease	Natural
841	Control	2	M	W	70	21.2	7.18	Hypertrophic cardiomyopathy	Natural
510	Control	3	M	W	63	12.4	6.51	GI hemorrhage	Natural
685	Control	4	M	W	56	14.5	7.06	Hypoplastic coronary artery	Natural
604	Control	5	M	W	39	19.3	7.08	Hypoplastic coronary artery	Natural
585	Control	6	M	W	26	16.0	6.67	Trauma	Accidental
694	Control	7	M	W	38	20.7	6.73	Subarachnoid hemorrhage	Natural
615	Control	8	M	W	62	7.2	6.39	Ruptured aortic aneurysm	Natural

Subject number	Group	Pair	Sex	Race	Age	PMI (hours)	pH	Cause of death	Manner of Death
567	Control	9	F	W	46	15.0	6.77	Mitral valve prolapse	Natural
902	Control	10	M	W	60	23.6	6.74	Arteriosclerotic cardiovascular disease	Natural
700	Control	11	M	W	42	26.1	6.95	Arteriosclerotic cardiovascular disease	Natural
10003	Control	12	M	W	49	21.2	6.54	Trauma	Accidental
871	Control	13	M	W	28	16.5	6.33	Trauma	Accidental
1047	Control	14	M	W	43	13.8	6.63	Arteriosclerotic cardiovascular disease	Natural
643	Control	15	M	W	50	24.0	6.23	Arteriosclerotic cardiovascular disease	Natural
789	Control	16	M	W	22	20.0	7.04	Asphyxiation	Accidental
1067	Control	17	M	W	49	6.5	6.55	Hypertensive heart disease	Natural
857	Control	18	M	W	48	16.6	6.54	Arteriosclerotic cardiovascular disease	Natural
1282	Control	19	F	W	39	24.5	6.84	Cardiac arrhythmia	Natural
818	Control	20	F	W	67	24.0	7.06	Anaphylaxis	Accidental

[a]Abbreviations: M, male; F, female; PMI, postmortem interval; ASCVD, arteriosclerotic cardiovascular disease.

Table 3.

5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} Western blot results for depressed and controls (means and standard deviation), and depressed percent matched controls (means and standard deviations)

Protein	Controls		Depressed		Percent matched controls	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
5-HT_{1A}	326.40	233.99	306.15	234.00	115.25	69.52
5-HT_{2A}	406.75	247.52	636.85*	277.62	217.30	153.40
5-HT_{2C}	326.25	127.77	328.05	134.57	110.65	55.18

Values expressed as relative density.

*P=0.009.

Table 4.

Correlations between PKA and PKC activity and 5-HT receptors

Group	5-HT receptor	PKA		PKC	
		Basal	Activated	Basal	Activated
Total	5-HT$_{1A}$	0.009	0.030	0.093	0.067
	5-HT$_{2A}$	−0.365 *	−0.348 *	−0.297	−0.230
	5-HT$_{2C}$	0.238	0.300 †	−0.045	−0.007
Depressed	5-HT$_{1A}$	0.210	0.213	0.341	0.255
	5-HT$_{2A}$	−0.421 †	−0.560 *	0.112	0.143
	5-HT$_{2C}$	0.432 †	0.437 †	−0.097	−0.134
Control	5-HT$_{1A}$	−0.362	−0.357	−0.123	−0.154
	5-HT$_{2A}$	−0.051	0.031	−0.515 *	−0.435 †
	5-HT$_{2C}$	0.090	0.175	−0.009	0.100

* $P<0.05$.

† $P>0.05$ and <0.10.