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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. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: 5-HT receptors, protein kinases, protein kinase A, protein kinase C, depression, postmortem.

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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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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). $5\text{-}HT_{2A}$ 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 $5\text{-}HT_{2A}$ 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 $5\text{-}HT_{2A}$ mRNA and protein expression in frontal cortex (Dwivedi et al., 2005).

Elevated availability of $5\text{-}HT_{2A}$ 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 $5\text{-}HT_{2A}$ receptors is complex and, under certain conditions, paradoxical (Van Oekelen et al., 2003). For example, both agonists and antagonists induce down-regulation in $5\text{-}HT_{2A}$ receptor availability (Van Oekelen et al., 2003). $5\text{-}HT_{2A}$ 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)