Neuropsychopharmacology (2004) 29, 2081–2087

www.neuropsychopharmacology.org

Decreased Serotonin 5-HT_{2A} Receptor-Stimulated Phosphoinositide Signaling in Fibroblasts from Melancholic Depressed Patients

Demet Akin[1,2], D Hal Manier[1,2], Elaine Sanders-Bush[1,2] and Richard C Shelton*[,1,2]

[1]Department of Psychiatry, Vanderbilt University School of Medicine, Nashville, TN, USA; [2]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA

Abnormalities in serotonin (5-HT) receptors and 5-HT receptor-mediated signal transduction systems have been widely reported in mood disorders. This study was intended to evaluate 5-HT_{2A} receptor-coupled activation of phosphatidylinositol (PI) hydrolysis in subtypes of depression. Samples for fibroblast culture were obtained from patients with major depression with or without melancholia, and normal controls. Dose response curves were determined for 5-HT-induced PI hydrolysis. PI response was determined for bradykinin and l-α-lysophosphatidic acid (LPA), alternative Gq-coupled receptor agonists. [^{125}I]LSD binding for 5-HT_{2A} also was conducted. Finally, Western blot analysis was performed for phospholipase $C_{\beta 1}$ ($PLC_{\beta 1}$) and $G\alpha_{q/11}$ proteins. The maximum PI response observed with 5-HT was significantly lower in melancholics but not nonmelancholic patients relative to controls. Activation of PI hydrolysis by bradykinin and LPA was not reduced in melancholic *vs* melancholics and controls; responses to both agonists actually were increased in the melancholic group. [^{125}I]LSD binding, $PLC_{\beta 1}$, and $G\alpha_{q/11}$ protein levels did not differ between groups. The data raise the possibility that the reduced 5-HT_{2A} receptor-induced PI hydrolysis is intrinsic to the receptor itself or its coupling to Gq protein, and is not related to altered availability of the 5-HT_{2A} receptor, Gq or PLC.
Neuropsychopharmacology (2004) **29,** 2081–2087, advance online publication, 9 June 2004; doi:10.1038/sj.npp.1300505

Keywords: depression; melancholia; fibroblast; serotonin; phosphoinositide; receptors; 5-HT_{2A}; phosphatidylinositol

INTRODUCTION

Abnormalities in serotonin (5-HT) receptors and 5-HT receptor-mediated signal transduction systems have been widely reported in mood disorders (Ressler and Nemeroff 2000). Of these, the 5-HT_{2A} receptor subtype is the most widely studied. Activation of Gq-coupled 5-HT_{2A} receptors results in phospholipase C (PLC) catalyzed hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5 bisphosphate, with the formation of two second messengers: inositol(1,4,5) triphosphate (IP_3) and diacylglycerol (DAG). IP_3 plays an important role in various physiological processes by releasing calcium from intracellular stores. DAG activates protein kinase C (PKC), which, in turn, phosphorylates target proteins in the cell. Therefore, abnormalities of 5-HT_{2A} receptors have the potential to alter a diverse set of cellular processes (Aghajanian and Sanders-Bush 2002), potentially contributing to psychiatric diseases such as major depressive disorder.

Differences in the density of 5-HT_{2A} receptors in depressives relative to normal controls have been reported in many studies; however, the results are controversial. For example, some studies of 5-HT_{2A} receptors in platelets have found significant elevations (Pandey *et al*, 1990, 1995; Hrdina *et al*, 1995, 1997; Rao *et al*, 1998; Alda and Hrdina, 2000), while others have not (McBride *et al*, 1994; Neuger *et al*, 1999; Rosel *et al*, 1999). Similar results have been shown in post-mortem brain specimens from depressed patients; in some studies, apparent 5-HT_{2A} binding was elevated (Stanley and Mann 1983; Mann *et al*, 1986; Hrdina *et al*, 1993; Arranz *et al*, 1994; Pandey *et al*, 2002a, b), but not in others (Cheetham *et al*, 1988; Rosel and Arranz, 1998; Gross-Isseroff *et al*, 1998; Rosel *et al*, 2000). Recent studies have also investigated the activity of the PLC/phosphoinositide (PI) second messenger system in mood disorders (Pacheco *et al*, 1996; Jope *et al*, 1996; Shimon *et al*, 1997; Soares and Mallinger, 1997; Pandey *et al*, 2002a, b). Pacheco *et al* (1996) found that GTPγS-stimulated PI hydrolysis is significantly lower in the brains of depressed persons who committed suicide relative to a normal control group. They also reported slightly lower PI responses to agonists such as 5-HT and ATP. Impairments of the PI signal transduction

*Correspondence: Dr RC Shelton, 1500 21st Avenue, South Suite 2200, Nashville, TN 37212, USA, Tel: +1 615 343 9669, Fax: +1 615 343 7868, E-mail: richard.shelton@vanderbilt.edu
Received 6 November 2003; revised 26 April 2004; accepted 2 May 2004
Online publication: 15 May 2004 at http://www.acnp.org/citations/Npp051504035l9/default.pdf

system have been reported in brains from bipolar depressive subjects as well. Pandey *et al* (1999) found significantly lower PI-PLC activity and expression of the PLC isozyme PLC$_{\beta 1}$ in the brains of teenage suicide subjects relative to normal controls. In contrast, hyperactive PI signaling has been shown in platelets from depressed patients (Karege *et al*, 1996; Alvarez *et al*, 1999; Pandey *et al*, 2001). Elevated 5-HT-stimulated intracellular calcium has also been reported in platelets from unipolar depression (Delisi *et al*, 1998) and bipolar disorder (Suzuki *et al*, 2001). Alternatively, Suzuki *et al* (2001) found no differences in the calcium response to 5-HT in platelets from patients with depressive disorder with or without melancholia in contrast to a variety of other psychiatric conditions. Together, these studies suggest that there may be an abnormality in the 5-HT$_{2A}$ receptor in depressed patients, but the exact nature remains uncertain.

These discordant results may be related to a variety of issues, including differences based on diagnostic subtypes, drug treatment at the time of death, or dissimilarities between depressive and controls in circulating neurotransmitters and hormones. Other factors such as marked differences between depressives and normals in the cause of death and post-mortem intervals complicate interpretation of post-mortem brain studies. The aim of the current study was to probe for differences in the 5-HT$_{2A}$ receptor-mediated signal transduction pathway, using an *ex vivo* fibroblast cell culture model system. Previous studies in our laboratory have demonstrated significant differences in the adenylyl cyclase signaling pathway in melancholic subtype of depression *vs* controls and depressives with the atypical subtype (Shelton *et al*, 1996, 1999; Manier *et al*, 2000). Since these results were found only in a subset of depressives, the differences could be masked in the total depressive patient population, underscoring the value of diagnostic subclassification. We therefore compared 5-HT$_{2A}$ receptor-mediated signaling in cultured fibroblasts from melancholic subtype of depression *vs* controls and nonmelancholic depressives. Fibroblasts and neurons share many of the same receptors and intracellular signaling molecules (Manier *et al*, 2000), including the 5-HT$_{2A}$ receptor–Gq–PLC–PKC–CREB pathway. Furthermore, fibroblasts are relatively simple to obtain and maintain in culture in a controlled, reproducible environment, thus eliminating potential differences in drug treatment, transmitter or hormone exposure, or agonal states.

MATERIALS, PATIENTS, AND METHODS

Human Subjects

All procedures involving human subjects were conducted after written informed consent was obtained. Participants include patients with major depression, with or without melancholic subtype, and normal volunteers. Subjects were evaluated using the Structured Clinical Interview for DSM-IV (First *et al*, 1996) and the 17-item Hamilton Rating Scale for Depression (HAM-D) (Hamilton 1960). A second diagnostic interview was conducted by a senior psychiatrist. Diagnostic assignment, including subtyping, was made based on a consensus between the two interviewers. All the patients had a principal diagnosis of major depression; secondary diagnoses were allowed. However, no patient had a current or past history of psychotic disorder. Normal volunteer controls were free of Axis I mental disorder by DSM-IV (American Psychiatric Association 1994).

Collecting and Culturing of Human Fibroblasts

Fibroblasts were cultured from skin biopsies, as described previously (Shelton *et al*, 1996, 1999). All samples have been subcultured for at least five passages in order to minimize any effect from exposure *in vivo* to factors such as hormones, transmitters, drugs, or other factors. Fibroblast cultures were grown at 37°C in a humidified atmosphere containing 5% CO_2. Growth medium consisted of Dulbecco's modified Eagle's medium, containing 10% fetal bovine serum (FBS) or 10% dialyzed FBS that lacks 5-HT, and an antibiotic/antimycotic solution at final concentrations of 30 U penicillin, 30 μg streptomycin, and 70 ng amphotericin B/ml. The medium was changed three times per week and the cultures were inspected daily. After reaching confluency (4×10^6 cells/75 cm^2), the samples were subcultured by exposure to 0.05% trypsin/0.02% EDTA solution.

Saucier *et al* (1998) reported that fibroblasts, including NIH-3T3 cells, lack 5-HT receptors since no specific binding or response to 5-HT was detected. However, when these cells are cultivated in medium containing 10% dialyzed FBS, which lacks 5-HT, the cells became responsive to 5-HT. In the current study, 5-HT-induced PI hydrolysis was measured in fibroblasts grown in medium supplemented with 10% normal FBS (5-HT containing) or dialyzed FBS (5-HT free). The cells were gradually changed to dialyzed serum over a 3-week period, since more rapid changes resulted in cell death. We replicated the results of Saucier *et al*; 5-HT responses were only obtained in cells that were maintained in dialyzed FBS, therefore only dialyzed serum was used for all further experiments.

PI Hydrolysis Assay

Cultured cells were prelabeled with [^{3}H]myo-inositol (NEN Life Science Products, Boston, MA 02118) for 16–20 h. The formation of [^{3}H]inositol monophosphate ([^{3}H]-IP) in the presence of 10 mM lithium and 10 μM pargyline was used as an index of PLC activation. [^{3}H]-IP was isolated by extraction and purified by ion exchange chromatography as described previously (Barker *et al*, 1994). Concentration–response curves were generated by incubation with 5-HT (100 fmol–10 μM) and were fit using Graph Pad Prism, a computer graphics/analysis program (Graph Pad, San Diego, CA) to calculate EC_{50} and maximum effect. In addition, PI hydrolysis was determined for bradykinin (10 μM) and l-α-lysophosphatidic acid (LPA) (10 μM).

[^{125}I]LSD Binding to 5-Ht$_{2a}$ Receptors

For each determination, twenty 75 mm plates were combined. Cells were homogenized in 5 ml hypotonic medium (50 mM Tris/10 mM $MgCl_2$), by using a polytron for 2 s and centrifuged at 13 K rpm for 20 min at 4°C. The pellet was resuspended in 50 mM Tris buffer (pH 7.5) and used for the determination of the 5-HT$_{2A}$ receptor level. The receptor binding assay was carried out using a single concentration

of [^{125}I]LSD (NEN Life Science Products, Boston, MA 02118), each data point replicated 6–10 times. A 250 µl aliquot of membrane suspension (~25 µg protein), 25 µl [^{125}I]LSD (3 nM), and 25 µl incubation buffer (50 mM Tris) or ketanserin (1 µM) were incubated 1 h at 37°C. The assay was terminated by filtration through Whatman GF/C filters. The filters were washed for 30 s with 50 mM Tris buffer (pH 7.7) containing 0.01% bovine serum albumin, dried and counted in γ counter. Specific binding was defined as total binding minus binding in the presence of 1 µM ketanserin.

Western Blots of PLC$_{\beta 1}$ Isozyme and G$\alpha_{q/11}$ Protein

Cells were homogenized in 5 ml hypotonic medium (50 mM Tris/10 mM $MgCl_2$), using a polytron for 2 s and centrifuged at 13 K rpm for 20 min at 4°C. The pellet was suspended in homogenizing buffer containing 10 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 1% Na orthovanadate, 1.5 mM pepstatin, 2 mM leupeptin, and 0.2 U/ml aprotinin. Equal volumes of membrane fraction (120 µl) were subjected to 10% polyacrylamide gel by using a gel electrophoresis apparatus. The proteins were subsequently transferred electrophoretically to nitrocellulose membrane using a protein transfer unit. Membranes were washed for 10 min and blocked with 3% milk powder in PBS. Then the blots were incubated with primary monoclonal antibodies (anti-PLCβ1 and anti-Gαq/11, Santa Cruz Biotechnology, Santa Cruz, CA 95060), diluted at 1 : 200 in the blocking solution for 90 min at room temperature. The membranes were then thoroughly washed and incubated with horseradish peroxidase-linked secondary antibody (anti-rabbit IgG, 1 : 3000 in blocking solution) for 60 min at room temperature. To reduce the interblot variability, membranes were stripped and reprobed with β-actin antibody (Sigma-Aldrich, St Louis, MO 63195) at a concentration of 1 : 1000 for 90 min at room temperature and then incubated with anti-mouse IgG as a secondary antibody (Upstate Biotechnology, Lake Placid, NY 12946) at a concentration 1 : 2000 for 90 min at room temperature. Bands on the autoradiogram were quantified using Image J image analysis system (NIH), and the optical density of each sample was corrected by dividing by the optical density of the corresponding β-actin band.

Statistical Analysis

Differences between the three groups were tested using a one-way analysis of variance (ANOVA) for independent groups, with *post hoc* Tukey HSD test. Two-group comparisons were conducted with Student's *t*-tests.

RESULTS

Participant Characteristics

The participants included patients with a diagnosis of major depression, melancholic subtype ($n = 8$, seven females), major depression without melancholia ($n = 10$, eight females), and normal volunteers ($n = 10$, nine females); mean age 49.1 years, (SD = 16.5), 41.9 years (SD = 18.1), and 36.9 years (SD = 7.9) (ANOVA, F = 1.538, df = 2,25, p = NS), respectively. The mean HAM-D score was 24.5 (SD = 2.9) for melancholics and 22.7 (SD = 4.2) for nonmelancholics ($t = 1.023$, df = 16, p = NS). Only one patient, a 69-year-old nonmelancholic depressed man, was on an antidepressant, venlafaxine at 150 mg/day for 6 weeks, at the time of the biopsy. Excluding him from the analyses did not change the outcomes reported below.

5-HT Stimulated PI Hydrolysis

5-HT dose response curves for activation of PI hydrolysis were determined in fibroblasts from normal controls, melancholics, and nonmelancholic patients (Figure 1). 5-HT increased PI hydrolysis with nearly identical −log EC_{50} values (6.5 for normal subjects, 6.8 for melancholics, and 6.4 for nonmelancholics). The 5-HT response was eliminated by pretreatment with 100 nM MDL-100907, a 5-HT$_{2A}$ receptor selective antagonist, suggesting that 5-HT-stimulated PI hydrolysis is mediated by 5-HT$_{2A}$ receptors in these cells (data not shown). To determine if the decrease in PI hydrolysis is specific for the 5-HT$_{2A}$ receptor or if it reflects a more general alteration in lipid labeling or intracellular signaling, the method was modified to eliminate potential differences in cell number or radiolabeling by expressing the data as a ratio of [^{3}H]PI over [^{3}H] total phospholipids, thus correcting for differences in labeling in the lipid fraction. The maximum signal was significantly lower in melancholic patients *vs* controls or nonmelancholics (ANOVA: F = 14.209, df = 2,15, $p < 0.001$; Tukey HSD: melancholics *vs* nonmelancholics and controls, $p = 0.001$) (Figure 2). In contrast, the maximum response did not differ between nonmelancholic subjects and normal controls (Tukey HSD, p = NS). These data suggest that the 5-HT$_{2A}$ receptor signal is blunted in fibroblasts from subjects diagnosed with melancholic depression, but not in patients with nonmelancholic depression.

An examination of the basal constitutive activity of the 5-HT$_{2A}$ receptor revealed a small [^{3}H]-IP signal that was reproducible in both normal control samples and melancholic patients (Figure 2). An unexpected increase in

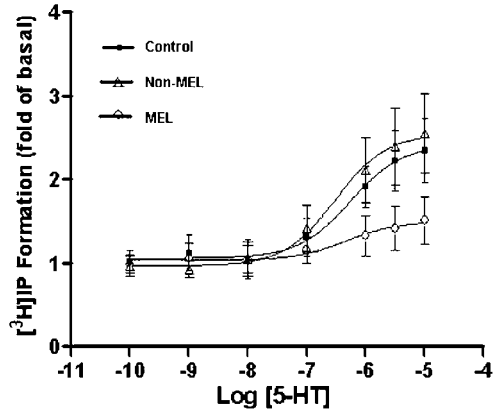

Figure 1 Phosphatidylinositol hydrolysis ([^{3}H]IP) in fibroblasts from representative control, nonmelancholic (nonMEL), and melancholic depressed (MEL) subjects. 5-HT produced a concentration-dependent increase in PI hydrolysis in fibroblasts from normal, nonmelancholic, and melancholic subjects. While maximum signal was significantly lower in melancholic patients, no differences were observed in nonmelancholics compared with controls.

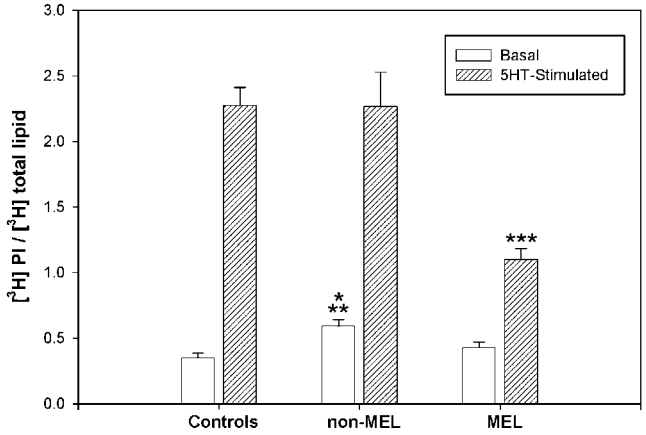

Figure 2 Basal and 5HT-stimulated phosphatidylinositol hydrolysis ([^{3}H]PI) in fibroblasts from control, nonmelancholic depressed (nonMEL), and melancholic depressed (MEL) subjects (mean ± SD). The basal signal was greater in nonmelancholics in contrast to controls (*$p = 0.002$) and melancholics (**$p < 0.03$). Maximum signal was significantly lower in melancholic patients *vs* both controls and nonmelancholics (***$p = 0.001$).

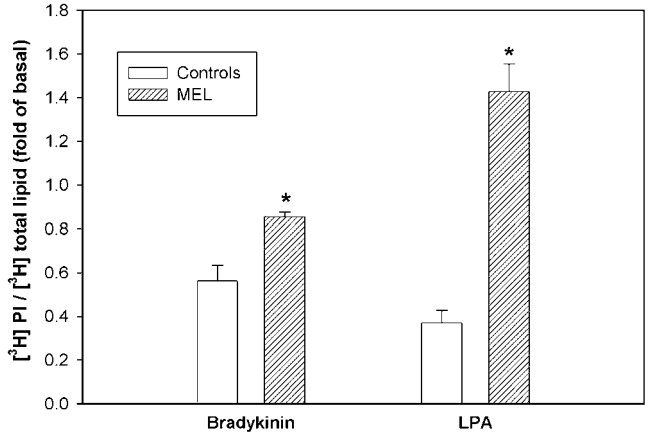

Figure 3 Phosphatidylinositol ([^{3}H]PI) hydrolysis with Gq-coupled receptor agonists in fibroblasts from control and melancholic depressed (MEL) subjects (mean ± SEM). The maximum bradykinin (10 μM) and LPA (10 μM) signal is greater in melancholic patients *vs* normal controls. $n = 10$ for normal controls and $n = 8$ for melancholic subjects. *$p < 0.05$ *vs* controls.

the basal [^{3}H]-IP formation was found in nonmelancholic patients relative to the other two groups (ANOVA: $F = 6.941$, $df = 2,57$, $p = 0.002$; *post hoc* Tukey HSD: nonmelancholics *vs* controls, $p = 0.002$; nonmelancholics *vs* melancholics, **$p < 0.03$; controls *vs* melancholics, $p =$ NS) (Figure 2). This difference was eliminated by the addition of clozapine (100 nM), a 5-HT$_{2A}$ receptor inverse agonist (data not shown), suggesting that the 5-HT$_{2A}$ receptor has constitutive activity in fibroblasts from nonmelancholic patients.

Bradykinin and LPA Stimulated PI Hydrolysis

To test whether or not the altered PI response was specific to the 5-HT$_{2A}$ receptors or, alternatively, related to the postreceptor mechanisms, we compared and examined the effects of bradykinin (10 μM) and LPA (10 μM), two other Gq-coupled receptor agonists, on PI hydrolysis (Figure 3). Unlike the response seen with 5-HT, the maximum bradykinin and LPA signals are actually increased in melancholic patients *vs* normal controls (bradykinin: $t = -3.733$, $df = 5$, $p < 0.02$; LPA: $t = -7.712$, $df = 6$, $p < 0.001$). This indicates that the decreased 5-HT signal is not related to alterations in postreceptor components of the signaling machinery.

[^{125}I]LSD Binding Studies

Preliminary radioligand binding experiments suggested a low density of 5-HT$_{2A}$ receptors in the cultured fibroblasts; this combined with the low yield of membrane protein from the fibroblasts cultured in dialyzed serum forced us to limit the binding experiments to a single concentration of [^{125}I]LSD. [^{125}I]LSD binding was performed in fibroblasts from normal, nonmelancholic, and melancholic patients that were used previously in the PI hydrolysis experiments; for each patient, twenty 75 mm plates were prepared and the specific binding of 3 nM of [^{125}I]LSD determined as described in methods, [^{125}I]LSD binding was not significantly different between the three groups ($F[2,15] = 2.06$, $p < 0.2$) (Figure 4). Although binding in the melancholic group was numerically higher than normal controls, this difference did not reach statistical significance.

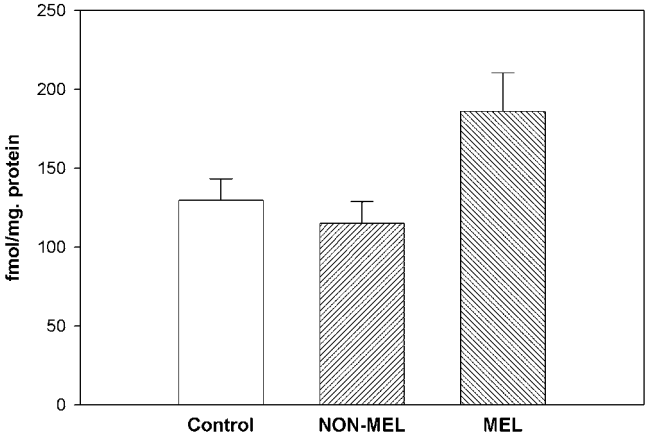

Figure 4 5-HT receptor density in fibroblasts from normal, nonmelancholic depressed (nonMEL) and melancholic depressed (MEL) subjects (mean ± SEM). Specific [^{125}I]LSD binding (3 nM) was determined in membrane preparations as described in Materials and Methods. The values are reported as fmol/mg protein. The density of binding sites was not different between the three groups ($n = 6$ for each group).

Immunolabeling of PLC$_{\beta 1}$ Isozyme and G$\alpha_{q/11}$ Protein

As an additional test of possible postreceptor alterations, we evaluated whether the low 5-HT-stimulated PI hydrolysis in fibroblasts from melancholics is due to altered expression of the PLC$_{\beta 1}$ isozyme or G$_q\alpha$ protein. Fibroblasts membranes were isolated and proteins determined by Western blot analyses using β-actin as an internal control. The level of PLC$_{\beta 1}$ isozyme and G$_q\alpha$ protein were not different in melancholic compared with normals and nonmelancholic patients ($n = 9$ for normals, $n = 7$ for nonmelancholics, and

$n = 8$ for melancholics). These results confirm that the decreased 5-HT signal is not related to a reduction in level of two key downstream mediators, PLC$_{\beta 1}$ isozyme and G$_q$ protein.

DISCUSSION

In the present study, the activity of the 5-HT-stimulated phospholipase C signaling pathway was investigated in fibroblasts from melancholic and nonmelancholic depressed patients and normal controls. Concentration response experiments revealed that PI hydrolysis was stimulated by 5-HT with a similar EC_{50} in the three groups, suggesting that these responses were the result of 5-HT interaction with receptors exhibiting similar binding characteristics and a similar degree of receptor reserve. MDL-100907, a highly specific 5-HT$_{2A}$ receptor antagonist, blocked 5-HT-stimulated PI hydrolysis, indicating that the effect was mediated via 5-HT$_{2A}$ receptors. The maximum 5-HT-stimulated PI hydrolysis response was markedly reduced in fibroblasts from melancholic subjects *vs* normal controls; however, this difference was not found in fibroblasts from nonmelancholic subjects. The maximum response of two other Gq-coupled receptors, bradykinin and LPA, was increased in melancholic subjects rather than decreased. The elevation of PI hydrolysis after activation by bradykinin and LPA is interesting in itself; the cause for this increase is unknown, and it needs to be explored further. However, the fact that the response via these two Gq-coupled receptor agonists is increased rather than reduced indicates that the effect seen with the activation of the 5-HT$_{2A}$ receptor is likely to be inherent in the receptor itself or its coupling to Gq protein. Furthermore, measurement of the radiolabeled lipid fraction showed that there were no differences in the level of substrates or lipid labeling between normals and melancholics. Thus, we conclude that the decrease in 5-HT-mediated PI hydrolysis is specific to the 5-HT$_{2A}$ receptor itself, rather than an alteration in the overall function of the cells. Since the blunting of the 5-HT$_{2A}$ receptor signal was maintained in subcultured cells, this change is not state dependent (ie secondary to drug treatment, hormone levels, etc.), but seems to be related to an underlying pathophysiological state.

One possible explanation for the blunted 5-HT$_{2A}$ receptor-mediated PI hydrolysis is a decrease in the density of receptors or other critical components of the 5-HT$_{2A}$ receptor-linked signal transduction pathway. The specific binding of [^{125}I]LSD did not differ between the three groups, suggesting that the reduced PI hydrolysis in melancholic participants was not dependent merely on altered receptor number. However, these data are very preliminary and certainly not definitive. Owing to the technical limitation of very low receptor protein, only a single concentration of [^{125}I]LSD was used; it is possible, then, that a difference would be masked if the density of receptors was decreased and the affinity for the ligand increased in parallel. Future studies in tissue subtypes with higher 5-HT$_{2A}$ protein concentration are needed in order to determine whether density or affinity are significantly changed.

PLC and Gq proteins are crucial components of the PI signaling pathway, and abnormalities in these components have been described in mood disorders in the past (Pandey *et al*, 1999, 2002a, b). However, in the current study, PI hydrolysis elicited by bradykinin and LPA actually was increased, not diminished, in melancholic patients relative to the other groups, suggesting that the abnormality in 5-HT$_{2A}$ receptor signaling was not related to the PLC-PI pathway *per se*. In addition, the levels of PLC$_{\beta 1}$ and Gq protein were not significantly decreased in fibroblasts from melancholic compared with normal and nonmelancholic subjects. Together, these results indicate that the abnormality in 5-HT-induced PI hydrolysis is directly related to the 5-HT$_{2A}$ receptor or its coupling to G proteins, and not other aspects of the intracellular signaling cascade.

These results must be viewed with caution, since the sample size was relatively small and a peripheral tissue was used. Replication in a larger sample size, and confirmation in human brain of the basic finding of a specific defect in melancholic *vs* nonmelancholic and controls is needed. However, it is more difficult to examine mechanism in postmortem human brain. Given that the reduction in 5-HT$_{2A}$ receptor signal was retained after many cell passages, a genetic alteration is likely, either in the receptor itself or key regulatory factors, such as multidomain scaffolding proteins. For example, recent evidence suggests that the 5-HT$_{2A}$ receptor (Xia *et al*, 2003) and its close relative, the 5-HT$_{2C}$ receptor (Parker *et al*, 2003), are regulated by interaction with a multifunctional PDZ domain protein. In addition, genetic studies have suggested that polymorphisms of the 5-HT$_{2A}$ receptor are associated with mood disorders, but the results have been mixed (Zhang *et al*, 1997; Spurlock *et al*, 1998; Turecki *et al*, 1999; Du *et al*, 1999; Hrdina and Du, 2001; Crawford *et al*, 2000; Arias *et al*, 2001). Four coding single-nucleotide polymorphisms (SNPS) that alter the amino-acid sequence of the 5-HT$_{2A}$ receptor have been identified, but have not yet been adequately evaluated in functional studies. An interesting possibility is that the blunted 5-HT$_{2A}$ receptor signal could be the result of different SNPs that produce a common functional deficit.

The current findings of alterations in 5-HT$_{2A}$ receptor response only in the melancholic subtype of depression may explain some of the earlier discrepancies in the literature. Melancholia is a subtype of depressive disorder that is characterized by specific clinical characteristics, especially so-called 'endogenous' features: markedly decreased sleep and appetite, psychomotor symptoms (eg motor retardation or agitation), loss of the capacity for pleasure and an absence of mood reactivity to stimuli that would be expected to elicit a reward response. As noted by Ressler and Nemeroff (2000), monoamines, including 5-HT, are critically involved in the modulation of neurobiologic systems that regulate these processes, and alterations in 5-HT function might be expected to contribute to these types of depressive symptoms. Further experiments of both the structure of the 5-HT$_{2A}$ receptor itself, as well as its interaction with potential intracellular binding partners, are needed to determine the mechanism of the reduced 5-HT$_{2A}$ receptor signal. Defining the mechanism of this abnormality of signal transduction could lead to new treatment strategies for melancholia.

ACKNOWLEDGEMENTS

This study was supported by NIMH Grants MH52339, MH01741, and MH34007.

REFERENCES

Aghajanian GK, Sanders-Bush E (2002). Serotonin In: Davis KL, Charney D, Coyle JT, Nemeroff C (eds). *Neuropsychopharmacology – The Fifth Generation of Progress*. Lippincott Williams & Wilkins: New York, NY. pp 15–25.

Alda M, Hrdina PD (2000). Distribution of platelet 5-HT 2A receptor densities in sucidal and non-suicidal depressives and control subjects. *Psychiatry Res* **94**: 273–277.

Alvarez JC, Glck N, Arnulf I, Quintin P, Leboyer M, Pecquery R *et al* (1999). Decreased platelet serotonin transporter sites and increased platelet inositol triphosphate levels in patients with unipolar depression. Effects of clomipramine and fluoxetine. *Clin Pharmacol Ther* **66**: 617–624.

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders* 4th edn. American Psychiatric Press: Washington, DC.

Arias B, Gasto C, Catalan R, Gutierrez B, Pintor L, Fananas L (2001). The 5-HT(2A) receptor gene 102T/C polymorphism is associated with suicidal behavior in depressed patients. *Am J Med Genet* **105**: 801–804.

Arranz B, Eriksson A, Mellerup E, Plenge P, Marcusson J (1994). Brain 5-HT1A, 5-HT1D, 5-HT2 receptors in suicide victims. *Biol Psychiatry* **35**: 457–463.

Barker EL, Westpal RS, Schmidt D, Sanders-Bush E (1994). Constitutively active 5-hydroxytryptamine 2C receptors reveal novel inverse agonist activity of ligands. *J Biol Chem* **269**: 11687–11690.

Cheetham SC, Crompton MR, Catona CLE, Horton RW (1988). Brain 5-HT2 receptor binding sites in depressed suicide victims. *Brain Res* **443**: 272–280.

Crawford J, Sutherland GR, Goldney RD (2000). No evidence for association of 5-HT2A receptor polymorphism with suicide. *Am J Med Genet* **96**: 879–880.

Delisi SM, Konopka LM, O'Connor FL, Crayto JW (1998). Platelet cytosolic calcium responses to serotonin in depressed patients and controls. *Biol Psychiatry* **43**: 327–334.

Du L, Faludi G, Palkovits M, Demeter E, Bakish D, Lapierre Y *et al* (1999). Frequency of long allele in serotonin transporter gene is increased in depressed suicide victims. *Biol Psychiatry* **46**: 196–201.

First MB, Spitzer RL, Gibbon M, Williams JBW (1996) Structured Clinical Interview for Axis I DSM-IV Disorders – Patient Edition (with Psychotic Screen) (SCID-I/P) (W/Psychotic Screen) (Version 2.0). Biometrics Research Department, New York State Psychiatric Institute: New York.

Gross-Isseroff R, Biegon A, Voet H, Weizman A (1998). The suicide brain: a review of postmortem receptor/transporter binding studies. *Neurosci Biobehav Rev* **22**: 653–661.

Hamilton M (1960). A rating scale for depression. *J Neurol Neurosurg Psychiatry* **23**: 56–62.

Hrdina PD, Bakish D, Chudzik J, Ravindran A, Lapierre YD (1995). Serotonergic markers in platelets of patients with major depression. Upregulations of 5-HT2 receptors. *J Psychiatry Neurosci* **20**: 11–19.

Hrdina PD, Bakish D, Ravindran A, Chudzik J, Cavazoni P, Lapierre YD (1997). Platelet serotonergic indices in major depression: up-regulation of 5-HT2A receptors unchanged by antidepressant treatment. *Psychiatry Res* **66**: 73–85.

Hrdina PD, Demeter E, Vu TB, Stonyi P, Palcovits M (1993). 5-HT uptake sites and 5-HT2 receptors in brain of antidepressant-free suicide victims/depressives. Increase in 5-HT2 sites in cortex and amigdala. *Brain Res* **614**: 37–44.

Hrdina PD, Du L (2001). Levels of serotonin receptor 2A higher in suicide victims? *Am J Psychiatry* **158**: 147–148.

Jope RS, Song L, Li PP, Young LT, Kish SJ, Pacheco MA *et al* (1996). The phosphoinositide signal transduction system is impaired in bipoar affective disorder brain. *J Neurochem* **66**: 2402–2409.

Karege F, Bovier P, Rudolph W, Gaillard JM (1996). Platelet phosphoinositide signaling system: an overstimulated pathway in depression. *Biol Psychiatry* **39**: 697–702.

Manier DH, Shelton RC, Ellis T, Peterson CS, Eiring A, Sulser F (2000). Human fibroblasts as a relevant model to study signal transduction in affective disorders. *J Affective Disord* **61**: 51–58.

Mann JJ, Stanley M, McBride PA, McEven BS (1986). Increased serotonin-2 and (-adrenergic receptor binding in the frontal cortex of suicide victims. *Arch Gen Psychiatry* **43**: 954–959.

McBride PA, Brown RP, De Meo M, Keilp J, Mieczkowski T, Mann JJ (1994). The relationship of platelet 5-HT2 receptor indices to major depression disorder, personality traits and suicidal behavior. *Biol Psychiatry* **35**: 295–308.

Neuger J, El Khoury A, Kjellman BF, Wahlund B, Aberg-Wistedt A, Stain-Malmgren R (1999). Platelet serotonin functions in untreated major depression. *Psychiatry Res* **85**: 189–198.

Pacheco MA, Stockmeier C, Meltzer HY, Overholser JC, Dilley GE, Jope RS (1996). Alterations in phosphoinositide signaling and G-protein levels in depressed suicide brain. *Brain Res* **723**: 37–45.

Pandey GN, Dwivedi Y, Pandey SC, Teas SS, Conley RR, Roberts RC *et al* (1999). Low phosphoinositide-specific phospholipase C activity and expression of phospholipase C β1 protein in the prefrontal cortex of teenage suicide subjects. *Am J Psychiatry* **156**: 1895–1901.

Pandey GN, Dwivedi Y, Rizavi HS, Ren X, Pandey SC, Pesold C *et al* (2002a). Higher expression of serotonin 5-HT2A receptors in the postmortem brains of teenage suicide victims. *Am J Psychiatry* **159**: 419–429.

Pandey GN, Dwivedi Y, SridharaRao J, Ren X, Janicak PG, Sharma R (2002b). Protein kinase C and phospholipase C activity and expression of their specific isozymes is decreased and expression of MARCKS is increased in platelets of bipolar but not in unipolar patients. *Neuropsychopharmacology* **26**: 216–228.

Pandey GN, Pandey SC, Dwivedi Y, Sharma RP, Janicak PG, Davis JM (1995). Platelet serotonin-2A receptors a potential biological marker for suicidal behavior. *Am J Psychiatry* **152**: 850–855.

Pandey GN, Pandey SC, Janicak PG, Marks RC, Davis JM (1990). Platelet serotonin-2 receptor binding sites in depression and suicide. *Biol Psychiatry* **28**: 215–222.

Pandey GN, Ren X, Pandey SC, Dwivedi Y, Sharma R, Janicak PG (2001). Hyperactive phosphoinositide signaling pathway in platelets of depressed patients: effect of desipramine treatment. *Psychiatry Res* **105**: 23–32.

Parker LL, Backstrom JR, Sanders-Bush E, Shieh BH (2003). Agonist induced phosphorylation of the serotonin 5-HT2C receptor regulates its interaction with multipl PDZ protein 1. *J Biol Chem* **278**: 215576–215583.

Rao ML, Hawellek B, Papassotiropoulos A, Deister A, Frahnert C (1998). Up-regulation of the platelet serotonin 2A receptor and low blood serotonin in suicidal psychiatric patients. *Neuropsychobiology* **38**: 84–89.

Ressler KJ, Nemeroff CB (2000). Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depress Anxiety* **12**(Suppl 1): 2–19.

Rosel P, Arranz B (1998). Variations in [^{3}H]-imipramine and 5-HT2A but not [^{3}H]-paroxetine binding sites in suicide brains. *Psychiatry Res* **82**: 161–170.

Rosel P, Arranz B, San L, Vallejo J, Crespo JM, Urretavizcaya M *et al* (2000). Altered 5-HT2A binding sites and second messenger inositol trisphosphate (IP3) levels in hippocampus but not in frontal cortex from depressed suicide victims. *Psychiatry Res* **99**: 173–181.

Rosel P, Arranz B, Vallejo J, Alvarez P, Menchon JM, Palencia T *et al* (1999). Altered [^{3}H] imipramine and 5-HT2 but not [^{3}H] paroxetine binding sites in platelets from depressed patients. *J Aff Disord* **52**: 225–233.

Saucier C, Morris SJ, Albert PR (1998). Endogenous serotonin-2A and -2C receptors in Balb/c3T3 cells revealed in serotonin-fee medium. Desensitization and down-regulation by serotonin. *Biochem Pharmacol* **56**: 1347–1357.

Shelton RC, Manier DH, Peterson CS, Ellis TC, Sulser F (1999). Cyclic AMP- dependent protein kinase in subtypes of major depression and normal volunteers. *Int J Neuropsychopharmacol* **2**: 187–192.

Shelton RC, Manier DH, Sulser F (1996). cAMP dependent protein kinase activity in major depression. *Neuropsychopharmacology* **15**: 55–61.

Shimon H, Agam G, Belmaker RH, Hyde TM, Kleinman JE (1997). Reduced frontal cortex inositol levels in postmortem brain of suicide victims and patients with bipolar disorder. *Am J Psychiatry* **154**: 1148–1150.

Soares JC, Mallinger AG (1997). Intracellular phosphatidylinositol pathway abnormalities in bipolar disorder patients. *Psycopharmacol Bull* **33**: 685–694.

Spurlock G, Heils A, Holmans P, Williams J, D'Souza UM, Cardno A *et al* (1998). A family based association study of T102C polymorphism in 5-HT2A and schizophrenia plus identification of new polymorphisms in the promoter. *Mol Psychiatry* **3**: 42–49.

Stanley M, Mann JJ (1983). Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet* **1**: 213–216.

Suzuki K, Kusumi I, Sasaki Y, Koyama T (2001). Serotonin-induced platelet intracellular calcium mobilization in various psychiatric disorders: is it specific to bipolar disorder? *J Aff Disord* **64**: 291–296.

Turecki G, Briere R, Dewar K, Antonetti T, Lesage AD, Seguin M *et al* (1999). Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. *Am J Psychiatry* **156**: 1456–1458.

Xia Z, Gray JA, Compto-Toth BA, Roth BL (2003). A direct interaction of PSD-95 with 5-HT2A serotonin receptors regulates receptor trafficking and signal transduction. *J Biol Chem* **278**: 21901–21908.

Zhang HY, Ishigaki T, Tani K, Chen K, Shih JC, Miyasato K *et al* (1997). Serotonin2A receptor gene polymorphism in mood disorders. *Biol Psychiatry* **41**: 768–773.