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Increased Gs α within Blood Cell Membrane Lipid Microdomains in Some Depressive Disorders: An Exploratory Study

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Abstract

The stimulatory guanine nucleotide binding protein Gs couples many cellular receptors to adenylyl cyclase, and the Gs α subunit activates all 9 isoforms of the adenylyl cyclase catalytic unit to produce the enzyme product cyclicAMP or cAMP. In prefrontal cortex and cerebellum of unipolar depressive suicides, Rasenick and colleagues have found increased concentrations of Gs α in membrane lipid microdomains (Donati et al, 2008), where the ensconced Gs α is less likely to activate adenylyl cyclase by receptor and postreceptor pathways (Allen et al, 2005 & 2009).

We report that a group of 7 depressed patients (DP-1) had (1) reduced activation of platelet receptor-stimulated adenylyl cyclase by both prostaglandins E2 and D2 compared to controls, and (2) reduced postreceptor stimulation of adenylyl cyclase by aluminum fluoride ion in both platelets and mononuclear leukocytes when compared to both another group of depressed patients (DP-2, n=17) and to controls (n=21). Our observations in the blood cells of the group DP-1 support the findings of Donati et al (2008), and they reflect the importance of this interaction between the activated Gs α subunit and membrane lipid microdomains in the pathophysiology and treatment of some major depressive disorders.

Keywords

major depressive disorder; adenylyl cyclase; Gs α subunit; membrane lipid rafts

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INTRODUCTION

Circulating blood platelets and mononuclear leukocytes have been used to study the receptor—Gs protein—adenylate cyclase (AC) enzyme catalytic unit complex in depressive disorders before and during treatment (Dwivedi & Pandey, 2008). The stimulatory guanine nucleotide binding protein Gs, which couples many cellular receptors to AC, activates all 9 isoforms of the AC catalytic unit, and Gs has a tissue distribution which is ubiquitous.

Gs is a heterotrimeric protein $G\alpha\beta\gamma$, composed of the alpha subunit $G\alpha$, and the Gs beta-gamma heterodimer, $G\beta\gamma$. The $G\alpha$ subunit is expressed by the complex imprinted locus *GNAS* on chromosome 20 (Kelsey, 2010; Mantovani, 2011), and there is a predominant maternal origin of *GNAS* transcripts in three seemingly unrelated tissues: renal proximal tubules, pituitary somatotrophs, and thyroid gland (Weinstein et al, 2000; Mantovani et al, 2002; Kelsey, 2010; Mantovani, 2011). The $G\alpha$ subunit is biallelically expressed in many other tissues, including leukocytes and platelets (Kelsey, 2010; Mantovani, 2011).

In the quiescent state, guanosine diphosphate (GDP) is bound to the $G\alpha$ subunit of Gs. Receptor activation of Gs leads to rapid replacement of GDP with guanosine triphosphate (GTP) and the binding of GTP is associated with conformational changes in so called 'switches' or regions of the $G\alpha$ subunit structure near the guanine nucleotide binding site. The activated $G\alpha$ subunit, $G\alpha\cdot GTP$, dissociates from both the receptor and $G\beta\gamma$, and stimulates the catalytic unit of membrane bound AC that converts ATP into cyclicAMP (cAMP) until the bound GTP is dephosphorylated back to GDP by the GTPase at the guanine nucleotide binding site.

The Gs protein can also be activated in postreceptor fashion by GTP analogues such as guanosine-5'-3-O-(thio)triphosphate ($GTP\gamma S$), or aluminum tetrafluoride ion (AlF_4 ion, herein abbreviated as AIF; Sternweis & Gilman, 1982). In the case of $GTP\gamma S$, the dissociation of $G\alpha$ -bound GDP is the rate-limiting step for $GTP\gamma S$ binding and activation (Ferguson et al, 1986). The steric configuration of AIF ion closely resembles a phosphate group (Bigay et al, 1985). Activation of Gs by AIF is very rapid since the AIF attaches adjacent to the cleft-bound GDP of $G\alpha$, forming $G\alpha-GDP-AIF-Mg^{+2}$ which slows the release of GDP, and mimics a transitional state of GTP (Higashijima et al, 1987; Coleman et al, 1994). Thus, $GTP\gamma S$ and AIF activate Gs by different postreceptor mechanisms.

Dwivedi & Pandey (2008) noted that basal levels of cAMP in plasma, cerebrospinal fluid, and blood cells were not altered in mood disorders. In platelets, AC activity is regulated by both the stimulatory Gs protein and the inhibitory G protein G_i (Katada, et al, 1984a). Several laboratories observed that cAMP formation was diminished in platelets from depressed patients following AC activation by prostaglandins through Gs. Using intact leukocytes, Mann et al (1997) found that in depressed patients, cAMP production was reduced in the presence of either the beta-adrenergic agonist isoproterenol or prostaglandin E_1 . In mononuclear leukocytes, AC activity is regulated by Gs but not by the inhibitory guanine nucleotide protein G_i , although G_i is present in mononuclear leukocytes (Motulsky et al, 1986; Maisel, et al, 1990). This suggests that diminished signaling through Gs rather than enhanced inhibitory signaling through G_i is responsible for the reductions in cAMP formation in leukocytes from depressed patients.

Activated $G\alpha$ can accumulate in membrane lipid microdomains, resulting in reductions in AC signaling (Allen et al, 2005 & 2009; Head et al, 2006; Donati et al, 2008). Allen et al (2009) has drawn attention to a study of platelet AC in 1,481 subjects with lifetime histories of alcohol and/or drug dependence, where subjects who also had lifetime histories of

depression (n=226) had reductions in the stimulation of platelet AC by both cesium fluoride and forskolin (Hines & Tabakoff, 2005). Allen et al (2009) suggested that these attenuated signals may reflect an increased proportion of Gs α being localized to lipid rafts. Donati et al (2008) found that there was a greater fraction of brain Gs α ensconced in lipid microdomains in the prefrontal cortex and cerebellum of unipolar depressed suicides compared to controls. Such lipid-rich regions are abundant in platelets (Dorahy et al, 1996; Jayachandran & Miller, 2003) and leukocytes (Szö r et al, 2010).

Here we describe a subgroup of untreated depressed patients DP-1 who had evidence of reduced AC signaling in both leukocytes and platelets. We propose that our findings in blood cells from the depressed DP-1 support the observations of Donati et al (2008) in unipolar depressed suicides.

MATERIALS & METHODS

The study protocol was approved by institutional review boards (IRB) at McLean Hospital (Belmont, MA), Massachusetts Mental Health Center, and Harvard Medical School (Boston, MA). All subjects were recruited from newspaper advertisements or by referral from the clinical staff at McLean Hospital during the period 1990–1993. We published an earlier report on these subjects using a de-identified data base (Mooney et al, 1998). The re-analysis of the same de-identified database for the present study does not meet the definition of human subjects research (Beth Israel Deaconess Medical Center IRB, Boston, MA; 2012).

On admission to the study in 1990–1993, all subjects were explained the nature and purpose of the study and gave their signed informed consent. The subjects were 24 (14 male and 10 female) depressed patients, and 21 (15 male and 6 female) nondepressed control subjects. Depressed patients met full DSM-III-R criteria for unipolar major depressive disorder based on a clinical interview using the Structured Clinical Interview for DSM-III-R (SCID) by a clinical research assistant who trained and achieved interrater reliability (kappa greater than .7) with standard raters at the Depression Research Facility of McLean Hospital. Untreated at intake, depressed patients scored a minimum of 15 on the 21-item Hamilton Depression Rating Scale (HDRS). The mean HDRS score was 23 ± 5 (range: 15–36). The duration of the “current episode” is known for 19 patients. The median duration of the current episode was 18 months (range: 1–240 months). Nine episodes were less than or equal to 12 months in duration, and 10 current episodes were greater than 12 months in duration. Control subjects were staff members or acquaintances of staff members at McLean Hospital who showed no current Axis I or Axis II disorders on the SCID and had a HDRS score less than 8. The mean age for the depressed patients was 38.4 ± 10.6 years, and for control subjects, 32.8 ± 8.0 years ($t = 1.95$, $df = 43$, NS).

All subjects had been free of all psychoactive medication, aspirin, and nonsteroidal anti-inflammatory agents for a minimum of two weeks prior to study (6 months for serotonergic agents and monoamine oxidase inhibitors), showed no drug or alcohol abuse in the previous 6 months, and evidenced no major medical disorders. There were 12 depressed patients with remote histories of alcohol or drug abuse. The mean values for AC activities in platelets and leukocytes were not significantly different when those depressed patients with histories of alcohol and/or drug abuse in the remote past were compared to those depressed patients who did not have remote histories of alcohol or drug abuse (data not shown). Patients with remote histories of alcohol or drug abuse did not have alcohol or drug dependence.

From the original group of 24 depressed patients, we identified a subgroup of 7 depressed patients, DP-1, who had abnormalities of blood cell adenylate cyclase signaling described in Results. These observations on DP-1 (mean age = 37 ± 10 years; 4 females, 3 males) were

compared to the remaining 17 depressed patients DP-2 (mean age = 39 ± 11 years; 6 females, 11 males) and the 21 control subjects (mean age = 32.8 years; 6 females, 15 males). The mean ages for DP-1 and DP-2 did not differ significantly from controls (data not shown). Mean HDRS scores at intake for DP-1 (22.3 ± 6.7) and DP-2 (23.6 ± 3.8) were not significantly different ($t = .61$, $df = 22$, NS). In the group DP-1, 4 members out of 7 had remote histories of alcohol or drug abuse, and in the group DP-2, 8 members out of 17 had remote histories of alcohol or drug abuse.

Adenylate Cyclase (AC) Assays in Platelet and Mononuclear Leukocyte Lysates

The isolation of mononuclear leukocytes and platelets from whole blood samples is described in Mooney et al (1998). Basal AC activities in platelet or mononuclear leukocyte lysates were measured by the method of Salomon et al (1974) using [γ - 32 P]cyclic adenosine monophosphate (cAMP; Mooney et al, 1998). All tissue assays were performed on the same day the blood sample was drawn from the subject. The final concentration of Mg^{+2} ion in the assay system was 3mM. The stimulation of AC by prostaglandins D2 and E2 in platelet lysates employed final concentrations of 10^{-4} mol/L of either prostaglandin D2 or prostaglandin E2. To determine the capacity of the full $\alpha 2$ -adrenergic agonist epinephrine (10^{-4} mol/L, final concentration) to inhibit platelet AC activity stimulated by 10^{-4} mol/L prostaglandin E2, we have reported the differences between the activities of prostaglandin E2-stimulated platelet AC in the presence and absence of epinephrine (Mooney, et al, 1998). The post-receptor stimulation of AC in platelet or mononuclear leukocyte lysates was also measured in the presence or absence of either 10^{-5} mol/L GTP γ S (final concentration) or aluminum fluoride ion (AlF; 5×10^{-3} mol/L NaF and 5×10^{-5} mol/L Al^{+3} , final concentrations; Sternweis & Gilman, 1982; Mooney et al, 1998). All AC activities in platelet and mononuclear leukocyte lysates were expressed in picomoles cAMP formed per mg protein per min. Protein was determined by the method of Lowry et al (1951) using bovine albumin as the standard. Basal AC activities were subtracted from other AC measures.

Urinary and Plasma Catecholamine Procedures

The methods for the analyses of norepinephrine and its metabolites in urine and plasma are provided in Supplementary Materials.

Statistical Methods

The data are expressed as mean $\pm$ 1SD. One-way analysis of variance and two-tailed t tests were used to compare AC values in mononuclear leukocytes and platelets from depressed patients and control subjects. Adenylate cyclase values were natural log-transformed prior to analysis of variance for comparisons across the three subject groups (DP-1, DP-2, and controls). Using the log-transformed data, post-hoc pairwise comparisons with appropriate corrections for multiple comparisons were made if the group main effect was significant. Statistical significance required either two-tailed $p < .01$ in Table 1 or two-tailed $p < .005$ in Table 2, employing the Bonferroni correction procedure for each data set in these tables. There were 19 (2.2%) missing observations out of a total of 855 study measures (45 subjects $\times$ 19 measured variables).

RESULTS

When the group of control subjects ($n = 19-21$) were compared to the entire group of depressed patients ($n = 22-24$; see Supplementary Material Table 1), there were some trends in the data bases from platelets and mononuclear leukocytes suggesting that the depressed patients as a group had lower AlF-stimulation of adenylate cyclase (AC) in mononuclear

leukocytes ($t=2.34$, $df=42$, $p<.025$) and platelets ($t=2.26$, $df=40$, $p<.025$), as well as reductions in prostaglandin E2 stimulation of platelet AC ($t=2.43$, $df=41$, $p<.01$).

Following Jones et al (1990), who studied the activation efficiency of the stimulatory G-protein Gs, we measured in platelets the relative efficiencies of receptor-mediated AC activities to postreceptor AC activities using prostaglandin E2 and A1F. In mononuclear leukocytes, we measured the relative efficiencies of two postreceptor AC activities using GTP γ S and A1F which activate AC by different postreceptor mechanisms (see Introduction).

Using these methods of procedure, a group of 7 depressed patients DP-1 was distinguished from both the 17 remaining depressed patients, group DP-2, and the 21 controls by having significantly greater values of the ratio mGTP γ S/mA1F for mononuclear leukocytes (Table 1) and the ratio PGE2/pA1F for platelets (Table 2).

In mononuclear leukocytes, analysis of variance for the ratio mGTP γ S/mA1F showed a significant group effect for DP-1, DP-2, controls: $F = 33.2$, where $F(.01,2,41) = 5.15$. The effect size η^2 is 0.62. The mean ratio was significantly greater in the group DP-1 than DP-2 or controls (see Table 1).

In platelets, analysis of variance for the ratio PGE2/A1F showed a significant group effect for DP-1, DP-2, controls: $F = 25.6$, where $F(.01,2,39) = 5.19$. The effect size η^2 is 0.57. The mean ratio was significantly greater in DP-1 than in DP-2 or controls (see Table 2).

The analysis of variance for the stimulation of mononuclear leukocyte AC by A1F (mA1F) showed a significant group effect: DP-1, DP-2, controls, $F = 22.5$ where $F(.01,2,41) = 5.15$. The effect size η^2 is 0.52. The reduction in the stimulation of leukocyte AC by A1F was significantly greater in DP-1 compared to either DP-2 or controls (see Table 1).

The analysis of variance for the stimulation of platelet AC by A1F (pA1F) showed a significant group effect: DP-1, DP-2, controls: $F = 23.4$, where $F(.01,2,39) = 5.19$. The effect size η^2 is 0.54. As observed in mononuclear leukocytes, A1F stimulation of AC in platelets was also significantly lower in DP-1 than in DP-2 or controls (see pA1F in Table 2). Although platelet AC activity is regulated by both Gs and the inhibitory G-protein Gi (Katada et al, 1984a), it is important to note that at the concentrations of A1F and Mg⁺² ion used here, A1F does not activate Gi in human platelets during the stimulation of basal AC activity (Katada et al, 1984b).

There was a significant group effect for the stimulation of platelet AC by prostaglandin E2: DP-1, DP-2, controls, $F = 7.16$, where $F(.01,2,39) = 5.19$. The effect size η^2 is 0.27. A significant reduction was observed in group DP-1 (but not group DP-2) when compared to controls (see Table 2).

There was a significant group effect for the stimulation of platelet AC by prostaglandin D2: DP-1, DP-2, controls, $F = 6.09$, where $F(.01,2,38) = 5.19$. The effect size η^2 is 0.24. A significant reduction in the stimulation of platelet AC by prostaglandin D2 was observed for group DP-1 (but not DP-2) when compared to controls (see Table 2).

There was no significant group effect for the basal AC activities (mBasal; Table 1) in mononuclear leukocytes across the three groups of subjects: DP-1, DP-2, controls: $F = 1.20$, where $F(.01,2,42) = 5.13$. There was no significant group effect for the postreceptor activation of mononuclear leukocyte AC by GTP γ S (mGTP γ S; Table 1): DP-1, DP-2, controls: $F = 3.25$ where $F(.01, 2, 42) = 5.13$.

There was no significant group effect for the basal AC activities (pBasal; Table 2) in platelets across the three groups of subjects: DP-1, DP-2, controls: $F = 2.84$, where $F(01,2,40) = 5.17$. There was no significant group effect for the postreceptor activation of platelet AC by GTP γ S (pGTP γ S, Table 2): DP-1, DP-2, controls: $F = 2.97$, where $F(01,2,40) = 5.17$.

Two Measurements of Platelet Gi Activity

The postreceptor stimulation of AC by GTP γ S in platelets was substantially lower in all three groups of subjects when compared to the GTP γ S-stimulated activities in mononuclear leukocytes where Gi does not regulate AC activity (compare mGTP γ S in Table 1 with pGTP γ S in Table 2; see Mooney et al, 1998). There were no significant changes across the three groups of subjects in an estimate of platelet Gi activity, delta-GTP γ S, which is the difference between GTP γ S-stimulated AC activities in mononuclear leukocytes and GTP γ S-stimulated AC activities in platelets (DP-1: 72 ± 38 ; DP-2: 76 ± 30 ; controls: 85 ± 36 ; see Mooney, et al, 1998). Secondly, we examined the suppression of PGE2-stimulated platelet AC activity by the α_2 -adrenergic agonist epinephrine which is mediated by platelet Gi, and the mean percent suppression of PGE2-stimulated platelet AC by epinephrine was comparable across all three groups of subjects (controls: $52 \pm 11\%$, range 31–69%; DP-1: $50 \pm 11\%$, range 33–70%; DP-2: $53 \pm 8\%$, range 38–61%). Together, these findings suggest that the reduction in prostaglandin E2 receptor-mediated stimulation of platelet AC activity in group DP-1 was not related to changes in platelet Gi activity.

Measurements of Urinary and Plasma Catecholamines and Metabolites

We examined the 24 hr outputs of urinary norepinephrine and its major urinary metabolites (NMN, MHPG, and VMA), and the 8 am plasma levels of norepinephrine and its metabolite MHPG in controls and the untreated depressed patients (see Supplementary Material Table 2). We found no significant differences in any of these measures across the three groups of subjects.

In summary, we have identified a group of 7 depressed patients DP-1 with reduced activation of mononuclear leukocyte AC during postreceptor stimulation of Gs by AIF, while basal AC activity and postreceptor stimulation of leukocyte AC by GTP γ S were comparable in DP-1, DP-2, and controls. Group DP-1 had significant reductions in the activation of platelet AC during postreceptor-mediated stimulation by AIF as well as reductions in the receptor-mediated stimulation of platelet AC by prostaglandins E2 and D2 which act through Gs. Basal platelet AC activities and the postreceptor stimulation of platelet AC by GTP γ S were comparable in DP-1, DP-2, and controls. Two measures of platelet Gi activity were unchanged across the three groups of subjects.

DISCUSSION

The diminished activation of AC during both prostaglandin receptor and postreceptor AIF stimulation in the platelets of the depressed patients in DP-1 (Table 2) raises the possibility of heterologous or agonist–nonspecific desensitization of the Gs-AC enzyme catalytic unit complex in the DP-1 patients. We also found reduced AIF stimulation of AC activity in mononuclear leukocytes from the group DP-1 (Table 1), but the postreceptor stimulation of AC by GTP γ S was unchanged in mononuclear leukocytes from DP-1 (Table 1). Studies using parallel AC activations by either guanine nucleotides (such as GTP γ S) or AIF during heterologous desensitization have noted that heterologous desensitization is associated with significant decreases in AC activity by both guanine nucleotides and AIF (Garrity et al, 1983; Edwards et al, 1987; Jaschonek et al, 1988; Yamashita et al, 1989). Thus, our finding reduced AIF-stimulation in the absence of reductions during GTP γ S stimulation in

mononuclear leukocytes from the group DP-1, suggests that a mechanism other than heterologous desensitization accounts for our observations in this group of depressed patients. This interpretation is also supported by our finding that the mean 24 hr urinary catecholamine outputs and the mean morning plasma catecholamine measures were comparable across the three groups of subjects, and all three groups of subjects (DP-1, DP-2, controls) had mean 24 hr urinary MHPG outputs in the intermediate range (1951–2500 micrograms of MHPG per 24 hr; see Supplementary Material Table 2; Schatzberg et al, 1982; Schildkraut et al, 1983).

Allen et al (2005 & 2009) have reported that both receptor-mediated activation of Gs and postreceptor activation of Gs by AIF can localize portions of Gs α to caveolae/membrane lipid microdomains which remove Gs α from membrane signaling cascades and “dampen globally Gs α /adenylyl cyclase/cAMP signaling” (Allen et al, 2009). Importantly, Rasenick and colleagues have found that there was an increased retention of Gs α in lipid microdomains isolated from prefrontal cortex and cerebellum of unipolar depressed suicides when compared to controls (Donati et al, 2008).

We found several studies from the US, UK, and Australia during the 1960’s–70’s which reported that untreated unipolar depressed patients excreted less urinary cAMP [see Sinanan et al (1975) and Jarrett et al (1977) and their bibliographies]. These culminated in the study of Jarrett et al (1977), who noted that the reductions in urinary cAMP content in untreated depressed patients were not accompanied by decrements in plasma cAMP or CSF cAMP levels. Parathormone, vasopressin, glucagon, and calcitonin can regulate renal excretion of cAMP by acting through Gs (Weinstein et al, 2000; Chase et al, 1969). However, we have found no reports describing the effects of challenge testing with these hormones on urinary cAMP levels in depressed patients.

Growth hormone releasing hormone (GHRH) regulates the release of growth hormone (GH) through activation of Gs α in pituitary somatotrophs (Germaine-Lee et al, 2003). Several studies have noted the blunted release of GH after GHRH infusion in depressed adults (Lesch et al, 1989; Contreras et al, 2007; Owashi et al, 2008), and in children/adolescents with depression (Dahl et al, 2000; Birmaher et al, 2000).

Thyroid-stimulating hormone (TSH) regulates the release of thyroid hormone through activated Gs α in thyroid tissue (Mantovani et al, 2002). Brouwer et al (2005 & 2006) have observed greater serum TSH values in the presence of normal thyroid hormone levels in a large group of depressed patients lacking both antithyroid antibodies and subclinical hypothyroidism.

The presence of blunted responses to GHRH and TSH in different groups of depressed patients could result from enhanced localization of activated Gs α in membrane lipid microdomains leading to reduced signal transduction through AC in pituitary somatotrophs and thyroid.

Antidepressants localize to lipid microdomains (Eisensamer et al, 2005) and chronic antidepressant treatment prevents the accumulation of Gs α in these microdomains, thereby enhancing receptor and postreceptor signaling by AC (Donati & Rasenick, 2005; Zhang & Rasenick, 2010).

Suzdak & Gianutsos (1986) observed that the receptor-mediated stimulation of AC significantly augmented cAMP accumulation in cerebral cortex slices from mice who received imipramine for two weeks *in vivo* when compared to controls.

Sinanan et al (1975) and Jarrett et al (1977) reported that successful antidepressant treatments for three weeks or more were associated with the recovery of urinary cAMP output to the levels seen in their controls, and Brouwer et al (2006) have observed that TSH resistance in their depressed patients was correlated with clinical responsiveness to the antidepressant paroxetine. The findings of Sinanan et al (1975), Jarrett et al (1977), and Brouwer et al (2006) suggest that the blunted formation of cAMP in kidney and thyroid may be relieved during chronic antidepressant treatment which restores AC signaling. Allen et al (2009) noted that disruption of lipid microdomains/caveolae in C6 glioma cells transfected with a TSH receptor enhanced TSH-stimulated cAMP production.

Chronic antidepressant treatment can overcome these functional impairments by reducing the entrapment of activated Gsα in membrane lipid microdomains, thereby enhancing Gsα-AC signaling which is temporally associated with clinical improvement. Thus, the functional abnormalities we observed in the DP-1 group may not preclude such patients from achieving successful treatment outcomes. (We note anecdotally that three DP-1 patients participated in 6-week trials of either fluoxetine or desipramine, and their 21-item HDRS scores decreased by 46%, 69%, and 81% respectively.) Our findings in blood cells from the depressed patient group DP-1 support the potential importance of the enhanced accumulation of activated Gsα in membrane lipid microdomains in the pathophysiology (Donati et al, 2008) and treatment (Donati & Rasenick, 2005; Zhang & Rasenick, 2010) of some major depressive disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Mononuclear leukocyte adenylate cyclase (AC) activities in two groups of depressed patients (DP-1, DP-2) and controls

Subjects (n)	Mononuclear Leukocyte AC (mAC) *			
	mBasal	mGTP γ S	mAlF ^a	[mGTP γ S/mAlF] ^b
Controls (21)	13.4 $\pm$ 4.9	110 $\pm$ 29	73 $\pm$ 26	1.56 $\pm$ 0.35
DP-1 (7)	10.8 $\pm$ 3.8	85 $\pm$ 43 (77% $\pm$ 39) ⁺	29 $\pm$ 20 (39% $\pm$ 27) ⁺	3.28 $\pm$ 0.81
DP-2 (16-17)	11.8 $\pm$ 3.8	96 $\pm$ 24 (88% $\pm$ 22) ⁺	67 $\pm$ 18 (89% $\pm$ 25) ⁺	1.49 $\pm$ 0.28

* GTP γ S = guanosine 5'-3-O- (thio) triphosphate; AlF = aluminum fluoride ion.

⁺The mean percentages of the mean mAC activity (mGTP γ S or mAlF) observed in the control group.

Data are presented as means $\pm$ SD with mAC activities expressed as pmol cAMP produced per min per mg protein.

Statistical significance required two-tailed p .01 (see Methods).

^a mAlF values:

DP-1 vs. Controls t=5.84, df=26, p<.0002

DP-1 vs. DP-2 t=5.05, df=21, p<.0002

^b [mGTP γ S/mAlF] ratios:

DP-1 vs. Controls t=7.02, df=26, p<.0002

DP-1 vs. DP-2 t=7.88, df=21, p<.0002

Table 2

Platelet adenylate cyclase (AC) activities in two groups of depressed patients (DP-1, DP-2) and controls

Subjects (n)	Platelet AC (pAC) [*]				
	pBasal	pGTPγS	pAIF ^a	PGE ₂ ^b	PGD ₂ ^d
Controls (19)	7.6±3.0	24±13	47±22	301±61	214±51
DP-1 (6-7)	4.8±2.3	13±7	11±12 (24%±25) ⁺	192±93	134±70
DP-2 (16-17)	7.1±4.8	20±11	41±18 (88%±30) ⁺	266±75	185±52

^{*} GTPγS=guanosine 5'-3-O- (thio) triphosphate; AIF = aluminum fluoride ion; PGE₂ = prostaglandin E₂; PGD₂ = prostaglandin D₂.

⁺The mean percentages of the mean AC activity observed in controls.

Data are presented as means±SD with pAC activities expressed as pmol cAMP produced per min per mg protein.

Statistical significance required two-tailed p .005 (see Methods).

^a pAIF mean values:

DP-1 vs. Controls t=6.32, df=24, p<.0002

DP-1 vs. DP-2 t=5.45, df=21, p<.0002

^b PGE₂ mean values:

DP-1 vs. Controls t=3.94, df=23, p<.001

^c [PGE₂/pAIF] mean values:

DP-1 vs. Controls t=6.22, df=24, p<.0002

DP-1 vs. DP-2 t=7.10, df=21, p<.0002

^d PGD₂ mean values:

DP-1 vs. Controls t=3.57, df=23, p<.002