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mRNA and Protein Expression of Selective Alpha Subunits of G Proteins are Abnormal in Prefrontal Cortex of Suicide Victims

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The present investigation was undertaken to examine whether there is an abnormality in the expression of α and $\beta\gamma$ subunits of G proteins both at the transcriptional and translational level in postmortem brain of adult and teenage suicide subjects and whether these abnormalities are related to mental disorders or suicide per se. In addition, an attempt has been made to investigate whether these abnormalities are similar or dissimilar in teenage and adult suicide, because the etiology of teenage suicide may be different than that of adults.

A significant decrease in both mRNA and protein levels of $G_{i2}\alpha$ and $G_O\alpha$ and a significant increase in levels of $G_s\alpha_{-S}$ were observed in prefrontal cortex of suicide subjects ($n = 43$) compared with non-psychiatric control subjects ($n = 38$). When subjects were grouped according to age, a significantly decreased expression of $G_{i2}\alpha$ and $G_O\alpha$ and significantly increased expression of $G_s\alpha_{-S}$ were observed in adult suicide subjects (age $\geqslant$ 20 yrs; $n = 20$) as compared with age-matched controls ($n = 27$). These changes were present in all adult suicide subjects regardless of psychiatric diagnosis. On the other hand, although there were no significant differences in any α or $\beta\gamma$ subunits in teenage suicide subjects (age $\leqslant$ 19 yrs; $n = 16$) when compared with matched control subjects ($n = 18$); however, mRNA and protein levels of $G_{i2}\alpha$ and $G_O\alpha$ were significantly decreased and of $G_s\alpha_{-S}$ were significantly increased only in those teenage suicide subjects who had a history of mental illness ($n = 11$). Our results suggest that there are defects in the expression of selective G protein α subunits in prefrontal cortex of adult and teenage suicide subjects, which appear to be related to mental disorders.

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KEY WORDS: *G proteins; Suicide; Postmortem brain; Human; Immunolabeling; mRNA*

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Suicide is a major public health concern. In the past several years there have been a number of studies suggesting an association of suicidal behavior with neurobiological abnormalities. The precise molecular mechanisms associated with suicidal behavior, however, remain unclear. Several studies have implicated various neurotransmitter receptors, such as serotonin $(5HT)_{2A}$, $5HT_{1A}$, α_1-, α_2-, and β adrenergic, as well as GABA, in the pathophysiology of suicide (reviewed by Gross-Isseroff et al. 1998; Pandey et al. 2002). The functional properties of these receptors, however, lie in their ability to relay extracellular messages through various signal transduction mechanisms. In this respect, guanine nucleotide binding proteins (G proteins) occupy a cen-

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tral position and play a critical role in transducing extracellular messages from cell surface receptors to effectors. About 80% of the receptors for neurotransmitters, hormones, and neuromodulators have been shown to elicit their responses through G proteins (Birnbaumer et al. 1990; Spiegel et al. 1990).

Structurally, G proteins are composed of three subunits, α, β, and γ, each encoded by a specific gene. β and γ subunits bind tightly to each other. Whereas the β subunit contains a common binding site for α subunit recognition, the α subunit binds to guanosine trisphosphate (GTP) and confers receptor effector specificity to G proteins. The γ subunit has been reported to have a G protein-specific receptor recognition site (Tamir et al. 1991). Receptor-mediated activation of G proteins causes the release of guanosine diphosphate (GDP) from the α subunit, allowing GTP to bind and induce the dissociation of the G protein α subunit from the βγ subunits. The α and the βγ subunits can then activate various effectors to modulate cellular responses (Neer 1995; Clapham and Neer 1997; Hamm 1998; Freissmuth et al. 1999).

On the basis of cDNA sequencing and the similarity of amino acid sequences, Gα subunits have been classified into four major classes: G_s, G_i, G_q, and G_{12}. More than 16 distinct genes encode the G protein α subunits with a splice variant from at least two genes (Gilman 1987; Simon et al. 1991; Neer 1995; Clapham and Neer 1997; Hamm 1998). Five distinct β subunit genes and 12 α subunit genes have also been identified (Clapham and Neer 1997; Hildebrandt 1997). Once dissociated, both α and βγ subunits can activate or inhibit multiple effectors to modulate cellular responses. These effectors include adenylyl cyclase, phospholipase C, phosphodiesterases, phospholipase A_2, and phosphoinositide 3 kinase, thereby inhibiting or activating a variety of second messengers, such as cAMP, cGMP, diacylglycerol, inositol (1,4,5) trisphosphate, phosphatidylinositol (3,4,5) trisphosphate, arachidonic acid, and phosphatidic acid, in addition to promoting increases in intracellular Ca^{2+} and the opening and closing of a variety of ion channels (Marinissen and Gutkind 2001). Recent studies demonstrate that different classes of G proteins can couple to a single receptor. This occurs through molecular switching of one G protein:effector system to a different G protein:effector system, triggered by agonist-mediated phosphorylation of receptors (Daaka et al. 1997; Lefkowitz 1998; Luo et al. 1999).

Because of the critical role of G proteins in signaling systems, studies in recent years have focused on their involvement in the pathophysiology of mental disorders, including affective disorders (Ozawa et al. 1993; Garcia-Sevilla et al. 1997, Young et al. 1993; Manji et al. 1995; Friedman and Wang 1996; Vawter et al. 2000), panic disorder (Stein et al. 1995; Gurguis et al. 1999a,b), Parkinson's disease (Avissar et al. 1997), schizophrenia (Jope et al. 1998), alcoholism (Hoffman and Tabakoff 1990; Jope et al. 1998), and also in the mechanisms of action of psychoactive drugs (reviewed by Manji 1992; Dwivedi and Pandey 1997). The role of G proteins in suicidal behavior, however, has not been evaluated in greater detail. There are a few studies that have examined abnormalities in the expression of G protein subunits in postmortem brain of suicide subjects (Cowburn et al. 1994; Pacheco et al. 1996; Garcia-Sevilla et al. 1999). However, the findings of these studies are inconsistent and do not clarify whether the alterations in G proteins are associated with mental disorders or suicide per se. Furthermore, from these studies it is not clear whether the alterations in levels of G protein subunits are due to a defect at the translational level or to a defect in gene transcription.

In the present investigation, we comprehensively studied the role of G proteins in suicidal behavior by examining the gene transcription as well as the protein levels of various α and βγ subunits of G proteins in postmortem brain of a large number of suicide subjects, which include subjects with and without a history of mental disorders.

An interesting aspect of this study is the inclusion of postmortem brain samples from teenage suicide subjects (age $\leqslant$ 19 yrs). Although the neurobiology of adult suicide has been studied, the neurobiology of teenage suicide has been virtually unexplored. Psychological and psychosocial studies suggest that some factors associated with teenage suicide may be different than in adult suicide. Whereas teenage suicide is thought to be driven primarily by impulsive, aggressive, and violent behaviors (Brent et al. 1993), adult suicide appears to be associated with chronic mental disorders. It is, thus, quite possible that the alterations in expression of G protein subunits may be different in postmortem brain of teenage suicide subjects than of adult suicide subjects.

Our present study thus clarifies several issues, namely whether: (1) there are any difference in levels of G protein subunits in postmortem brain of suicide subjects; (2) alterations in levels of G protein subunits occur at the transcriptional level; (3) alterations in expression of G protein are related to mental disorders; and (4) alterations in G protein subunit expression are different in teenage than in adult suicide.

MATERIALS AND METHODS

Postmortem Brain Samples

Postmortem brain samples from 43 adult and teenage suicide and 38 non-psychiatric control subjects, herein referred to as control subjects, were collected by the Brain Collection Program of the Maryland Psychiatric Research Center, Baltimore, MD, in collaboration with the Medical Examiner's Office of the State of Maryland.

The present studies were performed in prefrontal cortex (Brodmann's area (BA) 8/9) obtained from the right hemisphere of the brain. All the cases were examined neuropathologically prior to inclusion in the study. Cases with signs of atrophy, infarcts or gross anatomical abnormality were excluded from this study. These brain samples were also negative to HIV antibodies. To make sure that tissues were taken from the same anatomic level, sections were prepared from tissue blocks and Nissl stained. The tissue sections were matched across the control and suicide groups. Toxicology data of subjects were obtained by analysis of urine and blood samples. The biochemical determinations were performed in parallel between control and suicide subjects. Each determination was measured in duplicate per subject.

We elected to analyze BA 8/9 since most of the previous G protein studies have been performed in this brain area (Pacheco et al. 1996; Garcia-Sevilla et al. 1999). Furthermore, we have a long-standing interest in the dorsolateral prefrontal cortex since this brain area has been shown to play a relevant role in mood regulation (George et al. 1994) and has been implicated in the pathophysiology of affective disorders and suicide in a number of neurochemical studies (reviewed by Rajkowska 1997; Sastre et al. 2001). In our own studies, we have observed several interesting findings in BA 8/9 of suicide victims, such as a higher number of $5HT_{2A}$ receptors, increased expression of mRNA and protein of $5HT_{2A}$ receptors (Pandey et al. 2002), and abnormalities in phosphoinositide (Pandey et al. 1997, 1999), adenylyl cyclase-cAMP (Dwivedi et al. 2002), and mitogen-acivated protein kinase (Dwivedi et al. 2001) pathways.

All subjects in this study were diagnosed in the following manner: after giving written informed consent, at least one family member was interviewed, based on the Diagnostic Evaluation After Death (DEAD) (Salzman et al. 1983), the Schedule for Clinical Interviews for the DSM-IV (SCID) (Spitzer et al. 1992), or a teenage diagnostic instrument, the Kiddie Schedule for Affective Disorders and Schizophrenia (KIDDISADS). Family members gave permission for clinical records to be obtained from mental health treatment providers when there was a history of mental health treatment, and in all cases of suicide. An attempt was made to collect all the available records on each case, and then the appropriate data were extracted from the records and collated using the DEAD. Two senior psychiatrists provided independent DSM-IV diagnoses. These diagnoses were compared and discrepancies were resolved by means of a consensus conference. Similarly, controls were verified as free from mental illness using such consensus diagnostic procedures. Data on suicide cases were collected and the circumstances of the suicide were determined using the DEAD form during the same interview process. The protocols for tissue sampling and retrospective assessments were approved by the Institutional Review Board (IRB) of the University of Maryland. This study was also approved by the IRB of the University of Illinois at Chicago.

The demographic characteristics of suicide and control subjects are provided in Table 1. There were 31 males and 7 females in the control group and 27 males and 16 females in the suicide group. The age range was 12–87 years and the postmortem interval (PMI) was in the range of 5–35 h. We observed that there were no significant differences in age ($t = 0.05$, $df = 79$, $p = .96$) or PMI ($t = 0.10$, $df = 79$, $p = .91$) between suicide and control subjects. To determine the agonal state, brain pH was measured by homogenizing a piece of cerebellum in 15 volumes of distilled water, and the acidity was measured by a pH meter. There were no significant differences in pH of the brain between control and suicide subjects ($t = 0.002$, $df = 79$, $p = .99$), as it ranged from 5.35 to 6.85. The tissue storage time was in the range of 3.9–7.6 years and did not differ between control and suicide subjects ($t = 1.5$, $df = 79$, $p = .13$).

When control and suicide subjects were subdivided on the basis of age (adult $\geqslant$ 20 yrs; teenage $\leqslant$ 19 yrs), there were 27 adult suicide subjects and 20 adult controls, and 16 teenage suicide subjects and 18 teenage control subjects. There were no significant differences in age ($t = 0.63$, $df = 45$, $p = .53$) or PMI ($t = 0.07$, $df = 45$, $p = .95$) between adult suicide and adult control subjects, as well as in age ($t = 1.7$, $df = 32$, $p = .08$) or PMI ($t = 0.02$, $df = 32$, $p = .98$) between teenage suicide and teenage control subjects. There were 16 male and 4 female subjects in the adult control group and 17 male and 10 female subjects in the adult suicide group. In the teenage control group, there were 15 male and 3 female subjects, whereas in the teenage suicide group, there were 10 male and 6 female subjects. The distribution of races in the adult and teenage populations was as follows: 8 black and 12 white in adult control; 4 black and 23 white in adult suicide; 12 black and 6 white in teenage control; and 3 black, 11 white, and 2 of other races in teenage suicide group.

Quantitation of G Proteins by Western Blot

Gel electrophoresis and immunolabeling of G protein subunits ($G_s\alpha_{-S}$, $G_s\alpha_{-L}$, $G_{i1}\alpha$, $G_{i2}\alpha$, $G_O\alpha$, $G_{q/11}\alpha$, Gβ and Gγ) were performed by Western blot as previously reported (Dwivedi and Pandey 1997). Equal amounts of protein samples (25 μg protein in each lane) were loaded onto 12% (w/v) acrylamide gel and subsequently transferred electrophoretically to enhanced chemiluminescent (ECL) nitrocellulose membranes (Amersham Pharmacia, Piscataway, NJ). The blots were incubated overnight at 4°C with primary antibody for $G_s\alpha$, $G_{q/11}\alpha$, $G_O\alpha$ (NEN Research Products, Boston, MA)

Table 1. Demographic Characteristics of Suicide Control Subjects

Group & Subject	Age (Yrs)	Race	Gender	PMI (hrs)	Brain pH	Tissue Storage Time (Yrs)	Cause of Death	Drug Toxicity	Psychiatric Diagnosis
Suicide Subjects									
1.	22	Black	Female	16	5.3	4.2	Drug overdose	Propranolol	Major depression
2.	24	White	Male	7	5.6	6.2	GSW	None	Major depression
3.	21	White	Male	17	6.1	5.7	GSW	None	Major depression, adjustment disorder
4.	27	White	Male	24	6.4	6.8	GSW	None	Major depression, alcohol abuse
5.	38	White	Male	24	6.3	4.5	Drug overdose	Ethanol, Diphenhydramine	Major dpression, alcohol abuse
6.	36	White	Female	10	6.5	5.8	GSW	Butalbital, Diphenhydramine, Acetaminophen	Major depression
7.	41	White	Female	27	5.9	6.9	Drug overdose	Amitriptyline, Desipramine, Diphenhydramine, Nortriptyline, Pseudophedrine, Salicylate, Ethanol	Major depression, alcohol abuse
8.	44	White	Female	11	5.6	6.8	Drug overdose	Nortriptyline	Major depression, alcohol abuse
9.	46	White	Female	16	6.1	5.9	Drug overdose	Nortriptyline	Major depression, agoraphobia
10.	46	White	Female	21	5.3	3.8	Drug overdose	Amitriptyline, Desipramine, Ethanol	Major depression
11.	53	White	Male	23	6.1	4.9	Jump	None	Major depression
12.	24	White	Male	22	6.5	4.4	Hanging	None	Schizoaffective disorder
13.	21	White	Male	23	6.4	5.5	Hanging	None	Adjustment disorder
14.	40	White	Male	26	5.6	5.8	GSW	Ethanol	Adjustment disorder
15.	68	White	Female	26	6.1	6.1	GSW	None	Schizoaffective disorder
16.	37	Black	Male	NA	5.8	6.5	CO_2 intoxication	CO	NA
17.	26	Black	Male	NA	6.5	6.6	Hanging	Cocaine	NA
18.	50	White	Male	7	6.1	6.5	GSW	None	No psychiatric illness
19.	24	White	Male	22	6.6	6.2	GSW	None	Schizoaffective disorders
20.	75	White	Male	18	6.7	5.6	GSW	None	Adjustment disorder/conduct disorder
21.	71	White	Female	24	6.4	6.1	Drug overdose	Pseudophedrine, Acetaminophen	Adjustment disorder
22.	20	White	Female	11	6.5	5.2	Jump	None	Schizophrenia
23.	21	White	Male	24	6.4	6.4	Drug overdose	Acetaminophen, Norpropoxyphen, Propoxyphene	Schizophrenia
24.	40	White	Male	17	6.2	6.1	Jump	None	Schizophrenia
25.	41	Black	Male	12	6.3	5.5	Multiple injuries	None	No psychiatric illness
26.	87	White	Male	16	6.2	6.6	GSW	None	Adjustment disorder
27.	36	White	Female	18	NA	7.0	GSW	None	Schizoaffective
28.	15	White	Female	7	5.5	5.8	GSW	Ethanol	NA
29.	12	Black	Male	10	5.9	4.8	Hanging	None	Major Depression
30.	15	White	Female	11	6.4	6.2	Drug overdose	Phenylpropanolamine, chlorpheniramine, codeine, salicylate, acetaminophen	No psychiatric illness
31.	13	White	Male	18	6.0	5.4	Hanging	Ritalin	Hyperactivity, attention deficit disorder
32.	13	Black	Male	11	5.4	6.1	Hanging	None	No psychiatric illness

continued

Table 1. *(continued)*

Group & Subject	Age (Yrs)	Race	Gender	PMI (hrs)	Brain pH	Tissue Storage Time (Yrs)	Cause of Death	Drug Toxicity	Psychiatric Diagnosis
Suicide Subjects									
33.	15	White	Male	11	5.3	5.1	Asphyxia	None	Major depression
34.	15	White	Female	17	5.6	5.8	Drug overdose	Imipramine, desipramine	Major depression, hyperactivity, attention deficit disorder
35.	16	Other	Male	20	6.2	6.3	Hanging	None	No psychiatric illness
36.	17	White	Female	25	5.6	4.6	Drug overdose	Verapamil	Adjustment disorder
37.	15	White	Male	27	6.1	5.5	GSW	Pseudoephedrine, phenylpropanolamine	Adjustment disorder with depressed mood
38.	16	White	Female	18	6.3	4.6	GSW	Amitriptyline	No psychiatric illenss
39.	16	White	Female	33	6.6	4.4	GSW	None	Adjustment disorder
40.	16	White	Male	24	6.8	6.8	Hanging	None	Adjustment disorder, conduct disorder
41.	19	White	Male	12	NA	7.0	Hanging	None	No psychiatric illness
42.	17	Other	Male	7	NA	7.1	GSW	Ethanol	No psychiatric illness
43.	16	Black	Male	12	NA	6.6	GSW	None	No psychiatric illness
Mean	31	34 White/ 7 Black/	27 Male/	17.7	6.05	5.8			
SD	19	2 Other	16 Female	6.7	0.41	0.85			
Control Subjects									
44.	45	White	Male	22	6.5	5.0	ASCVD	None	No psychiatric illness
45.	22	Black	Male	19	6.2	3.9	GSW	None	No psychiatric illness
46.	83	White	Male	20	5.6	6.3	ASCVD	None	No psychiatric illness
47.	63	White	Female	30	5.7	7.6	Ovarian cancer	None	No psychiatric illness
48.	31	Black	Male	8	5.6	6.6	GSW	None	No psychiatric illness
49.	35	White	Male	24	5.6	6.5	Crash injury	None	No psychiatric illness
50.	33	White	Male	15	6.0	5.6	GSW	Acetaminophen	No psychiatric illness
51.	37	Black	Male	5	6.6	5.6	ASCVD	None	No psychiatric illness
52.	37	White	Male	24	6.3	5.3	ASCVD	None	No psychiatric illness
53.	65	Black	Female	23	5.6	4.5	ASCVD	None	No psychiatric illness
54.	38	Black	Male	16	5.8	5.2	Lung sarcoidosis	None	No psychiatric illness
55.	40	White	Female	7	6.5	3.9	ASCVD	None	No psychiatric illness
56.	23	Black	Male	15	6.7	4.6	GSW	None	No psychiatric illness
57.	42	White	Female	23	6.2	6.5	Pneumonia	None	No psychiatric illness
58.	46	Black	Male	9	6.2	7.5	Multiple injuries	None	No psychiatric illness
59.	48	White	Male	26	6.1	4.4	ASCVD	None	No psychiatric illness
60.	52	White	Male	30	6.3	4.1	ASCVD	None	No psychiatric illness
61.	37	Black	Male	9.5	6.1	4.4	ASCVD	None	No psychiatric illness
62.	43	White	Male	17.5	5.7	4.1	NA	None	No psychiatric illness
63.	41	White	Male	24	6.2	4.3	NA	None	No psychiatric illness
64.	19	Black	Male	6	6.1	6.8	GSW	None	No psychiatric illness
65.	16	Black	Male	6	6.5	5.8	GSW	None	No psychiatric illness
66.	19	Black	Male	12	5.9	5.8	GSW	None	No psychiatric illness

continued

Table 1. (continued)

Group & Subject	Age (Yrs)	Race	Gender	PMI (hrs)	Brain pH	Tissue Storage Time (Yrs)	Cause of Death	Drug Toxicity	Psychiatric Diagnosis
67.	17	Black	Male	10	5.9	5.6	GSW	None	No psychiatric illness
68.	16	White	Male	10	5.4	4.4	Stab wound	None	No psychiatric illness
69.	18	Black	Male	11	6.2	6.4	GSW	None	No psychiatric illness
70.	17	Black	Male	11	6.5	5.4	GSW	None	No psychiatric illness
71.	13	Black	Male	22	6.1	6.4	GSW	None	No psychiatric illness
72.	14	Black	Male	18	5.7	4.9	GSW	None	No psychiatric illness
73.	18	Black	Male	27	6.1	5.8	Drowning	None	No psychiatric illness
74.	16	White	Male	21	6.0	5.6	Hanging	None	Adjustment disorder
75.	18	White	Female	35	6.0	6.1	Accident (multiple injuries)	None	Adjustment disorder, conduct disorder
76.	17	Black	Female	26	6.2	5.3	Accident (multiple injuries)	None	No psychiatric illness
77.	19	White	Female	10	6.2	4.6	Cardiac arrhythmia	None	No psychiatric illness
78.	18	White	Male	19	6.0	6.8	Drowning	None	No psychiatric illness
79.	16	Black	Male	8	5.6	4.9	GSW	None	No psychiatric illness
80.	19	Black	Male	9	6.8	3.3	GSW	None	No psychiatric illness
81.	13	White	Male	NA	5.2	2.1	Accidental hanging	None	No psychiatric illness
Mean	31	18 White/	31 Male/	17.5	6.05	5.4			
SD	17	20 Black	7 Female	8.2	0.37	1.0			

ASCVD = atherosclerotic cardiovascular disease, GSW = gunshot wound, NA = not available.

$G_{i1}\alpha$, $G_{i2}\alpha$ or $G\beta\gamma$ (Calbiochem, San Diego, CA) at a dilution of 1:3000 to 1:5000 (depending on the antibody used) and with horseradish-peroxidase-linked secondary anti-rabbit antibody (Amersham Pharmacia) for 3 to 5 h at room temperature. The signals were detected with the ECL Western Blot Detection System (Amersham), followed by exposure to ECL-autoradiographic film (Amersham). The membranes were stripped using stripping solution (Chemicon International, Temecula, CA), and probed with β-actin monoclonal primary (1:5000 for 1 h, Sigma Chemical Co., St. Louis, MO) and anti-mouse secondary antibody (1:5000 for 1 h). The bands on the autoradiograms were quantified using the Loats Image Analysis System (Westminster, MD). The optical density of each G protein subunit was corrected by the optical density of the corresponding β-actin band.

Determination of mRNA Levels of $G_S\alpha$, $G_{i1}\alpha$, $G_{i2}\alpha$, and $G_O\alpha$ by Competitive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total RNA from BA 8/9 was isolated by $CsCl_2$ ultracentrifugation as described earlier (Dwivedi et al. 2001). The primer pairs were designed to allow amplification for $G_S\alpha$ (518–841 bp; Bray et al. 1986; GenBank accession # M14631), $G_{i1}\alpha$ (487–882 bp; Bray et al. (1987); GenBank accession # NM002069), $G_{i2}\alpha$ (483–884 bp; Didsbury et al. (1987); GenBank accession # 31743), and $G_O\alpha$ (506–818 bp; Zigman et al. (1994); GenBank accession # L10665). Each primer contained a comparable G/C content to minimize the variability in hybridization efficiency at the annealing temperature. The sequences and the positions of external primers and the percent of G/C content of each primer are given in Table 2. The specificity of $G_s\alpha$, $G_{i1}\alpha$, $G_{i2}\alpha$, and $G_O\alpha$ products was checked by sequencing the amplified area with the Sequenase Version 2.0 DNA Sequencing Kit.

Internal standards (cRNA) for the various G protein subunits were prepared by site-directed mutagenesis using PCR overlap extension, as described earlier (Dwivedi et al. 2001). Each standard was designed to introduce a *Bgl*II or *Xho*I restriction site midway between the amplification primers so that the digestion of the amplicon would generate two fragments of approximately equal molecular size. The designs for each internal primer are provided in Table 3. The internal standard templates were linearized with *Ssp*I. The cRNA corresponding to sense strand was synthesized with linearized template and Sp6 RNA polymerase by means of an in vitro transcription kit.

To quantitate the mRNA levels of the various G protein subunits, decreasing concentrations of internal standard cRNA were added to 1 μg of RNA and the mixture of total RNA and RNA/cRNA was reverse-transcribed with cloned Moloney murine leukemia virus (M-MLV) and reverse-transcriptase (200 U). The RT mixture was incubated at 37°C for 60 min to promote cDNA synthesis. The reaction was terminated by heating the tissue samples at 98°C for 5 min. In all assays, as a control, one RT reaction was performed in the absence of RNA.

The PCR mixture was amplified for 30 cycles (94°C, 15 s; 60°C, 30 s; 72°C, 30 s) using 0.5 μM specific primer pairs, 200 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM Tris/HCl (pH = 9.0), 20 mM ammonium sulfate, 15 mM KCl, 1 μ Ci of ^{32}PdCTP and 2.5 U of Hot *Tub* DNA polymerase. Following amplification, aliquots were digested with restriction enzyme in triplicate and run by 1.5% agarose gel electrophoresis. To quantitate the amount of product, the ethidium bromide-stained bands were excised and counted. The results were calculated as the counts incorporated into the amplified cRNA standard divided by the counts incorporated into the corresponding mRNA amplification product versus the known amount of internal standard (cRNA) added to the test sample

Statistical Analysis

Data analyses were performed using the SPSS 8.0 (Chicago, IL) statistical software package. All values re-

Table 2. External Primer Sequence of $G_S\alpha$, $G_{i1}\alpha$, $G_{i2}\alpha$ and $G_O\alpha$ for Amplification

	Primers	% (C + G)	Nucleotide Position
$G_S\alpha$	F: 5′ACG TGA TCA AGC AGG CTG ACT	52	548–568
	R: 5′GGA ACA GGA TCA CAG AGA TGG	52	851–871
$G_{i1}\alpha$	F: 5′ GCT CAA CCA AAT TAC ATC CCG	47	487–507
	R: 5′GTT TGA TCC TGC ATA TTC TGG	43	862–882
$G_{i2}\alpha$	F: 5′GCG TAT TGC ACA GAG TGA CTA	47	483–503
	R: 5′TTG GCC CCT GTG TAC TCA GGG	61	864–884
$G_O\alpha$	F: 5′TCG ACA GCG TCA GCT TGG TTG	61	506–526
	R: 5′GAA ATG GTC CGT AAC CAC CTG	52	798–818

F = Forward; R = Reverse.

Table 3. Internal Primer Sequence of $G_S\alpha$, $G_{i1}\alpha$, $G_{i2}\alpha$ and $G_O\alpha$ for Site-directed Mutagenesis

Primers		Restriction endonuclease	% (C + G)	Nucleotide position
$G_S\alpha$	5′AGT GGA TCC AGA *TCT* TCA ACG ATG	*Bgl*II	46	668–691
	5′CAT CGT TGA AGA *TCT* GGA TCC ACT		46	
$G_{i1}\alpha$	5′TAC GAC CTG G*CT* CGA GCT GAA GAT	*Xho*I	54	688–711
	5′ATC TTC AGC *TCG* AGC CAG GTC GTA		54	
$G_{i2}\alpha$	5′CTT CTG CGT AGA *TCT* GAG CGC CTA	*Bgl*II	54	669–692
	5′TAG GCG CTC AGA *TCT* ACG CAG AAG		54	
$G_O\alpha$	5′GAA AAT GGC TCG AGT GCT TTAACG	*Xho*I	46	656–679
	5′CGT TAA AGC ACT CGA GCC ATT TTC		46	

Italicized letters indicate the mutated bases. Underlined bases indicate cleavage site.

ported are the mean ± the standard deviation (S.D.). The differences in mRNA and protein levels of the various subunits of G protein, age, and PMI between suicide and control subjects were analyzed using the independent-sample *t*-test. The relationships between the mRNA and protein levels of G protein subunits, and PMI, age, and gender were determined by Pearson product-moment correlation analysis. *p* values were 2-tailed. The statistical differences in levels of $G_s\alpha_{-S}$, $G_{i1}\alpha$, $G_{i2}\alpha$, $G_O\alpha$, $G_{q/11}\alpha$, Gβ, and Gγ between various subgroups of suicide subjects and normal controls were evaluated by 1-way analysis of variance (ANOVA). During analysis of the data, we included race as a potential confounding variable. Multiple comparisons were conducted and we report unadjusted *p* values. We have indicated in the tables the Bonferroni adjusted α level and the rationale for its adjustment.

RESULTS

Immunolabeling of G Protein Subunits in Prefrontal Cortex of Control and Suicide Subjects

Representative autoradiograms of expressed levels of $G_s\alpha$, $G_{i1}\alpha$, $G_{i2}\alpha$, $G_{q/11}\alpha$, $G_O\alpha$, and Gβγ subunits in the prefrontal cortex of control and suicide subjects are given in Figure 1. The molecular weights of $G_{i1}\alpha$, $G_{i2}\alpha$, $G_O\alpha$, and $G_{q/11}\alpha$ were 42 kDa, which is consistent with those reported earlier by us (Dwivedi and Pandey 1997) and in the literature (Spiegel et al. 1990). It has been reported that $G_s\alpha$ resolves into two bands, i.e., 52 and 45 kDa (Jones et al. 1990), which is due to the splicing of a single gene (Robishaw et al. 1986). In our experiments, we also observed two bands (52 and 45 kDa); therefore both bands (low-molecular-weight $G_s\alpha_{-S}$ and high-molecular-weight $G_s\alpha_{-L}$) were quantified separately. Immunolabeling of Gβγ subunits using a common Gβγ antibody resolved into two bands: one at 35 kDa (β) and another at 10 kDa (γ). The apparent molecular weight of β-actin, which was used as a housekeeping protein, was 46 kDa.

Comparison studies showed that levels of $G_{i2}\alpha$ and $G_O\alpha$ were significantly decreased and the level of $G_s\alpha_{-S}$ was significantly increased in BA 8/9 of suicide subjects (n = 43) compared with control subjects (n = 38) (Figure 2). No significant differences were observed in the levels of $G_s\alpha_{-L}$, $G_{q/11}\alpha$, $G_{i1}\alpha$, Gβ, or Gγ subunits between suicide and control subjects.

In order to examine if the levels of G protein subunits were differentially affected in teenage and adult suicide, we subdivided the total suicide population into teenage (⩽ 19 years) and adult (⩾ 20 years) groups and analyzed the levels of G protein subunits in these two populations separately. Representative Western blots of various subunits of G proteins from two adult and two teenage suicide subjects and their respective age-matched control subjects are shown in Figure 1, and scattergrams of protein levels of G protein subunits from 27 adult suicide and 20 adult controls, and 16 teenage suicide and 18 teenage controls are given in Figures 3 and 4, respectively. It was observed that the expressed protein levels of $G_{i2}\alpha$ and $G_O\alpha$ were significantly decreased whereas the protein level of $G_s\alpha_{-S}$ was significantly increased in BA 8/9 of adult suicide subjects compared with adult control subjects. We did not observe any significant changes in protein levels of $G_s\alpha_{-L}$, $G_{i1}\alpha$, $G_{q/11}\alpha$, Gβ or Gγ subunits between adult control and adult suicide subjects (Figure 3).

When the teenage suicide group was compared with the teenage control group, there were no significant differences in protein levels of any of the G protein subunits studied, i.e., $G_s\alpha_{-S}$ $G_s\alpha_{-L}$, G_O G_{i1} G_{i2} $G_{q/11}$ Gβ, or Gγ (Figure 4).

mRNA Levels of G Protein Subunits in Prefrontal Cortex of Suicide and Control Subjects

In order to examine whether the altered protein levels of $G_s\alpha_{-S}$, $G_{i2}\alpha$, and $G_O\alpha$ were due to an altered expression of their respective transcripts, we determined the mRNA levels of $G_s\alpha$, $G_{i2}\alpha$, and $G_O\alpha$ subunits in the prefrontal cortex of control and suicide subjects using the

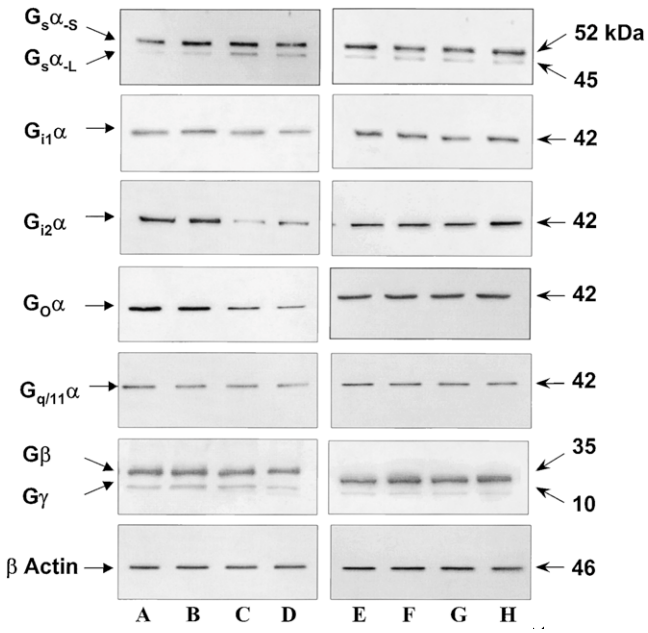

Figure 1. Representative western blots showing the immunolabeling of $G_s\alpha_{-S}$, $G_s\alpha_{-L}$, $G_{i1}\alpha$, $G_{i2}\alpha$, $G_O\alpha$, $G_{q/11}\alpha$, Gβ, Gγ, and β actin in BA 8/9 of two adult suicide subjects (C, D) and two matched adult control subjects (A, B), as well as two teenage suicide subjects (G, H), and two matched teenage control subjects (E, F). The ages of the adult suicide subjects (C and D) were 36 and 87 yrs, and PMI were 10 and 16 h. Subject C was female and subject D was male. The psychiatric diagnosis in subject C was major depression and subject D had adjustment and conduct disorder. The ages of the adult controls (A and B) were 37 and 83 yrs and PMI were 10 and 20 h. The ages of the teenage suicide subjects (G and H) were 15 and 16 yrs and PMI were 11 and 20 h. Subject G was female and subject H was male. Both subjects had no history of mental disorders. The ages of the teenage control subjects (E and F) were 16 and 17 yrs and PMI were 20 and 26 h. Equal concentrations of protein samples (25 μg) were subjected to 12% polyacrylamide gel electrophoresis and transferred to ECL-nitrocellulose membranes, which were then incubated with primary antibodies specific for $G_S\alpha$, $G_{i1}\alpha$, $G_{i2}\alpha$, $G_O\alpha$, $G_{q/11}\alpha$, Gβγ, or β actin, and secondary anti-rabbit or anti-mouse antibodies. The bands were quantified as described in Materials and Methods. The ratios of the optical densities of $G_s\alpha_{-S}$, $G_{i1}\alpha$, $G_{i2}\alpha$, $G_O\alpha$, $G_{q/11}\alpha$, Gβ and Gγ to that of β actin were calculated. Arrows indicate the molecular weights of Gα and βγ subunits and β actin.

quantitative RT-PCR technique. Although we observed that the protein level of $G_s\alpha_{-S}$ but not $G_s\alpha_{-L}$ was increased, during mRNA estimation we could not determine the levels of these two subunits separately because they are splice variants and show a high homology in DNA sequences. Further, because we observed that the protein level of only $G_{i2}\alpha$ but not $G_{i1}\alpha$ was altered in prefrontal cortex of suicide subjects, we measured the mRNA levels of both $G_{i1}\alpha$ and $G_{i2}\alpha$ subunits separately to ascertain whether the transcription of only one subtype of $G_i\alpha$ protein, i.e. $G_{i2}\alpha$, is changed. Figure 5 shows representative gel electrophoreses of the competitive RT-PCR of $G_s\alpha$ (Figure 5, panel A), $G_{i1}\alpha$ (Figure 5, panel C), $G_{i2}\alpha$ (Figure 5, panel E), and $G_O\alpha$ (Figure 5, panel G) in prefrontal cortex from one control subject. We observed that amplification product arises from the $G_s\alpha$ mRNA template at 324 bp and the corresponding digestion product from cRNA at 162 + 162 bp. Similarly, the amplification products for $G_{i1}\alpha$, $G_{i2}\alpha$, and $G_O\alpha$ were at 396 bp, 402 bp and 312 bp, respectively, whereas the corresponding digestion products arising from cRNA for $G_{i1}\alpha$, $G_{i2}\alpha$, and $G_O\alpha$ were at 214+182, 204+198, and 161+152, respectively. Representative competitive RT-PCR analyses for $G_s\alpha$, $G_{i1}\alpha$, $G_{i2}\alpha$, and $G_O\alpha$ are given in Figures 5, panels B, D, F, and G, respectively, where the point of equivalence represents the amount of mRNA for the respective G protein subunit.

As we observed with the protein levels, mRNA level of $G_{i2}\alpha$ and $G_O\alpha$ were significantly decreased whereas $G_s\alpha$ was significantly increased in prefrontal cortex of suicide subjects as compared with control subjects, without any change in the mRNA level of $G_{i1}\alpha$ (Figure 6).

When the suicide population was subdivided on the basis of age, we again observed that the mRNA levels of $G_{i2}\alpha$ and $G_O\alpha$ were significantly decreased and $G_s\alpha$ was significantly increased in prefrontal cortex of adult suicide subjects as compared with adult control subjects, without any change in mRNA levels of $G_{i1}\alpha$ (Figure 7). On the other hand, there were no significant differences in mRNA levels of any of the G protein subunits between our total teenage control and teenage suicide subjects (Figure 8).

Effects of Potential Confounding Variables

The effects of potential confounding variables such as PMI, age, gender, and brain pH were evaluated with respect to mRNA and protein levels of G protein subunits. During data analysis, race was included as a confounding variable, because of an uneven distribution in control and suicide populations. The PMI for suicide subjects and control subjects varied between 5 and 35 h. Western blot analysis of expressed G protein levels in prefrontal cortex revealed that PMI had no significant effect on protein levels of $G_s\alpha_{-S}$ ($r = 0.11$, $p = .32$), $G_s\alpha_{-L}$ ($r = 0.009$, $p = .93$), $G_{i1}\alpha$ ($r = 0.10$, $p = .37$), $G_{i2}\alpha$ (0.12, $p = .27$), $G_O\alpha$ ($r = 0.13$, $p = .26$), $G_{q/11}\alpha$ ($r = 0.10$, $p = .37$), Gβ ($r = 0.11$, $p = .32$), or Gγ ($r = 0.05$, $p = .67$) subunits. Similarly, there was no effect of PMI on mRNA levels of $G_s\alpha$ ($r = 0.10$, $p = .36$), $G_{i1}\alpha$ ($r = 0.12$, $p = .28$), $G_{i2}\alpha$ ($r = 0.10$, $p = .35$) or $G_O\alpha$ ($r = 0.13$, $p = .22$) subunit.

Correlation analysis between pH and protein levels of the various G protein subunits revealed no significant effects of pH on $G_s\alpha_{-S}$ ($r = 0.09$, $p = .40$), $G_s\alpha_{-L}$ ($r = 0.03$, $p = .79$), $G_{i1}\alpha$ ($r = 0.09$, $p = .41$), $G_{i2}\alpha$ ($r = 0.06$, $p = .58$), $G_O\alpha$ ($r = 0.03$, $p = .76$), $G_{q/11}\alpha$ ($r = 0.02$, $p = .86$),

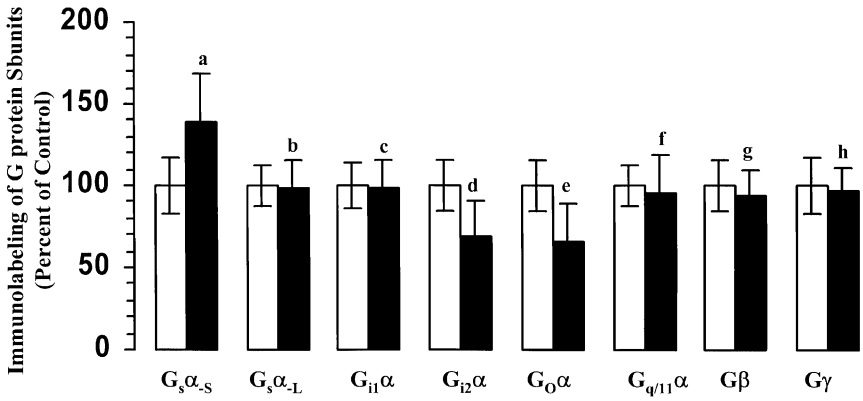

Figure 2. Immunolabeling of G protein subunits in BA 8/9 of total control and total suicide subjects. Data are the mean ± SD. Suicide group was compared with control group.

[a]$t = 7.3$, df = 79, $p < .0001$

[b]$t = 0.42$, df = 79, $p = .67$

[c]$t = 0.48$, df = 79, $p = .63$

[d]$t = 7.2$, df = 79, $p < .0001$

[e]$t = 7.4$, df = 79, $p < .0001$

[f]$t = 1.05$, df = 79, $p = .29$

[g]$t = 1.4$, df = 79, $p = .16$

[h]$t = 0.75$, df = 79, $p = .45$

Gβ ($r = 0.04$, $p = .70$), or Gγ ($r = 0.07$, $p = .50$) subunits. Similarly, pH did not affect mRNA levels of $G_s\alpha$ ($r = 0.09$, $p = .41$), $G_{i1}\alpha$ ($r = 0.04$, $p = .73$), $G_{i2}\alpha$ ($r = 0.19$, $p = .07$), or $G_O\alpha$ ($r = 0.04$, $p = .74$) subunits. Since in some cases the pH was lower than 6.1, we subdivided the total population into those with pH < 6.1 and those with pH > 6.1. Comparison of the mRNA levels of G protein subunits revealed no significant differences between these two groups (data not shown).

There were 22 female and 59 male subjects in the total study population. Our correlation analysis showed that there were no significant effects of gender on protein levels of $G_s\alpha_{-S}$ ($r = 0.15$, $p = .17$), $G_s\alpha_{-L}$ ($r = 0.17$, $p = .13$), $G_{i1}\alpha$ ($r = 0.05$, $p = .65$), $G_{i2}\alpha$ ($r = 0.11$, $p = .30$), $G_O\alpha$ ($r = 0.14$, $p = .19$), $G_{q/11}\alpha$ ($r = 0.10$, $p = .38$), Gβ ($r = 0.07$, $p = .50$) or Gγ ($r = 0.11$, $p = .31$) subunits. Similarly, gender did not affect mRNA levels of $G_s\alpha$ ($r = 0.16$, $p = .45$), $G_{i1}\alpha$ ($r = 0.04$, $p = .74$), $G_{i2}\alpha$ ($r = 0.19$, $p = .07$) or $G_O\alpha$ ($r = 0.18$, $p = .11$) subunits.

The ages of control and suicide subjects were in the range of 12–87 years. No significant correlation was observed between age and protein levels of $G_s\alpha_{-S}$ ($r = 0.13$, $p = .23$), $G_s\alpha_{-L}$ ($r = 0.02$, $p = .87$), $G_{i1}\alpha$ ($r = 0.01$, $p = .92$), $G_{i2}\alpha$ ($r = 0.17$, $p = .14$), $G_O\alpha$ ($r = 0.18$, $p = .10$), Gβ ($r = 0.20$, $p = .07$) or Gγ ($r = 0.14$, $p = .23$) or between age and mRNA levels of $G_S\alpha$ ($r = 0.20$, $p = .07$), $G_{i1}\alpha$ ($r = 0.20$, $p = .07$), $G_{i2}\alpha$ ($r = 0.17$, $p = .13$) or $G_O\alpha$ ($r = 0.004$, $p = .97$), however, immunolabeling of $G_{q/11}\alpha$ was negatively correlated with age ($r = -0.44$, $p < .0001$).

Effects of Diagnosis and Antidepressant Treatment

Since earlier studies found alterations in the levels of G proteins in depressed suicide victims (Pacheco et al. 1996; Garcia-Sevilla et al. 1999), we also examined the effect of major depression on protein and mRNA levels of G protein subunits. For this purpose, we subdivided the adult suicide subjects into those who had a history of major depression and those who had a history of other psychiatric disorders. As mentioned in Methods, in the adult suicide group, 11 subjects had a history of major depression and 14 subjects had other psychiatric disorders (two had no mental illness)., The diagnoses of two suicide subjects were not available, so we excluded those two subjects from our analysis. When we analyzed the levels of G protein subunits of the adult suicide subjects with a history of major depression and those with other mental disorders, both groups showed

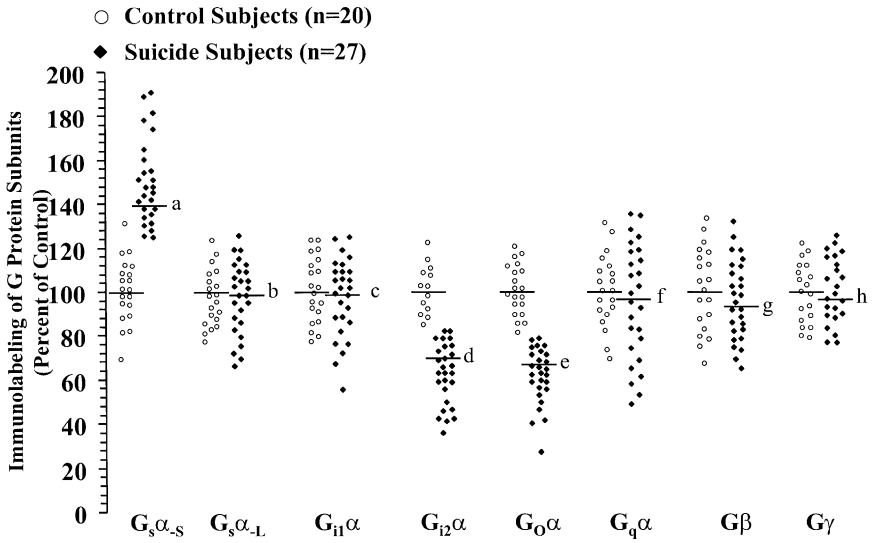

Figure 3. Scattergram of immunolabeling of G protein subunits in BA 8/9 of adult control and adult suicide subjects. Adult suicide group was compared with adult control group.

[a]$t = 10.5$, df = 45, $p < .001$

[b]$t = 0.35$, df = 45, $p = .73$

[c]$t = 0.58.$, df = 45, $p = .56$

[d]$t = 13$, df = 45, $p < .0001$

[e]$t = 14.06$, df = 45, $p < .0001$

[f]$t = 0.71$, df = 45, $p = .48$

[g]$t = 0.88$, df = 45, $p = .38$

[h]$t = 53$, df = 45, $p = .59$

significantly decreased levels of $G_{i1}\alpha$ and $G_O\alpha$ and a significant increase in protein levels of $G_S\alpha_{-S}$ as compared with control subjects (Table 4). Similarly, the mRNA level of $G_{i2}\alpha$ and $G_O\alpha$ significantly decreased and of $G_s\alpha$ was significantly increased in adult suicide subjects, both those with a history of major depression and those with a history of other mental disorders as compared with control subjects (Table 4). Interestingly, mRNA and protein levels of these G protein subunits of the two suicide subjects who had no mental illness were similar to that of control subjects.

In view of previous studies showing that chronic administration of antidepressants may modify the levels of G proteins in rat brain, we examined the effect of antidepressant toxicology on the levels of G protein subunits. In the adult suicide population, four subjects showed positive antidepressant toxicology. The mean values of mRNA and protein levels of G protein subunits were very similar between suicide subjects with positive antidepressant toxicology and suicide subjects with no antidepressant toxicology (data not shown).

To further examine whether the presence of psychopathology had any effect on the levels of G protein subunits in teenage suicide subjects, we subdivided teenage suicide subjects into those who had a history of psychiatric disorders and those who did not have a history of psychiatric disorders. The analysis of the effects of diagnosis on the levels of α subunits of G proteins in teenage suicide subjects is given in Table 5. In the teenage suicide group, seven subjects had no history of mental illnes and eight subjects had a history of mental illness. Interestingly, we observed that the protein level of $G_{i2}\alpha$ and $G_O\alpha$ were significantly decreased and that the levels of $G_s\alpha_{-S}$ was significantly increased in those teenage suicide subjects who had a history of mental illness, both when compared with control subjects or with suicide subjects with no history of mental illness. On the other hand, the levels of these G protein subunits

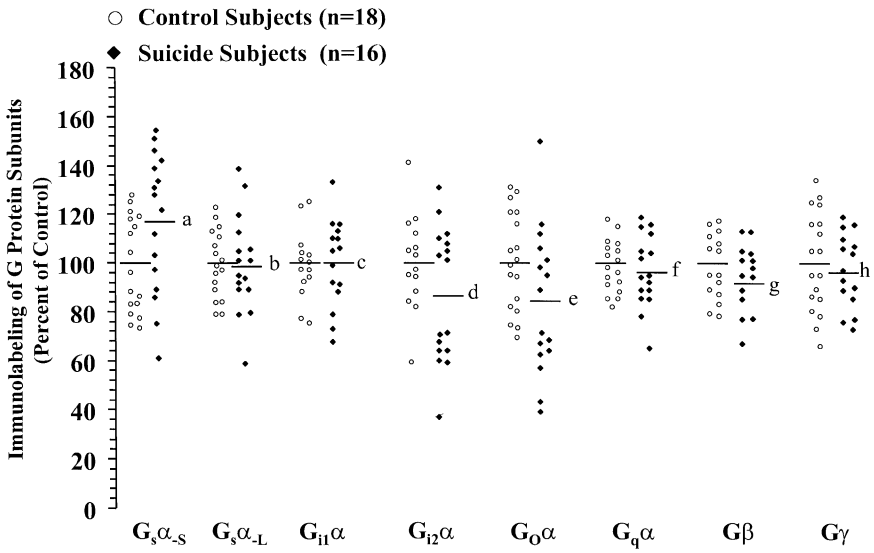

Figure 4. Scattergram of immunolabeling of G protein subunits in BA 8/9 of teenage control and teenage suicide subjects. Teenage suicide group was compared with teenage control group.

[a]$t = 1.9$, df = 32, $p = .06$

[b]$t = 0.24$, df = 32, $p = .81$

[c]$t = 0.01$, df = 32, $p = .99$

[d]$t = 1.76$, df = 32, $p = .87$

[e]$t = 1.9$, df = 32, $p = .07$

[f]$t = 0.89$, df = 32, $p = .38$

[g]$t = 1.3$, df = 32, $p = .20$

[h]$t = 0.53$, df = 32, $p = .59$

were not statistically different between teenage suicide subjects who had no history of mental illness and normal teenage control subjects. None of the subgroups of teenage suicide subjects showed any significant changes in the levels of $G_s\alpha_{-L}$, $G_{q/11}\alpha$, or Gβ or Gγ subunits compared with control subjects. Out of eight teenage suicide subjects who had a history of psychiatric disorders, three showed positive antidepressant toxicology. The mean values of mRNA and protein levels of G protein subunits from those suicide subjects who showed positive antidepressant toxicology were similar to those suicide subjects who did not show positive antidepressant toxicology (data not shown).

DISCUSSION

The present study revealed several interesting observations in the prefrontal cortex of suicide subjects: (1) protein levels of $G_{i2}\alpha$ and $G_O\alpha$ were significantly decreased, whereas that of $G_s\alpha_{-S}$ was significantly increased; (2) no changes were observed in protein levels of $G_s\alpha_{-L}$, $G_{q/11}\alpha$, $G_{i1}\alpha$, G_β and G_γ subunits; (3) changes in protein levels of $G_{i2}\alpha$, $G_O\alpha$, and $G_s\alpha_{-S}$ were accompanied by parallel changes in their respective mRNA levels; (4) changes in the expression of $G_{i2}\alpha$, $G_O\alpha$, and $G_s\alpha_{-S}$ were present in all adult suicide subjects who had a history of mental disorders; (5) no significant changes in the expression of G protein subunits were observed when teenage suicide subjects were compared with teenage control subjects; however, expression of $G_{i2}\alpha$ and $G_O\alpha$ was significantly decreased and expression of $G_s\alpha_{-S}$ was significantly increased in those teenage suicide subjects who had a history of mental disorders.

In the past, there have been a few studies examining expression levels of G protein subunits by the immunolabeling technique in the postmortem brain of suicide subjects. For example, Cowburn et al. (1994) reported

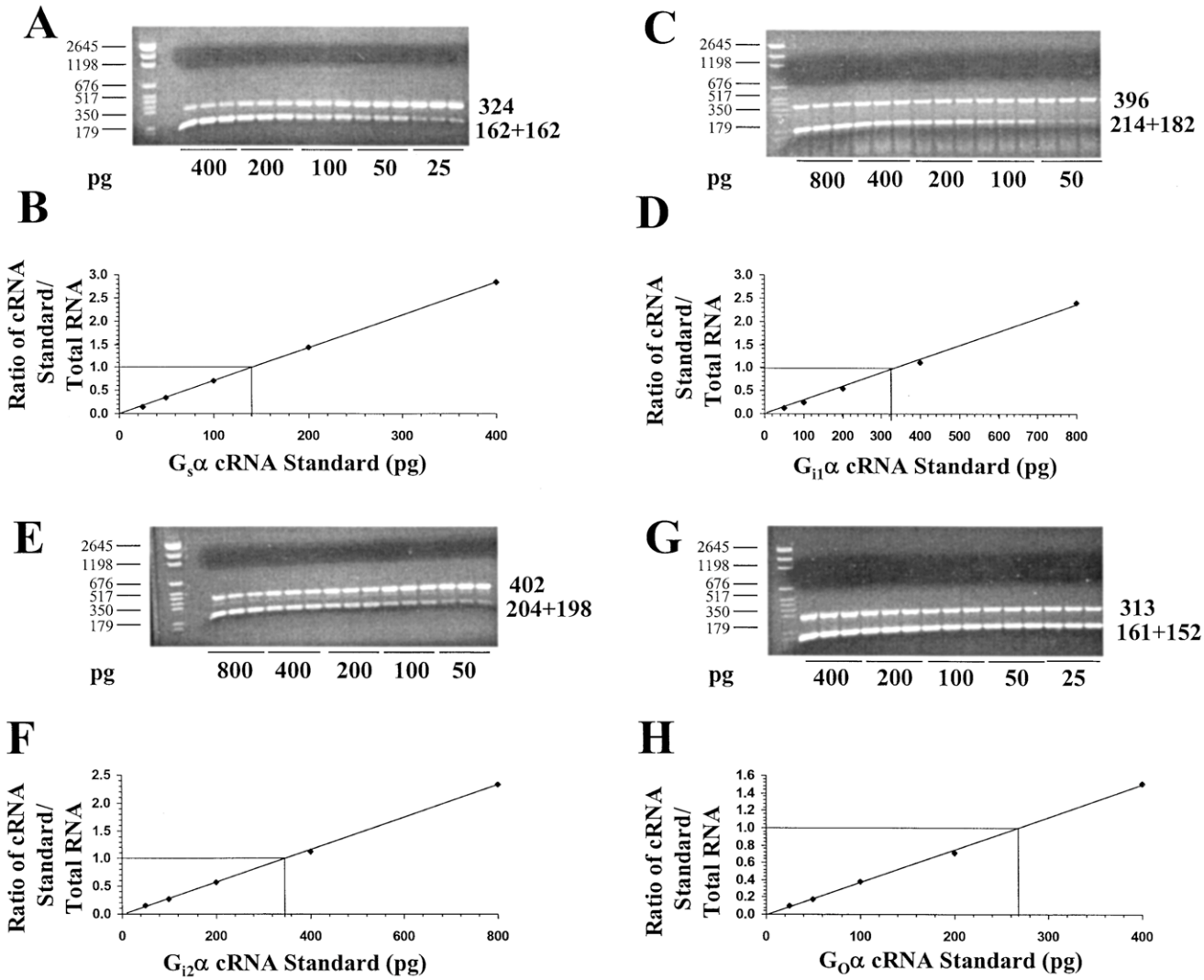

Figure 5. Representative experiments showing competitive PCR analysis for $G_s\alpha$ (A), $G_{i1}\alpha$ (C), $G_{i2}\alpha$ (E), and $G_O\alpha$ (G) mRNA contents in BA 8/9 obtained from one control subject. Decreasing concentrations of corresponding cRNAs were added to 1 μg of total RNA. The mixtures were reverse transcribed and PCR-amplified in the presence of trace amounts of [^{32}P]dCTP; aliquots were digested by *Bgl*II (for $G_s\alpha$ and $G_{i2}\alpha$) or *Xho*I (for $G_{i1}\alpha$ and $G_O\alpha$) and electrophoresed on 1.5% agarose gel. The higher molecular size bands correspond to the amplification products arising from mRNA, whereas the lower bands arise from cRNA generated from the internal standard digested by *Bgl*II or *Xho*I. Data derived from agarose gels were plotted as the counts incorporated into the amplified cRNA standard divided by the counts incorporated into the corresponding subunit mRNA amplification product verses the known amount of internal standard cRNA added to the test sample. The point of equivalence represents the amount of $G_s\alpha$ (B), $G_{i1}\alpha$ (D), $G_{i2}\alpha$ (F), or $G_O\alpha$ (H) mRNA

an increase in the immunolabeling of $G_s\alpha_{-S}$ in frontal cortex of suicide subjects without any change in $G_s\alpha_{-L}$, or $G_{i1/2}\alpha$ protein. In the prefrontal cortex (BA 8/9) of depressed suicide, Pacheco et al. (1996) reported an increase in the immunolabeling of $G_s\alpha_{-S}$ and a decrease in that of $G_{i2}\alpha$. Contrary to that, Garcia-Sevilla et al. (1999) reported an increase in the immunolabeling of $G_{i1/2}\alpha$ in prefrontal cortex (BA 9) of suicide subjects. In the present study, we found that not only the immunolabeling of $G_s\alpha_{-S}$ is increased and that of $G_{i2}\alpha$ and $G_O\alpha$ is decreased, but that their respective mRNA levels are also altered in a parallel direction, which suggests that there are defects in the transcription of these genes in prefrontal cortex of suicide subjects. Our finding of an increased expression of $G_s\alpha_{-S}$ is thus consistent with that of Cowburn et al. (1994) and Pacheco et al. (1996). On the other hand, contrary to the reports of Garcia-Sevilla et al. (1999) but similar to Pacheco et al. (1996), we found decreased expression specifically of only $G_{i2}\alpha$, without any change in $G_{i1}\alpha$. Additionally, we found decreased expression of $G_O\alpha$ protein in prefrontal cortex of suicide subjects, which is contrary to the re-

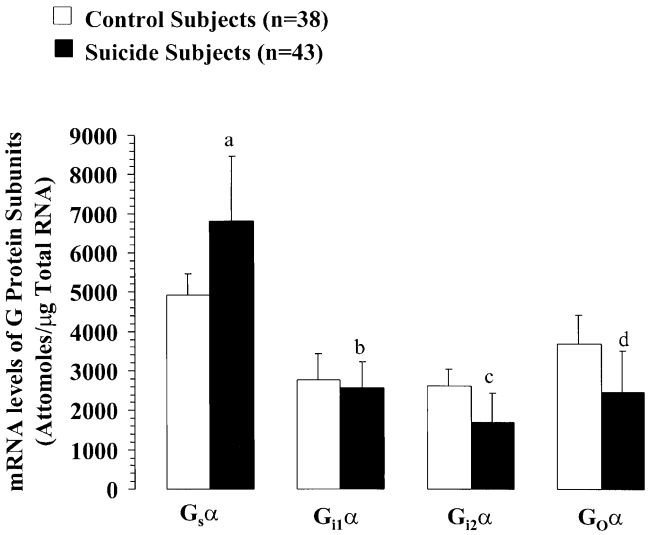

Figure 6. mRNA levels of G protein subunits in BA 8/9 of total control and total suicide subjects. Data are the mean ± SD Suicide group was compared with control group.

[a]t = 6.7, df = 79, $p < .0001$

[b]t = 1.35, df = 79, $p = .18$

[c]t = 7.2, df = 79, $p < .0001$

[d]t = 7.4, df = 79, $p < .0001$

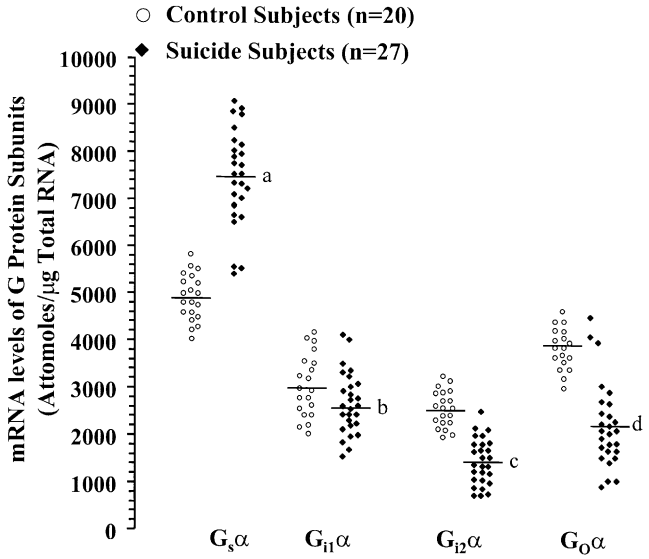

Figure 7. Scattergram of mRNA levels of G protein subunits in BA 8/9 of adult control and adult suicide subjects. Adult suicide group was compared with adult control group.

[a]t = 10.7, df = 45, $p < .0001$

[b]t = 1.9, df = 45, $p = .06$

[c]t = 10.1, df = 45, $p < 0.001$

[d]t = 7.6, df = 45, $p < .0001$

port of Pacheco et al. (1996), who did not find any significant change in the expression of this G protein subunit in prefrontal cortex of depressed suicide subjects. The reasons behind these discrepancies between our results and those of other investigators are not clear; however, this cannot be attributed to the brain area studied, since all these studies were performed in the prefrontal cortex (BA 8/9). The differences in the methodology used in determining the levels of G protein subunits cannot be ruled out. It is worth mentioning that we found differences in the expression of selective G protein subunits both at the transcriptional and the translational level, thus ruling out the possibility of any artifact.

The observed changes do not appear to be related to potential confounding variables such as age, gender, race, PMI, or pH of the brain, as these variables did not show any effects on mRNA or protein levels of G protein subunits, with the single exception that mRNA and protein levels of the $G_{q/11}\alpha$ subunit were negatively correlated with age. It is pertinent to mention that there are two reports on the effects of age on expression levels of G protein subunits. Li et al. (1996) reported that levels of $G_{q/11}\alpha$ and $G_O\alpha$ are decreased with an increase in age, whereas Young et al. (1991) found that the immunoreactivity of $G_i\alpha$ is decreased in the parietal cortex after the age of 40 years, and also that levels of $G_O\alpha$ and $G_{q/11}\alpha$ proteins are inversely correlated with age in the prefrontal cortex. In our study population, the age range was between 12 and 87 years, and as reported by Li et al. (1996), we found decreased mRNA and protein levels of $G_{q/11}\alpha$ protein with an increase in age; however, contrary to this report, we did not observe any significant effects of age on $G_O\alpha$ proteins. Moreover, we did not observe any significant effects of age on $G_i\alpha$ proteins, as observed by Young et al. (1991).

An interesting aspect of our study was the inclusion of a teenage suicide population. As mentioned at the beginning of this article, it has been suggested that some factors associated with teenage suicide may be different from those of adult suicide. For example, teenage suicide is driven primarily by impulsive, aggressive and violent behaviors. There is an age related decline in aggressivity (Mann et al. 1989), but there is increased seriousness of suicidal behavior with an increase in age. Brent et al. (1993) reported that teenage suicide completers had more impulsive and aggressive personality disorders and higher aggression ratings than controls. Apter et al. (1995) also reported that adolescents with aggression and conduct disorders may be suicidal even in the absence of depression. In contrast, most of the

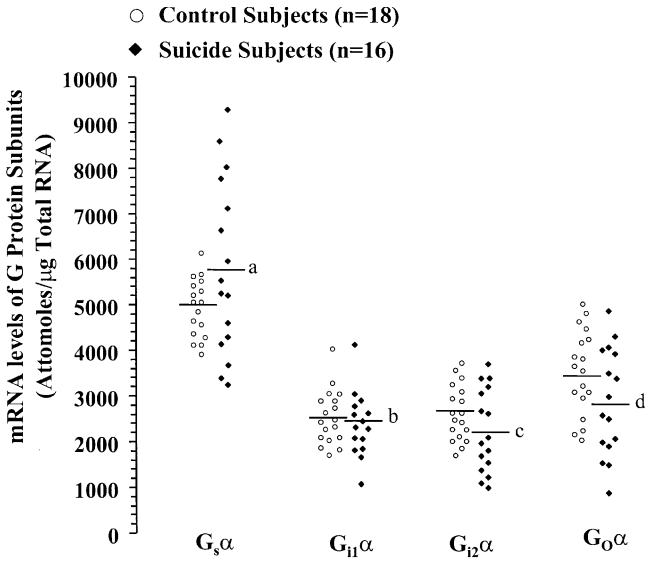

Figure 8. Scattergram of mRNA levels of G protein subunits in BA 8/9 of teenage control and teenage suicide subjects. Teenage suicide group was compared with teenage control group.

[a]t = 1.6, df = 32, $p = .12$

[b]t = 0.27, df = 32, $p = .79$

[c]t = 1.9, df = 32, $p = .07$

[d]t = 1.7, df = 32, $p = .09$

adult suicide subjects have a history of some form of mental illness. Thus it is interesting to examine whether there are any differences in altered expression of G protein subunits between teenage and adult suicide subjects. As mentioned above, we found that in prefrontal cortex of suicide victims, expression of $G_s\alpha_{-S}$ is increased and of $G_{i2}\alpha$ and $G_O\alpha$ is decreased. When the total suicide population was subdivided into teenage and adult groups, interestingly, it was observed that the expression of G protein subunits remained unaltered in teenage suicide subjects as compared with age-matched teenage control subjects. On the other hand, adult suicide subjects showed a significant increase in the expression of $G_s\alpha_{-S}$ and a significant decrease in the expression of $G_{i2}\alpha$ and $G_O\alpha$ as compared with age-matched adult control subjects. More interestingly, when the teenage suicide population was subdivided into those who had a history of mental disorders and those who did not have mental disorders, the expression of $G_s\alpha_{-S}$ was increased and the expression of $G_{i2}\alpha$ and $G_O\alpha$ was decreased in those teenage suicide subjects who had a history of mental disorders. Similarly, the levels of G protein subunits were unchanged in those adult suicide subjects who did not have a mental disorder, although the number of subjects in this group was too small for a meaningful conclusion. However, the levels of $G_s\alpha_{-S}$, $G_{i2}\alpha$, and $G_O\alpha$ were altered in those adult suicide subjects who had a history of major depression or other psychiatric disorders. This suggests that the alterations in levels of specific G protein subunits are related to mental disorders rather than suicide per se, or even whether it is adult or teenage suicide.

What could be the molecular mechanisms underlying the altered expression of $G_s\alpha_{-S}$, $G_{i2}\alpha$, and $G_O\alpha$ in prefrontal cortex of suicide subjects remains to be elucidated. In the past, several studies have demonstrated that the numbers of $5HT_{1A}$, $5HT_{2A}$, α_1-, α_2-, and β adrenergic receptors are altered in postmortem brain of suicide subjects (reviewed by Gross-Isseroff et al. 1998; Pandey et al. 2002). These receptors utilize different signaling mechanisms coupled to various G proteins to mediate their responses. $5HT_{2A}$ and α_1-adrenergic receptors are linked to the phosphoinositide signal transduction system via the $G_{q/11}\alpha$ subunit, whereas $5HT_{1A}$ and α_2-adrenergic receptors are linked to the adenylyl cyclase–cAMP signaling system via $G_i\alpha$ and $G_O\alpha$ subunits. On the other hand, β adrenergic receptors are linked to the adenylyl cyclase–cAMP signaling system via $G_s\alpha$ in a stimulatory fashion. It has been reported that many G protein-coupled receptors display the property of desensitization, which is the waning of signaling during continuous activation by agonists (Liggett 1997). This long-term agonist promoted-desensitization causes decreased expression of cellular G proteins. Such changes in levels of α subunits of G_s, G_i, and G_{11} have been reported earlier (Milligan et al. 1995). Although the concept is speculative in nature, it is quite possible, that continuous stimulation of receptors such as $5HT_{1A}$ and α_2-adrenergic receptors, as reported in postmortem brain of suicide subjects, may possibly be causing decreased expression of $G_i\alpha$ and $G_O\alpha$ as part of the desensitization. On the other hand, the mechanism of increased expression of $G_s\alpha_{-S}$ in prefrontal cortex of suicide victims, where increased binding to β adrenergic receptors has been shown, cannot be explained on the basis of agonist-stimulated desensitization. Similarly, expression of the $G_{q/11}\alpha$ subunit is not altered even though the $5HT_{2A}$ receptors coupled to this G protein subunit are upregulated in postmortem brain of suicide victims (Pandey et al. 2002; Gross-Isseroff et al. 1998). It is quite possible that the expression of G protein subunits is independent of receptor stimulation. The complexity of these signaling system is further illustrated by studies that demonstrate that the same effector can switch to different G protein subunits through the agonist-mediated phosphorylation of receptors by kinases such as protein kinase A (Daaka et al. 1997; Lefkowitz 1998; Luo et al. 1999; Lawler et al. 2001). On the other hand, interestingly, in cells expressing different receptors that couple to the same G protein, a G protein downregulation of one receptor but not the other has also been reported (McKenzie et al. 1991). Thus, the regulation of expres-

Table 4. Effect of mental Disorders on Protein and mRNA Levels of G Protein Subunits in Prefrontal Cortex of Adult Control and Adult Suicide

			Suicide victims* (n = 25)									
	Control subjects (n = 20) 1		With a history of Major depression (n = 11) 2		With a history of other psychiatric disorders (n = 14) 3		Overall ANOVA			Multiple comparison†		
Variables	Mean	SD	Mean	SD	Mean	SD	df	F	P	1 vs 2	1 vs 3	2 vs 3
Protein levels¶												
$G_s\alpha_{-S}$	100	14	160	19	144	14	2,42	64.85	<.0001	<.0001	<.0001	.02
$G_s\alpha_{-L}$	100	11	98	15	101	18	2,42	0.18	.83	.69	.79	.54
$G_{i1}\alpha$	100	15	101	16	90	17	2,42	1.8	.16	.77	.10	.09
$G_{i2}\alpha$	100	11	61	9	56	12	2,42	82.93	<.0001	<.0001	<.0001	.19
$G_O\alpha$	100	11	55	10	60	9	2,42	95.97	<.0001	<.0001	<.0001	.22
$G_{q/11}\alpha$	100	16	101	24	91	27	2,42	0.98	.38	.9	.22	.24
G_β	100	18	93	16	98	18	2,42	0.51	.60	.32	.85	.45
G_γ	100	13	101	14	93	14	2,42	1.52	.23	.73	.15	.12
mRNA§												
$G_S\alpha$	4907	368	7201	1085	7623	1015	2,42	54.61	<.0001	<.001	<.001	.20
$G_{i1}\alpha$	3018	642	2811	637	2593	634	2,42	1.83	.17	.39	.006	.40
$G_{i2}\alpha$	2562	349	1332	321	1452	462	2,42	51.86	<.0001	< .0001	<.0001	.44
$G_O\alpha$	3878	408	2182	880	2330	984	2,42	25,96	<.0001	< .0001	<.0001	.63

*In two cases, psychiatric diagnosis was not available. ¶Percent of control; § Attomoles/μg total RNA; †For multiple comparison, the *p* values were compa ccompared with a Bonferroni adjusted $\alpha = .05/12 = .004$. Multiple comparison test with $p < .004$ was considered significant.

sion of G proteins appears to be quite complex and needs further investigation.

The pathophysiological significance of the differences in levels of $G_s\alpha_{-S}$, $G_{i2}\alpha$, and $G_O\alpha$ proteins found in the prefrontal cortex of suicide subjects and control subjects is currently not known. However, the alterations in expression of $G_s\alpha_{-S}$, $G_i\alpha$, and $G_O\alpha$ have significant functional importance because many receptors modulate their responses through the activation of these G proteins, which then activate or inhibit various effectors. These effectors regulate cell functions through the production of second messengers, which then lead to the phosphorylation of various membrane and cytosolic proteins, which not only induce intracellular instantaneous reactions, like neuronal excitability and secretion of hormones, but also lead to the regulation of gene expression by activating a variety of transcription factors. It has been suggested that even small alterations in levels of G protein may lead to a manyfold amplification of signal transduction (Ross 1989). In our study, we found that the expression of $G_s\alpha_{-S}$, which is more effectively coupled to adenylyl cyclase in a stimulatory fashion than $G_s\alpha_{-L}$ (Walseth et al. 1989), is increased and that of $G_{i2}\alpha$ and $G_O\alpha$ is decreased in prefrontal cortex of suicide subjects, which suggests the activation of adenylyl cyclase and therefore increased formation of cAMP in suicide brain. On the other hand, $G_O\alpha$ is the most abundant heterotrimeric G protein in mammalian brain, and one of its primary functions is the regulation of several ion channels (Gilman 1987). A decrease in the expression of $G_O\alpha$ protein indicates abnormal G protein-mediated regulation of ion channels in prefrontal cortex of suicide subjects. Interestingly, it has been reported that antidepressants cause a decrease in the levels of cyclic AMP (reviewed by Manji 1992), and chronic administration of antidepressants to rats causes a decrease in the level of $G_s\alpha$ protein in brain (Lesch and Manji 1992). Thus, our present study, which shows that the levels of $G_{i2\alpha}$ and $G_O\alpha$ are decreased and of $G_s\alpha_{-S}$ are increased in the postmortem brains of those suicide subjects who had a history of mental illness, together with observations by other investigators that psychoactive drugs cause changes in the levels of G proteins that are directly the opposite of our observations, suggests that these alterations in $G_s\alpha_{-S}$, $G_{i2\alpha}$, and $G_O\alpha$ proteins may have an important role in the pathophysiology of mental disorders.

In summary, this is the first study to examine mRNA and protein expression of various subunits of G proteins in postmortem brain of teenage and adult suicide subjects with and without mental disorders, and it suggests that expression of $G_O\alpha$ and $G_{i2}\alpha$ is decreased and that of $G_s\alpha_{-S}$ is increased in suicide subjects. These changes were present in all adult suicide subjects who had a history of mental disorders. Although we did not observe any significant differences in the levels of α subunits of G proteins in the total teenage suicide population, we found that the expression of $G_{i2\alpha}$ and $G_O\alpha$ was decreased and

Table 5. Effect of Mental Disorders on mRNA and Protein Levels of G Protein Subunits in Prefrontal Cortex of Teenage Control and Teenage Suicide Victims

			Suicide victims (n = 15)*									
	Control subjects (n = 18) 1		With a history of psychiatric disorders (n = 8) 2		With no history of psychiatric disorders (n = 7) 3		Overall ANOVA			Multiple comparison†		
Variables	Mean	SD	Mean	SD	Mean	SD	df	F	*p*	1 vs 2	1 vs 3	2 vs 3
Protein levels¶												
$G_s\alpha_{-S}$	100	20	135	21	98	22	2,30	8.8	.001	<.0001	.82	.002
$G_s\alpha_{-L}$	100	14	101	25	95	15	2,30	0.29	.75	.87	.51	.48
$G_{i1}\alpha$	100	15	102	11	101	23	2,30	0.03	.96	.81	.86	.97
$G_{i2}\alpha$	100	18	60	10	112	10	2,30	26.48	<.0001	<.0001	.07	<.0001
$G_O\alpha$	100	21	59	12	108	21	2,30	15.96	<.0001	<.0001	.35	<.0001
$G_{q/11}\alpha$	100	11	101	12	89	17	2,30	2.34	.11	.81	.06	.07
G_β	100	14	94	16	95	10	2,30	0.46	.64	.42	.48	.96
G_γ	100	21	94	17	98	14	2,30	0.25	.78	.49	.79	.73
mRNA§												
$G_S\alpha$	4964	661	7074	1876	4319	815	2,30	14.1	<.0001	<.0001	.19	<.0001
$G_{i1}\alpha$	2506	579	2516	864	2456	505	2,30	0.02	.98	.97	.86	.86
$G_{i2}\alpha$	2675	524	1433	316	2928	506	2,30	23.40	<.0001	<.0001	.25	<.0001
$G_O\alpha$	2499	928	1948	704	3711	767	2,30	10.99	<.0001	<.0001	.57	<.0001

*In one case the diagnosis was not available; ¶Percent of control; §Attomoles/μg total RNA; †For multiple comparison, the *p* values were compared with a Bonferroni adjusted $\alpha = .05/12 = .004$. Multiple comparison test with $p < .004$ was considered significant.

that of $G_s\alpha_{-S}$ was increased only in those teenage suicide subjects who had a history of mental illness. These results indicate an impairment in the expression of $G_s\alpha_{-S}$, $G_{i2\alpha}$, and $G_O\alpha$ subunits only in suicide subjects who had a history of mental disorders. Given the functional significance of G proteins in mediating various physiological functions, such alterations in the levels of $G_s\alpha_{-S}$, $G_{i2\alpha}$, and $G_O\alpha$ subunits may be of relevance in the pathophysiology of mental disorders.

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