Reduced G Protein Functions and Immunoreactive Levels in Mononuclear Leukocytes of Patients With Depression

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Objective: *Heterotrimeric G proteins play a pivotal role in postreceptor information transduction. These proteins were previously implicated in the biochemical mechanism underlying lithium action and in the pathophysiology of mood disorders. The present study sought to quantitatively and functionally evaluate G proteins in patients with major depression.* *Method:* *G proteins were measured in mononuclear leukocytes of 37 untreated patients with major depression and 31 comparison subjects. Receptor-coupled G protein function was evaluated through β-adrenergic and muscarinic-agonist-induced increases in guanine nucleotide binding capacity, which were substantiated by quantitative measures of G proteins through immunoblot analyses that used polyclonal antibodies against stimulatory ($G_s\alpha$) and inhibitory ($G_i\alpha$) G proteins.* *Results:* *Mononuclear leukocytes of depressed patients showed significantly reduced immunoreactive quantities of $G_s\alpha$ and $G_i\alpha$ together with markedly hypofunctional G_s and G_i. The reductions in both function and quantity of G_s and G_i were significantly correlated with the severity of depressive symptoms. Moreover, simultaneous quantitative and functional measurements in a large number of patients showed significant correlations between the function and the quantity of mononuclear leukocyte G_s and G_i proteins.* *Conclusions:* *These findings lend further support to the implication of G proteins in the pathophysiology of mood disorders. G protein functional and quantitative measurements in mononuclear leukocytes of patients with mood disorders may potentially serve as a biochemical marker for the affective state of these patients.*

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In recent years, research on the pathophysiological mechanisms of mood disorders and the biochemical bases of treatments used to combat these disorders has focused on information transduction mechanisms beyond receptors. Heterotrimeric G proteins play a pivotal role in postreceptor information transduction (for review see references 1 and 2). An important characteristic of G proteins is their greater guanine nucleotide binding following agonist stimulation, which in turn leads to their activation. Tritiated guanine nucleotide binding following agonist stimulation is an established test for G protein function. This test has been used in membrane preparations (3, 4) and for the purification of G proteins from crude membrane preparations (5) and has been both kinetically and mechanistically analyzed by using various purified G proteins (6) in reconstituted systems (7, 8). More recently, this method was used by Evans et al. (9) to show specific GTP binding and hydrolysis activities in lymphocyte membranes following interleukin-2 stimulation.

We have adapted this method to measure early events in signal transduction beyond receptors through activated receptor-coupled guanine nucleotide exchange on G proteins (10, 11). Through this method the function of the family of receptor-coupled G proteins was found to be differentially attenuated by lithium (10–15), other antibipolar treatments (14–16), and antidepressant drugs (15, 16). Studies by other groups that used a variety of techniques and were generally in agreement with these results implicate the involvement of G proteins in the mechanism of lithium action (17–23).

The discovery of the involvement of G proteins in the mechanism of action of antibipolar and antidepressant treatments suggests alterations in members of the G protein family in patients with mental disorders (24). Indeed, lithium-sensitive hyperfunctional stimulatory (G_s) and in-

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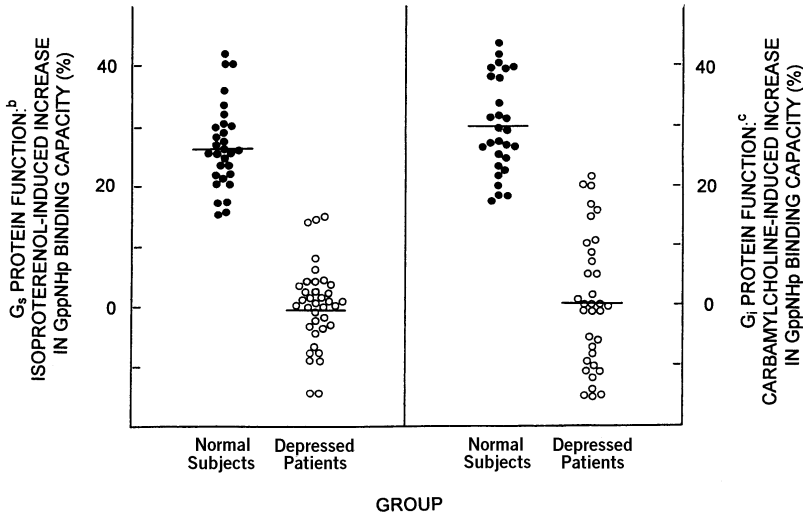
FIGURE 1. G_s and G_i Protein Function in Mononuclear Leukocyte Membranes of Normal Subjects and Patients With Depression[a]



[a]Horizontal lines represent mean values.
[b]Significant difference between depressed patients and normal subjects (Mann-Whitney U test: t=6.99, df=66, p<0.001).
[c]Significant difference between depressed patients and normal subjects (Mann-Whitney U test: t=6.58, df=60, p<0.001).

hibitory (G_i) G proteins were detected in mononuclear leukocytes of patients with mania (15, 25), while in a preliminary study (26) decreases in the function and level of G_s protein have been found in mononuclear leukocytes of patients with major depressive disorder. In accordance with our findings, Friedman et al. (27, 28) have shown that lithium inhibits the association of various receptors with membrane G proteins, and binding of labeled GTPγS enhanced by agonists is elevated in postmortem cerebral cortical membranes of patients with bipolar disorder. Although Young et al. (29) reported no changes in the immunoreactivities of $G_s\alpha$ and $G_i\alpha$ proteins in mononuclear leukocytes of patients with unipolar depression, they did report higher immunoreactive levels and function of $G_s\alpha$ in postmortem cerebral cortices of patients with bipolar disorder (30, 31). G protein quantitative and functional measures in human mononuclear leukocytes were found to be age-independent (32).

In the present study we simultaneously examined, in a larger group of patients, functional and quantitative immunoreactive measures of G_s and G_i proteins to test whether, in contrast with the higher values previously reported in manic patients, mononuclear leukocytes of depressed patients show hypofunctional and reduced immunoreactive quantities of $G_s\alpha$ and $G_i\alpha$. We also sought to determine whether the reductions in function and in quantity of both G_s and G_i were significantly correlated with the severity of depressive symptoms and whether these simultaneous quantitative and functional measurements showed a significant correlation between the function and the quantity of mononuclear leukocyte G_s and G_i proteins.

METHOD

Patients

Thirty-seven patients with major depression (26 with unipolar and 11 with bipolar depression), diagnosed according to DSM-III-R criteria by at least two senior psychiatrists, were included in the study. A physical examination, an ECG, and laboratory tests for renal, hepatic, hematologic, and thyroid function were administered to all subjects, and no physical abnormalities were reported. After complete description of the study to the subjects, written informed consent was obtained for a 60-ml blood donation. In all cases blood was drawn between 8:00 a.m. and 10:00 a.m. The patients were given the Beck Depression Inventory (33) before blood donation. The study was approved by the institutional review board.

The group of 37 depressed patients consisted of 19 female and 18 male subjects whose average age was 45.1 years (SD=15.4, range=19–78). Seventeen were outpatients, and 20 were hospitalized. Before the initiation of treatment, an examination of the patient histories confirmed that all subjects had not received treatment for at least 1 month. A healthy volunteer comparison group was recruited from the staff of the Beer-Sheva Mental Health Center and their families. This group consisted of 31 subjects, 16 men and 15 women, whose average age was 41.4 years (SD=15.7, range=20–77).

Mononuclear Leukocyte Isolation

Mononuclear leukocytes were isolated from 60 ml of heparinized fresh blood of adult donors by using Ficoll-Paque gradient, as reported by Boyum (34). Cells were homogenized in 25-mM Tris HCl and 1 mM dithiotreitol, pH=7.4. The homogenate was passed through two layers of cheesecloth to remove debris, and membranes were collected by further centrifugation at 18,000 *g* for 10 minutes. Membranes were then either freshly used for the functional binding measures or suspended in a homogenization buffer that contained 1 mM EGTA and 30% sucrose weight per volume, which was frozen at –70°C until assayed by the quantitative measures. Aliquots were taken for protein concentration determination by using Bradford's assay.

Guanine Nucleotide Binding Assay

Binding reactions were carried out for 15 minutes at room temperature in a final volume of 200 µl. The reaction buffer consisted of 25 mM Tris HCl, 1 mM ATP, 1 mM Mg^{2+}, 1 mM EGTA, and 1 mM dithiotreitol, pH=7.4, with varying concentrations of (^{3}H)GppNHp (0.05 µM–5 µM). Reactions were started by adding 50 µg of membrane protein and terminated with 5 ml of ice-cold buffer (10 mM Tris HCl and 100 mM NaCl; pH=7.4), followed by rapid filtration. Filters were subsequently washed twice with 3 ml of cold buffer and taken for scintillation counting. Agonist effects on (^{3}H)GppNHp binding were assessed by adding isoproterenol (25 µM) or carbamylcholine (50 µM) to the reaction mixture. These represent the minimal concentrations that result in maximal effect of the agonists.

Immunoblot Analysis

On the day of assay, membranes were thawed, and aliquots of 10 µg membrane G proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% acrylamide). The resulting proteins were transferred to nitrocellulose paper by use of electroblotting apparatus. Blots were washed in Tris-buffered saline that

contained 3% Tween-20 and blocked by incubation with 5% bovine serum albumin for 1 hour in Tris-buffered saline that contained 0.1% Tween-20. After two washes in Tris-buffered saline that contained 0.1% Tween-20, blots were incubated overnight in antisera (NEN-DuPont, Mississauga, Ont., Canada) that was directed specifically against $G_s\alpha$ (dilution 1:2,500), $G_{i1,2}\alpha$ (dilution 1:5,000), and Gβ (dilution 1:1,000), followed by subsequent incubation with goat anti-rabbit IgG labeled with horseradish peroxidase. Immunoreactivity was detected with the Enhanced Chemiluminescence Western Blot Detection System (Amersham, Oakville, Ont. Canada) followed by exposure to X-ray film.

A preliminary assay that used healthy volunteer mononuclear leukocyte membranes was carried out for the determination of the range of linearity of the assay with respect to protein concentration. Linearity was found between 2.5 μg–15 μg membrane protein. Peak heights of immunoreactive bands were determined with an image analysis system. The optical density of the immunoreactive bands was normalized against 10-μg rat cortical membranes run in each blot as a standard value. Although anti-$G_s\alpha$ detects both 52- and 45-kDa $G_s\alpha$ species, only the 45-kDa species is detected in leukocytes, while rat cortex membranes show predominantly the 52-kDa species. The other subunits assayed in leukocytes were found to migrate at the expected molecular weights similarly to those labeled in the rat cortex membranes.

Statistical Analysis

Correlations between functional and quantitative measures of G proteins in human mononuclear leukocytes as well as correlations between these G protein measures in mononuclear leukocytes of depressed patients and score on the Beck Depression Inventory were conducted by using the Spearman rank correlation. Comparisons of each agonist-induced increase in GppNHp binding capacities, as well as of the immunoreactivities of the various G protein subunits, between the depressed patients and the healthy comparison subjects were conducted by using the nonparametric Mann-Whitney rank-sum test (35).

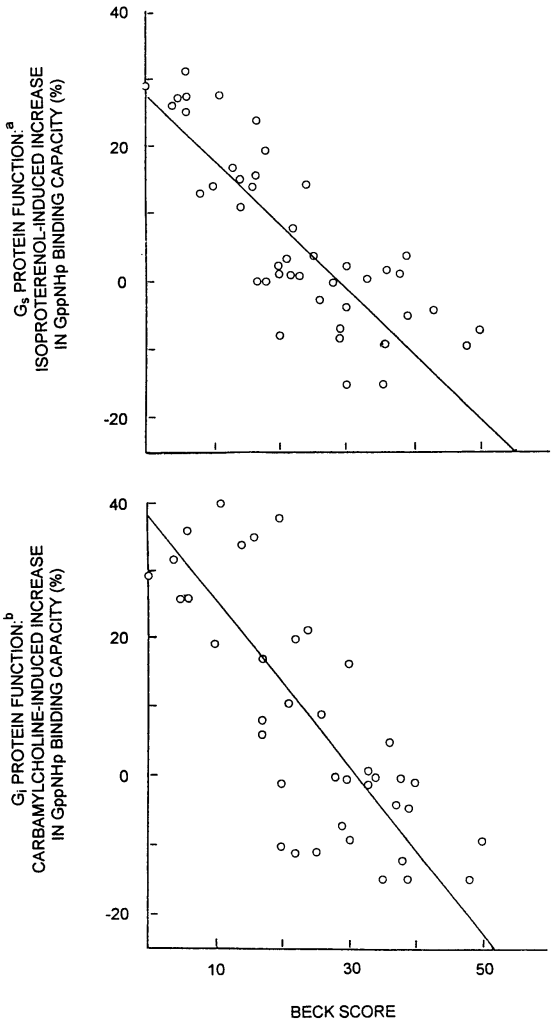
RESULTS

G protein function was measured through agonist-induced increases in GppNHp binding capacity: isoproterenol and carbamylcholine were used as β-adrenergic and muscarinic receptor agonists, respectively. Both agonists induced significant increases in GppNHp binding capacity without substantially affecting the affinity of binding. Isoproterenol-induced increases in GppNHp binding capacity were blockable by pretreatment with cholera toxin, with no effect of pertussis toxin, which indicates a specific effect through G_s protein. Carbamylcholine exerted its effects on guanine nucleotide binding in a specific pertussis toxin-sensitive manner, which suggests coupling with non-G_s proteins (e.g., G_i) (15, 16, 25, 26).

Figure 1 shows the functional measures of G_s and G_i following agonist stimulation for the normal and depressed subjects. The group of depressed patients showed statistically significant reductions in β-adrenergic-receptor-coupled G_s protein function and in muscarinic-receptor-coupled G_i protein function. These reductions for the depressed patients in G_s and G_i protein function were similar for unipolar and bipolar depression.

We compared depressive symptom severity as assessed by the Beck Depression Inventory with receptor-coupled G protein function measurements and found

FIGURE 2. Correlation Between G_s and G_i Protein Function and Score on the Beck Depression Inventory

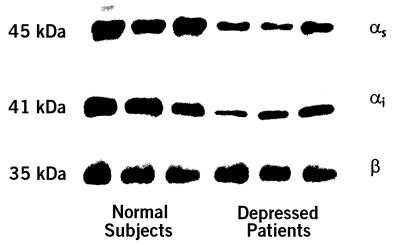


[a]Significant negative correlation (r_s=–0.78, N=44, p<0.001).
[b]Significant negative correlation (r_s=–0.75, N=39, p<0.001).

that depression of even mild to moderate severity could be detected in our assay system as partially inhibiting isoproterenol- and carbamylcholine-induced increases in GppNHp binding capacity. Patients with more severe depression showed complete inhibition of receptor-coupled G_s protein function. Significant negative correlations were found between the score on the Beck Depression Inventory and both β-adrenergic-receptor-coupled G_s function and muscarinic-receptor-coupled G_i protein function (figure 2).

G protein levels in human mononuclear leukocytes were assessed through immunoblot analysis by using polyclonal antibodies against various Gα and Gβ subunits. Figure 3 is a representative example of immunoblots obtained for the quantitation of $G_s\alpha$, $G_i\alpha$, and Gβ subunit proteins from mononuclear leukocytes of patients with major depression and age- and sex-matched comparison subjects. Semiquantitative nor-

FIGURE 3. Representative Immunoblots Obtained With Antisera Against $G_s\alpha$, $G_i\alpha$, and $G\beta$ Subunits in Mononuclear Leukocytes of Normal Subjects and Patients With Depression



malization of the data concerning both Gα levels obtained from all subjects indicated that the mononuclear leukocyte levels of $G_s\alpha$ and of $G_i\alpha$ in depressed patients were significantly less than that of the normal comparison subjects (figure 4). Moreover, as was found for G_s and G_i protein function, mononuclear leukocyte $G_s\alpha$ and $G_i\alpha$ protein contents were negatively correlated with depression severity as assessed by the Beck Depression Inventory (figure 5). Immunoreactive Gβ protein levels in depressed patients were similar to those of the comparison subjects (data not shown). Simultaneous G protein quantitative and functional measurements conducted on the same mononuclear leukocyte membrane preparations in patients and comparison subjects resulted in significant positive correlations between β-adrenergic-receptor-coupled G_s protein function and $G_s\alpha$ immunoreactivity as well as between muscarinic-receptor-coupled G_i protein function and $G_i\alpha$ immunoreactivity (figure 6).

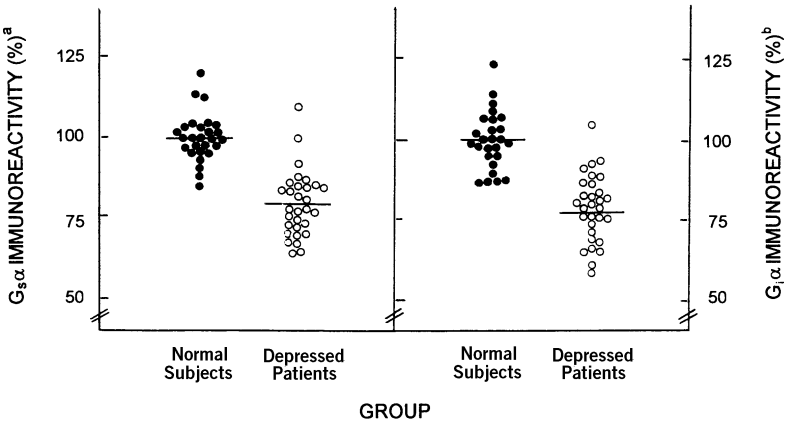
DISCUSSION

A role for monoaminergic and muscarinic cholinergic mechanisms in the pathogenesis of mood disorders has been largely suggested by and formulated in both the catecholamine hypothesis and the monoaminergic-cholinergic balance hypothesis (for a review see references 36 and 37). Biochemical research in studies of affective disorders has focused on the cascade of events involved in signal transduction: the primary messenger level (the catecholamine or acetylcholine neurotransmitter), the level of the neurotransmitter receptors, and, lately, information transduction mechanisms beyond receptors. The present study concentrated on alterations in early events in signal transduction beyond receptors that involve G proteins.

Signal transductions by G protein-coupled receptors are regulated by various mechanisms that act at the receptor level, e.g., β-adrenergic receptor kinase (38) or, at the G protein level, phosducin (39). It is possible that the reductions observed in receptor-coupled G protein function in the present study stem from alterations at the receptor level, at G protein level, or in regulatory factors that act at either levels. Since our functional G protein assay system measures activated receptor-enhanced guanine nucleotide binding and thus receptor coupling to a G protein, the reduced function measures may reflect reduced receptor levels, reduced coupling, or reduced G protein levels. Indeed, reduced levels of the β-adrenergic receptor were previously extensively described in mononuclear leukocytes of patients with unipolar and bipolar depression (40–44). However, our functional measures, together with the complementary immunoblot studies, suggest also direct, quantitative, and functional reductions in G_s protein in mononuclear leukocytes of depressed patients at the postreceptor level that are in accordance with functional findings of reduced β-adrenergic-receptor-coupled adenylate cyclase activity in patients with depression (40, 41, 45–47). Most evidence suggests that muscarinic receptor characteristics are not altered in patients with affective disorder (37). This information, together with our findings of reductions in both muscarinic-receptor-coupled G_i function and $G_i\alpha$ immunoreactivity, supports again a direct postreceptor, quantitative, and functional reduction in G_i protein in mononuclear leukocytes of patients with depression.

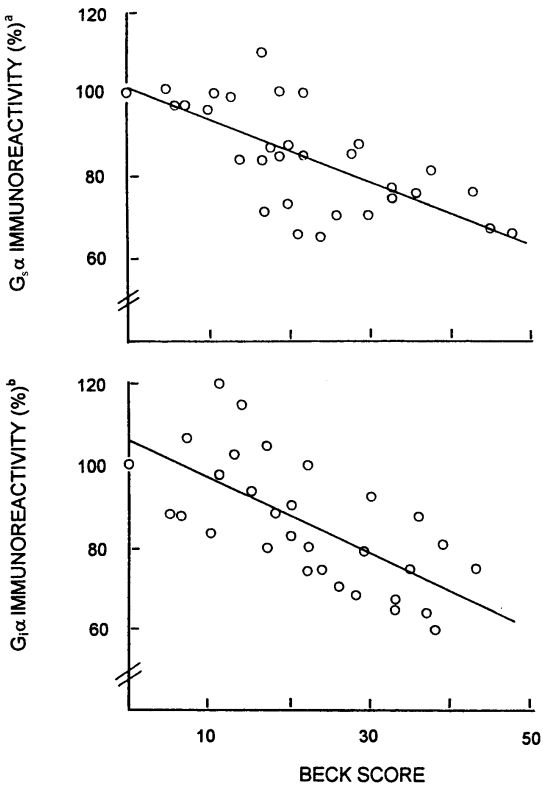
Higher $G_s\alpha$ and $G_i\alpha$ levels in mononuclear leukocytes of patients with bipolar depression and normal-like levels of these proteins in patients with unipolar depression were reported by Young et al. (29). These results contrast with the present findings of reduced immunoreactive and functional measures of β-adrenergic- and muscarinic-receptor-coupled G_s and G_i proteins, which correlated with depressive symptom severity as assessed by the Beck Depression Inventory. This discrepancy may stem from differences in the severity of illness in

FIGURE 4. $G_s\alpha$ and $G_i\alpha$ Relative Immunoreactivities in Mononuclear Leukocytes of Normal Subjects and Depressed Patients



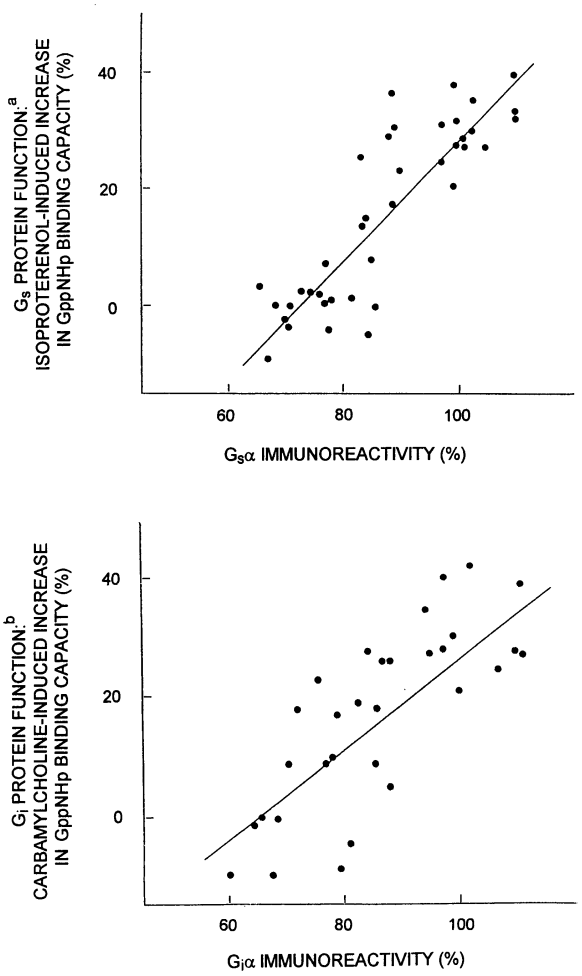
[a] Significant difference between depressed patients and normal subjects (Mann-Whitney U test: t=5.64, df=65, p<0.001).
[b] Significant difference between depressed patients and normal subjects (Mann-Whitney U test: t=5.67, df=54, p<0.001).

FIGURE 5. Correlation Between $G_s\alpha$ and $G_i\alpha$ Immunoreactivity and Score on the Beck Depression Inventory



[a]Significant negative correlation (r_s=–0.63, N=31, p<0.001).
[b]Significant negative correlation (r_s=–0.69, N=31, p<0.001).

FIGURE 6. Correlation Between G Protein Functional and Quantitative Measures in Human Mononuclear Leukocyte Membranes



[a]Significant positive correlation (r_s=0.83, N=39, p<0.001).
[b]Significant positive correlation (r_s=0.75, N=31, p<0.001).

the patients included in each study: almost all the depressed patients in Young et al.'s study were outpatients, with probably less severe depressive symptoms, while in the present study depressed inpatients formed more than 50% of the examined population. We have previously described hyperfunctional receptor-coupled G_s and G_i proteins in mononuclear leukocytes of patients with mania (15, 25). In the present study patients with bipolar depression (as well as unipolar depression) showed an inverse picture: hypofunctional G_s and G_i proteins. The finding of greater and reduced function of G proteins in mania and depression, respectively, suggests that G protein activity may be a biochemical marker indicative of the affective state rather than a trait marker of bipolar disorder. However, one should consider the possibility that both trait and state alterations may exist in G protein levels and that these may result in opposite changes and thus reveal different patterns in different patient populations.

We are aware that the involvement of G proteins in the pathophysiology of depression, as implicated from the data presented here, should be taken with considerable caution. Extrapolations from findings obtained in peripheral blood cells, which are exposed to circulatory catecholamines and hormones, to the CNS is not straightforward. In this regard, it should be mentioned that elevated immunoreactivities of G_s and G_i proteins were described in postmortem brains of patients with bipolar disorder (30). The mood state of these patients at the time of death was not described. One possibility is that these patients were manic at the time of death. In this case these results confirm our findings of hyperfunctional G_s and G_i proteins in peripheral white blood cells of patients with mania (25). However, it is far from clear that this is the correct explanation, and an alternative explanation might be that there are differences between the brain and peripheral cells. This would reduce the significance of the presently described findings of reduced function and levels of G proteins in patients with bipolar depression in terms of understanding the biochemical mechanisms that underlie bipolar depression.

Differences may exist in the tissue distribution of G proteins between CNS and peripheral tissues. For example, differences in the distribution of $G_s\alpha$ variants between CNS and peripheral tissues have been described (48). This heterogeneity might be the explana-

tion for selective lithium interactions with G_s proteins in the CNS (13). Also, since we used a mixed-cell mononuclear leukocyte preparation for our assays, the possibility remains that the alterations observed in G protein function or immunoreactivity reflect, at least in part, alteration in a white cell subpopulation induced by the depressive state. In this regard it should be noted that while the immunoreactivities of $G_s\alpha$ and $G_i\alpha$ proteins were found to be reduced in the group of depressed patients, the immunoreactivities of the Gβ subunit protein remained similar to those of the comparison group. Such differential alterations would not be expected to occur because of alterations in a white cell subpopulation.

If one considers our findings from a purely empirical point of view, G protein functional and immunoreactive measures may become useful as a biochemical peripheral assay for the diagnosis of depression, even if not directly connected to the pathophysiology of depression. If we determine that a ≤15% increase in GppNHp binding capacity by the agonist reflects G protein hypoactivity and a ≥15% decrease in $G_s\alpha$ or $G_i\alpha$ relative immunoreactivity reflects lower quantities of these proteins, we could calculate the sensitivity and selectivity of our diagnostic assays. The sensitivity and selectivity were found to be 0.89 and 0.90 for the functional assay and 0.73 and 0.81 for the quantitative assay, respectively. Besides the very good correlation between measures of receptor-coupled G protein function and immunoreactive levels, the present study showed that the extent of reductions in function and immunoreactivity of both G_s and G_i proteins were significantly correlated with depressive symptom severity as assessed by the Beck Depression Inventory. Although this assay may potentially provide biochemical information not only for the diagnosis of depression but also for evaluating its severity, one should be more cautious about such an implication of the findings. The reduced measures of G proteins observed in the depressed patients in this study may represent possible generalized effect of stress on G protein levels. Indeed, reductions in β-adrenergic receptor density and function in peripheral blood elements have been documented in panic disorder (49). However, our preliminary results show that mononuclear leukocytes of patients with panic disorder are characterized by hyper- rather than hypofunctional β-adrenergic-receptor-coupled G_s and hypofunctional muscarinic-receptor-coupled G_i (50), which lend support to the specificity of the present findings to depressive disorders.

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