The Effects of Seasons and Light Therapy on G Protein Levels in Mononuclear Leukocytes of Patients With Seasonal Affective Disorder

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Background: Information-transducing heterotrimeric G proteins have been implicated previously in the mechanism of action of mood stabilizers and in the pathophysiology of mood disorders. Mononuclear leukocytes of patients with unipolar and bipolar depression have been characterized by reduced measures of the stimulatory and inhibitory G proteins. In this study, patients with seasonal affective disorder (SAD) were measured for mononuclear leukocyte G protein levels while depressed during the winter, following light therapy, and in remission during the summer.

Methods: Twenty-six patients with SAD and 28 healthy subjects were assessed in the study. The immunoreactivities of Gsα, Giα, and Gβ subunit proteins were determined by Western blot analysis of mononuclear leukocyte membranes with selective polyclonal antibodies for the various G subunit proteins, followed by densitometric quantitation using an image analysis system.

Results: Untreated patients with SAD and winter, atypical-type depression showed significantly reduced mononuclear leukocyte immunoreactive levels of Gsα and Giα proteins, similar to previous observations in patients with nonseasonal major depression. The reduced G protein levels were normalized with 2 weeks of light therapy. The same patients while in remission during the summer had G protein levels that were similar to those of healthy subjects.

Conclusions: G protein–immunoreactive measures in patients with SAD are suggested as a state marker for winter depression, which is normalized by light treatment and during the summer. We speculate that light may exert its effects via normalization of transducin (Gt protein) levels, which are thought to be reduced in winter depression.

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SEASONAL AFFECTIVE DISORDER (SAD) is a mood disorder characterized by recurrent episodes of winter depression, with remission or hypomanic periods in the spring and summer.1 Patients with SAD differ from patients with melancholia in their clinical profiles (SAD patients overeat, crave carbohydrates, gain weight, sleep more, and are tired frequently), as well as in their biological characteristics (eg, normal dexamethasone suppression test responses, normal responses to thyrotropin-releasing hormone challenge tests, and normal rapid eye movement sleep latency), which resemble those of patients with atypical depression.1 The efficacy of phototherapy for SAD has been generally acknowledged.2,3

Although there is no consensus on the pathophysiology of SAD or on the mechanism of action of light therapy, the mechanisms that have been suggested to account for these phenomena generally involve altered primary messenger function: abnormal brain serotonergic transmission,4 reduced sympathetic system arousal,5 and underactive hypothalamic-pituitary-adrenal axis functioning.6

The family of heterotrimeric G proteins is a crucial point of convergence in the transmission of signals from a variety of primary messengers and their membrane receptors to a series of downstream cellular events, including intracellular second messenger effector enzymes and ionic channels7,8 (Figure 1). The increasing interest in the clinical perspective of altered G protein function has yielded important findings concerning the involvement of G proteins in the pathophysiology of mood disorders and in the biochemical mechanisms underlying the treatment of these disorders. We found that the function of receptor-coupled G proteins was altered by treatment with lithium9,10 and other antidepressant medications.11,12 Studies by other groups, generally in agreement with these results, implicate the involvement of G proteins in...
SUBJECTS AND METHODS

SUBJECTS

Patients and controls were recruited through newspaper and radio advertisements in the Washington, DC, metropolitan area, by word of mouth, and through referrals from physicians, therapists, friends, or family. Twenty-six patients (18 women) met the following inclusion criteria: (1) Rosenthal et al diagnostic criteria for SAD, a pattern of recurrent depressions, at least 1 of which met criteria for a major depressive episode, and at least 2 of which occurred in consecutive years; (2) a score of at least 15 on the Structured Interview Guide for the Hamilton Depression Rating Scale–Seasonal Affective Disorders Version (SIGH-SAD); (3) absence of any additional current Axis I disorders; (4) good physical health as determined by results of physical examination and routine blood work; and (5) no use of light therapy or medications for the current winter depression. The mean age of onset in cases where SAD could definitely be determined was 28 years. Thus, patients had SAD for an average of 13 years. Of the 26 patients, 17 (13 women) had unipolar and 9 (5 women) bipolar II characteristics. Twenty-eight healthy controls (20 women) were matched with patients on the basis of age, sex, body mass index (BMI or Quetelet index: calculated as weight in kilograms divided by the square of the height in meters), menstrual phase, and birth control status for women (4 women). Controls were required to have no personal or family history of Axis I psychiatric disorder and to be physically healthy. There were no significant differences (control vs SAD) in age (mean age, 46 years; range, 26-51 years vs mean age, 41 years; range, 26-60 years) and BMI (23.9 ± 1.1 vs 26.4 ± 1.2). Patients and healthy subjects were free of psychotropic medications for 1 month prior to the study, free of nonpsychotropic medications for 2 weeks prior to the study, and were allowed no more than 2 caffeinated beverages per day for 2 weeks prior to the study.

STUDY DESIGN

Subjects were studied during the winter (mean date, February 26, 1996 ± 29 days vs mean date, February 26, 1996 ± 32 days) in an untreated condition. Nineteen of the 26 patients with SAD were studied again after 2 weeks of standard light therapy. Of the 7 patients who did not receive light therapy, 3 missed the appointment during which blood for the study was scheduled to be drawn. The other 2 patients had poor treatment compliance, so we did not include them (blood was not withdrawn a second time) because the data would have been misleading. The patients received light therapy (10 000 lux) from a light box, 60 cm × 60 cm (Sun-Box Co, Gaithersburg, Md). The distance from the center of patient’s forehead to center of the box was 30.48 cm. Light therapy was administered at home for 45 minutes, twice per day, once between 6 and 9 AM, and once between 6 and 9 PM, as has previously been described by Terman et al. The summer group of the same patients included 22 patients, as 4 could not be reached. Remission was defined as not meeting criteria for major depression plus having SIGH-SAD scores of less than 10. Nonresponders were defined as meeting criteria for major depression plus having SIGH-SAD scores of more than 15. The same group of healthy volunteers was studied twice: during winter (28 subjects) and during summer (25 subjects; 2 could not be reached and 1 dropped out). Healthy volunteers did not receive light treatment in this study. Patients and healthy volunteers participated in the summer portion of the study between May 28 and July 25, 1996. For each study, 30 mL of blood was drawn in the morning (between 7 and 9 AM) at the Clinical Center of the National Institutes of Health, Bethesda, Md. The SIGH-SAD ratings were administered within 1 day of drawing the blood. Written informed consent was obtained after the procedures had been fully explained to the patients and the healthy subjects.

Several MNL preparations that were shipped from the United States to Israel could not be analyzed owing to technical problems in their preparations. These samples were either substantially contaminated with other cellular elements so that a sufficient quantity of MNL membrane protein could not be achieved in the MNL membrane preparations, or they were nonhomogeneous, forming large and/or dense cellular aggregates that could not be used for our measurements. Therefore, the results show similar Gα, Gβ, and Gγ immunoactivities for the control subjects during winter and summer.
summer: for Gαs, (100.0% ± 20.4%) vs (99.3% ± 18.3%), 2-tailed Wilcoxon statistic (W) = 4, n = 19, P > .05; for Gαi, (100.0% ± 29.5%) vs (97.2% ± 28.4%), W = 30, n = 17, not significant; for Gβb: (100.0% ± 24.1%) vs (102% ± 22.2%), W = −12, n = 15, not significant, Wilcoxon signed rank test).

Figure 2 shows that Gαs and Gαi immunoreactive levels in MNLs of patients with winter depression (71.9% ± 22.4% and 75.9% ± 23.2%, respectively), were significantly reduced in comparison with the respective levels in healthy subjects (100.0% ± 15.8% and 100% ± 7.2%), using both intrablot matched comparisons (for Gαs: W = 195, n = 21, P < .01; for Gαi: W = 221, n = 21, P < .01, Wilcoxon signed rank test) and interblot average comparisons (for Gαs: the Mann-Whitney test statistic [Us] = 319, t0 = 3.99, P < .01; for Gαi: Us = 274, t0 = 2.8, P < .01; Mann-Whitney test). In contrast, MNL Gβ levels of depressed patients with SAD (100.6% ± 16.6%) were similar to levels in healthy volunteers (100.0% ± 9.4%), as calculated using both interblot average comparison (Us = 170, t0 = 0.15, not significant, Mann-Whitney test), and intrablot matched comparisons (W = 37, n = 22, not significant, Wilcoxon signed rank test).

Two weeks of light therapy resulted in clinical remission in patients with SAD with decreases in typical, atypical, and total SIGH-SAD scores (Table). After 2 weeks of light therapy, the reduced Gαs and Gαi levels in the depressed patients with SAD were significantly elevated to normal levels for Gαs: 95.7% ± 24.2%, W = 85, n = 15, P < .02; for Gαi: 103.4% ± 23.6%, W = 90, n = 16, P < .02; Wilcoxon signed rank test), while Gβ levels, (100.3% ± 14.3%) remained similar to the control values obtained for depressed patients with SAD (W = 5, n = 18, not significant, Wilcoxon signed rank test) (Figure 2). The table shows that Gαs and Gαi protein normalization paralleled clinical remission in all treated patients as well as in the subgroups of responders and nonresponders.
During the summer, the SIGH-SAD scores show the patients with SAD to be in remission (Table), with levels of MNL Gα (102.3% ± 19.5%), Gα (102.9% ± 21.7%), and Gβ (101.5% ± 18.7%) similar to levels obtained for healthy subjects (for Gα: Us = 169, t14 = 0.24, not significant; for Gα: Us = 218, t35 = 1.11, not significant; for Us = 170, t35 = 0.25, Mann-Whitney test).

Figure 1. G protein as an information transducer from membrane receptor to intracellular effectors, the cycle of activation and inactivation. Heterotrimeric G proteins are located in the inner side of the cell membrane, playing a pivotal role in signal transduction beyond the receptor. The 3 G subunit proteins are α, β, γ. The α subunit contains the binding site for guanine nucleotides and possesses guanosine triphosphatase activity. The α subunit also contains the site for nicotinamide adenine nucleotide-dependent adenosine diphosphate–ribosylation catalyzed by cholera or pertussis bacterial toxins. The heterogeneity of α subunit serves to divide G proteins into the major classes (Gs, Gi, Gq, etc). The β and γ subunits, which have their functional roles in signal transduction, form a tightly associated complex, which contributes to the receptor recognition site on the G protein oligomer and facilitates the attachment of the oligomer to the inner face of the plasma membrane. When a hormone or a neurotransmitter (H) binds to its specific receptor (R), it forms an activated receptor-G protein (G) complex. This induces guanine nucleotide exchange on the α subunit of G protein so that guanosine diphosphate (GDP) is displaced by guanosine triphosphate (GTP). The binding of GTP induces the dissociation of the G protein. The GTP-bound α subunit interacts with the effector (E) molecule (ie, adenylyl cyclase or phospholipases) and affects its activity in producing respective second messengers (ie, cyclic adenosine monophosphate, inositol triphosphate, diacylglycerol). The GTP-bound α subunit has also intrinsic guanosine diphosphatase (GTPase) activity. The α subunit is then left in an inactive form tightly bound to GDP, and the G protein subunits reassociate. The rate-limiting step in this cycle is the release of GDP from the α subunit that is catalyzed through the activated receptor. Thus, G protein cycles between an inactive, GDP-liganded oligomeric form (“off” position), and an active, GTP-liganded monomeric state (“on” position).

Figure 2. The effect of light therapy on the immunoreactivity of various G protein subunits in the mononuclear leukocyte levels of patients with seasonal affective disorder. The relative immunoreactivities of Gα (left panel), Gα (middle panel), and Gβ (right panel) determined in the mononuclear leukocytes were obtained from patients with seasonal affective disorder while depressed; 21 were examined for Gα and Gα, and 22 for Gβ (open circles). After 2 weeks of light therapy, 15 were evaluated for Gα, 16 for Gα, and 19 for Gβ (closed circles) as compared with normal subjects for which 21 were assessed for Gα, 17 for Gα, and 15 for Gβ (open squares).

A major finding of this study is the description of reduced levels of Gα and Gα subunit proteins in MNLs of patients with SAD winter, atypical-type depression. These findings are compatible with previous studies in patients with typical major depression.24,28,30 The G protein abnormalities detected in this study in depressed patients with SAD seem to be a state rather than a trait marker of SAD since (1) the same patients examined for their MNL G protein levels in the summer, while in remission, did not show statistically significant alterations when compared with healthy control subjects; and (2) light therapy resulted in normalization of the reduced G protein immunoreactivity detected in the same patients while depressed. The results of this study are consistent with our earlier reports of MNL G protein measures as a state characteristic of mood disorders: (1) inverse picture of MNL G protein measures in bipolar mood disorder with respective increases in mania and decreases in bipolar depression23,24,28,30; and (2) normalization of MNL G protein measures in patients with mood disorders with lithium,23 antidepressants, and electroconvulsive therapy.33

The mechanisms underlying the alterations in G protein levels in MNL of depressed patients with SAD and their normalization by light are still unknown. Increasing evidence indicates the existence of neural-mediated immunomodulatory mechanisms26 involving the hypothalamic-pituitary-adrenal axis and the sympathetic and parasympathetic innervation of primary and secondary lymphoid organs.37 These mechanisms may modulate MNL G proteins. Thus, MNL G protein alterations may reflect secondary influences of circulatory primary messengers, altered by the depressive state, or secondary influences of altered sympathetic and parasympathetic innervation of lymphoid organs induced by the depressive state.
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: findings obtained in peripheral blood cells cannot be directly extrapolated to the central nervous system. We have discussed this issue at length previously. 

As we use a mixed-cell MNL preparation for our assays, the possibility remains that the alterations observed in G protein immunoreactivity reflect, at least in part, alteration in a white cell subpopulation induced by the depressive state and/or by light therapy. While Gs and Gα levels were reduced in the group of depressed patients with SAD, the Gβ levels remained similar to the control group. Such differential alterations would not be expected to occur owing to alterations in a white cell subpopulation.

If changes observed in MNL G protein levels in this study do reflect alterations in brain G proteins, a possible candidate may be transducin (Gt protein), which connects rhodopsin with a retinal phosphodiesterase regulating cyclic guanosine monophosphate, sodium permeability of the rod outer segment membrane, and, consequently, the electroretinogram (ERG). It has been shown that lithium can decrease ERG amplitude and that these effects are related to inhibition of transducin, similar to the inhibition of Gs and G protein reported previously. Indeed, ERG measurements have been conducted in SAD, indicating either subtle retinal changes in flash ERG or no changes in pattern ERG in depressed patients with SAD. The findings in this study of reduced MNL G protein levels in depressed patients with SAD may explain the reported subsensitivity to light in depressed patients with SAD by conjecturing reduced levels of transducin (Gt) in depressed patients with SAD. Light therapy found in this study to normalize the reduced levels of MNL G proteins in depressed patients with SAD may exert its effects centrally through possible normalization of sup- posed Gt protein hypofunction.

Another state marker of depressed patients with SAD is their abnormal response to the somewhat selective 5-hydroxytryptamine receptor subtype. The reported activation and euphoria seen in depressed patients with SAD, but not in healthy controls, following administration of meta-chlorophenylpiperazine is normalized both after effective light therapy and in the summer. In this regard, the meta-chlorophenylpiperazine findings resemble those in this study.

Most 5-hydroxytryptamine receptor subtypes are G protein-coupled, including 5-hydroxytryptamine receptor. It is possible that both light therapy and summer, which reverse the depressive symptoms of SAD, may also normalize G protein levels in both the brain and the periphery. Such putative normalization may be of relevance to the pathogenesis of symptoms.

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